A study of the WKY as a rat model of depression

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by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted to any university for a degree

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Signature Date

Dedicated to my parents who supported me through my study and God who gave me the opportunities to pursue my interest in research.

Abstract

Major depression is a heterogeneous neuropsychiatric disorder with a significant genetic-stress interaction. The Wistar-Kyoto (WKY) rat displays hypersensitivity to stress and depression-like behaviour and is used as a genetic model of major depression. However, the depression- and anxiety-like behaviour of WKY has not yet been compared between the WKY/NCrl and WKY/NHsd substrains when characterizing WKY as a rat model of depression. WKY rats respond to noradrenergic and dopaminergic drugs but not to selective serotonin reuptake inhibitors (SSRIs) and are therefore suggested to model treatment resistant depression. The early life stress of maternal separation (MS) has been used to produce a rodent model of depression in Sprague-Dawley (SD) rats but results have been variable. It was therefore considered that WKY subjected to MS might produce a more robust model of depression than either WKY or MS alone. The widely used MS SD rat model of depression, as well as MS SD rats subjected to restraint stress in adult life, were evaluated as appropriate comparator models of depression. Furthermore, changes in the biochemistry in relevant brain areas of MS SD rats exposed to restraint stress in adulthood is still elusive and was further explored. The glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, has been found to be clinically useful. The acute effects of ketamine have not been previously tested in male WKY or MS SD rats and it was therefore decided to study the behavioural effects of ketamine in these rat models of depression.

The aim of the first study was to characterize the WKY rat model of depression and to select the appropriate substrain of WKY best suited as a model of depression. The WKY/NCrl and WKY/NHsd substrains of WKY were tested for optimal depression-/anxiety-like behaviour in the forced swim test (FST), open field test (OFT) and elevated plus maze (EPM) and compared to the Wistar reference strain. Both WKY/NCrl and WKY/NHsd were less active than Wistar rats in the OFT and FST and WKY/NCrl were less active than WKY/ NHsd. Therefore, the initial study identified WKY/NCrl as the appropriate substrain of WKY to model depression. The WKY/NCrl rats were further characterized in terms of their response to an optimal dose of the antidepressant drug, desipramine in the FST. Desipramine has been shown to be effective in reducing the depression-like behaviour of WKY and was therefore chosen as the antidepressant drug for this study. A dose of 15 mg/kg desipramine attenuated the depression-like behaviour as evidenced by decreased immobility in the FST and was therefore used for subsequent experiments. Desipramine had no effect on opioid receptors (μ- and κ-opioid receptors,

MOR and KOR, respectively), and tyrosine hydroxylase in the nucleus accumbens (NAc) or prefrontal cortex (PFC) of WKY rats.

Since MS is a well-accepted animal model of depression, the aim of the second study was to create a more robust model of depression by subjecting WKY rats to MS and determining their depression-like behaviour, as well as its reversal with chronic desipramine treatment and the accompanying neurochemical changes following treatment. Depression-like behaviour was recorded in the OFT, FST, and isolation-induced ultrasonic vocalizations (USVs), in WKY/NCrl and MS (removal of the pups from the dam for 3 h per day from P2 to P14) WKY/NCrl rats. Wistar rats served as the reference strain. The normally reared WKY/NCrl, MS WKY/NCrl and Wistar rats were injected intraperitoneally with either saline or desipramine (15 mg/kg/day) for 15 days and their behaviour recorded. Similar to the first study, WKY/NCrl and MS WKY/NCrl rats displayed increased immobility and decreased active swimming and struggling behaviours in the FST, and decreased activity in the OFT compared to Wistar rats. In addition, MS WKY/NCrl spent more time in the closed arms of the EPM than normally reared WKY/NCrl, and WKY rats emitted more USVs than Wistar rats in response to removal of cage mate(s). Desipramine treatment decreased immobility and increased active swimming and struggling behaviour of WKY/NCrl in the FST and also decreased their USVs in response to removal of cage mate(s). Furthermore, MS WKY/NCrl responded to the anxiolytic effects of desipramine as evidenced by the increased amount of time spent in the open arms of the EPM. Therefore, MS WKY/NCrl rats displayed depression- and anxiety-like behaviour that responded to the antidepressant and anxiolytic effect of desipramine in the FST and EPM. This therefore implied that MS WKY/NCrl rats provided a more robust model of depression and anxiety. The increased number of USVs emitted by WKY/ NCrl compared to Wistar rats and decreased number of USVs after desipramine treatment, suggest that USVs occurred in response to an aversive situation (social isolation) that, in accordance with its communication function, may represent social signalling to re-establish social contact with the cage mate(s). In this study, USVs served as a useful marker of depression-/anxiety-like behaviour, with the rats responding to antidepressant treatment.

To determine the effect of chronic desipramine treatment on the neurochemistry in the brain of MS WKY rats, changes in dopamine, serotonin (determined by enzyme-linked immunosorbent assay (ELISA)), opioid receptor density (MOR and KOR), phosphorylated extracellular signal-regulated kinase (p-ERK) and phosphorylated glycogen synthase kinase 3β (p-GSK3β) (determined by polyacrylamide gel electrophoresis and western blotting) were measured in MS WKY/NCrl and normally

reared WKY/NCrl rats. Desipramine treatment increased the density of p-GSK3β in the PFC of normally reared WKY/NCrl rats but did not affect MS WKY/NCrl rats. This study provides novel evidence for the mechanism of action of desipramine and the possible role of p-GSK3β in mediating the reduction of depression-/anxiety-like behaviour of WKY/NCrl rats. However, desipramine had no effect on serotonin and p-ERK levels in the PFC or dopamine and opioid receptors in the NAc.

The aim of the third study was to determine if stress during adulthood can exaggerate the depression-/anxiety-like behaviour in the widely used MS SD rat model. Furthermore, to determine changes in brain-derived neurotrophic factor (BDNF) levels in the ventral hippocampus and protein profile in the PFC of MS SD rats subjected to stress in adulthood. Depression-like behaviour was measured in the EPM, OFT and FST in the MS SD rats exposed to chronic restraint stress (4 h per day for 5 days) in adulthood. Changes in BDNF concentration (measured with an ELISA) was measured in the ventral hippocampus in SD rats subjected to MS and restraint stress followed by proteomic analysis of the PFC. As expected, MS increased immobility of SD rats in the FST but restraint stress did not enhance the effect of MS on SD rats. MS on its own induced depression-like behaviour but restraint stress was unable to further potentiate the depression-like behaviour. It is suggested that since MS during early development causes a disruption in the hypothalamic-pituitary-adrenal axis (HPA axis) and long-term changes in the response to subsequent stress, it may have prevented restraint stress from exerting its depression-like behavioural effect. Furthermore, MS and restraint stress had no effect on BDNF levels in the ventral hippocampus but proteomic analysis of the PFC in SD rats revealed a decrease in actin-related proteins in MS rats and non-separated rats subjected to restraint stress as well as a general decrease in mitochondrial energy-related proteins in MS rats with or without subsequent exposure to restraint stress and non-separated rats subjected to restraint stress. Furthermore, a decrease in proteins involved in protein synthesis and an increase in proteins involved in protein degradation were found in rats subjected to both MS and restraint stress.

The aim of the final study was to determine whether the depression-like behaviour of the rodent models of depression could also be reversed by acute treatment with ketamine. The WKY/NCrl and Wistar rats as well as non-separated SD and MS SD rats were injected intraperitoneally with a single dose of either saline or ketamine (5, 10 or 15 mg/kg). Isolation-induced USVs (4 days before and 5 h and 29 h after injection of ketamine/saline) were recorded as well as their behaviour in the OFT (22 h and 46 h after injection of ketamine/saline) and FST (2 h, 48 h and 72 h after injection of ketamine/saline). Contrary to expectation, ketamine at a dose of 10 mg/kg increased

immobility and decreased swimming behaviour of WKY/NCrl in the FST but did not affect Wistar or MS SD rats. It is suggested that the ketamine-induced immobility in WKY/NCrl rats may be related to their reduced NMDA receptor density previously found in WKY rats.

In conclusion, this study is consistent with the use of the WKY/NCrl rat as a model of depression. MS increased the anxiety-like behaviour of the WKY/NCrl, thus providing a more robust model to study depression- and anxiety-related behaviour. WKY/NCrl rats responded to chronic desipramine treatment by normalizing their depression-/anxiety-like behaviour, decreasing their USVs and increasing p-GSK3β; an effect that was blocked by MS. The USVs emitted appeared to reflect signalling in an attempt to re-establish social contact with cage mate(s) that had been removed from the home cage. The MS SD rats showed depression-like behaviour in the FST that was unaffected by additional restraint stress. However, the decrease in structural proteins and proteins related to energy metabolism as well as the decrease in proteins related to protein synthesis and increase in proteins related to protein degradation in MS rats with or without subsequent restraint stress may not be related to their depression-like behaviour. Consistent with their selective response to antidepressant drug treatment, WKY/NCrl rats did not respond to the antidepressant effect of a single dose of ketamine which may be related to the dysfunction of the NMDA receptor.

Publications and conference proceedings

Some of the results in this study were published or presented at two national conferences and an international conference.

Publications

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Conference proceedings

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Abbreviations

ACTH	adrenocorticotropic hormone
BDNF	Brain-derived Neurotrophic Factor
CSF	cerebrospinal fluid
DAT	dopamine transporter
DOR	δ-opioid receptor
ELISA	enzyme-linked immunosorbent assay
EPM	elevated plus maze
ERK	extracellular signal-regulated kinase
FM	frequency modulated
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
FST	forced swim test
GSK	glycogen synthase kinase
HPA axis	hypothalamic-pituitary-adrenal axis
iTRAQ	isobaric tagging for relative and absolute quantification
KOR	κ-opioid receptor
MOR	μ-opioid receptor
MS	maternal separation or maternally separated
NAc	nucleus accumbens
NAcC	nucleus accumbens core
NAcS	nucleus accumbens shell
NMDA	N-methyl-D-aspartate

OFT	open field test
P	Postnatal day
p-ERK	phosphorylated extracellular signal-regulated kinase
PFC	prefrontal cortex
p-GSK	phosphorylated glycogen synthase kinase
SD	Sprague-Dawley
SSRIs	selective serotonin reuptake inhibitors
TH	tyrosine hydroxylase
USVs	ultrasonic vocalizations
WKY	Wistar-Kyoto

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Chapter 1

Introduction

1.1 Neurobiology of depression

1.1.1 Major depression

According to the Diagnostic and Statistical Manual of Mental Disorders V (DSM V), depressive disorders feature the presence of sad, empty or irritable mood accompanied by somatic and cognitive changes that significantly affect the individual‟s capacity to function (American Psychiatric Association 2013). Depressive disorders can be divided into groups according to duration, timing or presumed etiology. These include disruptive mood dysregulation disorder, major depressive disorder, persistent depressive disorder (dysthymia), premenstrual dysphoric disorder, substance/medication-induced depressive disorder, depressive disorder due to another medical condition, other specified depressive disorder and unspecified depressive disorder (American Psychiatric Association 2013). Major depressive disorder (unipolar) represents the characteristic condition in this group of disorders and will be the focus of the current study.

Major depression is a multifaceted and heterogeneous disorder that is highly prevalent and activated by a complex interaction between genetic, developmental and environmental factors (Millan 2006). The most characteristic symptoms are depressed mood (sadness) and the inability to experience pleasure (anhedonia) as discussed below (section 1.1.2).

1.1.2 Symptoms and diagnosis

People diagnosed with depression usually experience a combination of symptoms that can be divided into core symptoms, subsidiary symptoms and co-morbid symptoms/conditions (Fig 1.1). For major depression to be recognized according to the DSM V (2013), five (or more) of the symptoms in figure 1.1 (core symptoms and subsidiary symptoms) should be present during the same 2-week period and a change from previous functioning. At least one of the symptoms should reflect depressed mood or the loss of interest in pleasure (anhedonia) (American Psychiatric Association 2013). The rest of the symptoms should consist of subsidiary symptoms ranging from the inability to think or concentrate,

to recurrent thoughts of death. Despite the diagnostic symptoms, patients often present with non-diagnostic symptoms and conditions that further complicate diagnosis (see fig 1.1). In addition, to further complicate diagnosis, individual patients may experience different subsets of symptoms which may change over time (Millan 2006).

The various symptoms of depression therefore emphasize the multifaceted and heterogeneous nature of depression. This is further exemplified by the co-morbidity with other disorders such as anxiety (Brunello et al. 2000; Thaipisuttikul et al. 2014), psychotic disorder (Thaipisuttikul et al. 2014) and Parkinson‟s disease (Shen et al. 2013) that share certain symptoms of major depression. The co-morbid conditions are not only psychiatrically related but may also include diabetes mellitus, cardiovascular disease, osteoporosis, hepatitis and chronic pain (Millan 2006).

To differentiate between various mood disorders that require different treatment, the DSM V further divided major depression into nine subgroups according to different subsets of symptoms experienced (American Psychiatric Association 2013):

1 Anxious distress: Feeling keyed up or tense, unusually restless, difficulty concentrating because of worry, fear that something awful may happen and feeling that the individual might lose control of himself/herself.

2 Mixed features: Elevated mood, inflated self-esteem, more talkative, increased energy, increased or excessive involvement in activities that have a high potential for painful consequences, decreased need for sleep and flight of ideas or subjective experience.

3 Melancholic: The loss of pleasure in nearly all activities, unchanging emotional expression, feelings of excessive guilt, waking too early in the morning (terminal insomnia) and significant anorexia or weight loss.

4 Atypical features: Characterized by elevated mood in reaction to positive events and rejection sensitivity. These reactions lead to overreaction to criticism or rejection. Hypersomnia and increased appetite and consequently weight gain can also be present.

5 Psychotic features: Delusions and/or hallucinations are present.

6 Catatonia: Characterized by severe psychomotor retardation or excessive activity (agitation)

7 Peripartum onset: If onset of mood symptoms occurs during pregnancy or in the first 4 weeks following delivery of the baby

8 Seasonal pattern: Major depression occurs during a particular time of the year.

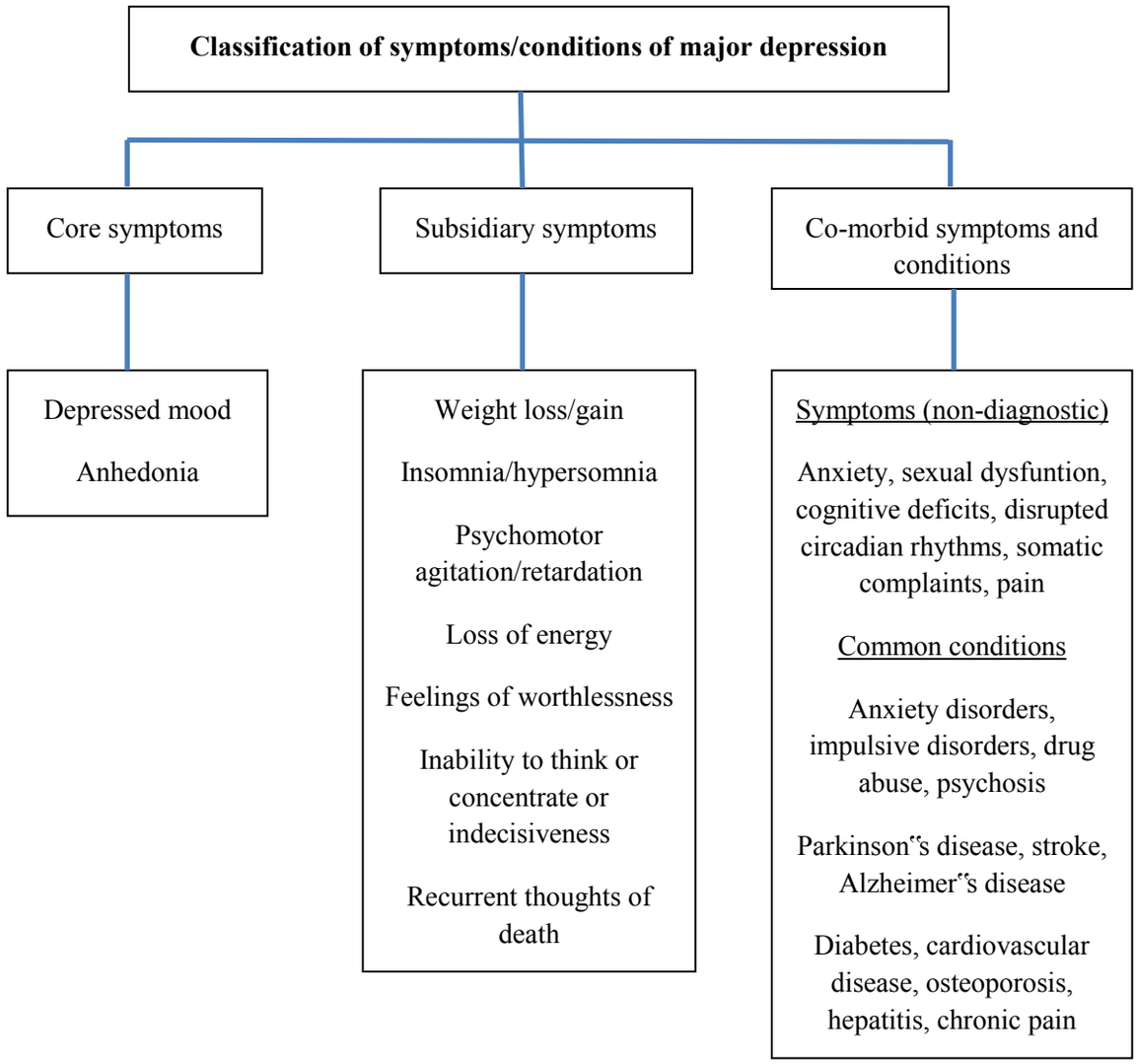


Figure 1.1: Classification of symptoms/conditions of major depression. Major depression is diagnosed by at least one core symptom and any four (or three if both core symptoms are present) subsidiary symptoms. The major symptoms of major depression are almost never present on their own and accompanied by additional non-diagnostic symptoms and underlying conditions. Adapted from American Psychiatric Association (2013) and Millan (2006).

1.1.3 Epidemiology and etiology

Major depression is the second leading cause of years of life lived with disability (YLD) and the eleventh leading cause of global burden of disability-adjusted life years (DALYs) according to the findings from the Global Burden of Disease study in 2010 (Ferrari et al. 2013; Murray et al. 2013). Moreover, it is projected to be the second leading cause of DALYs in the year 2030 (Mathers and Loncar 2006; Pike et al. 2013). According to an assessment of the National Comorbidity Survey Replication (Kessler and Merikangas 2004), major depression is highly prevalent with a lifetime prevalence of approximately 17 % and related to high morbidity and mortality (Kessler et al. 2005). The lifetime prevalence of major depression in high-income and low- to middle-income countries was estimated to be 6.6 % - 21 % and 6.5 % - 18.4 %, respectively (Kessler and Bromet 2013). Major depression is also more prevalent in younger people since the prevalence in 18 – 29 year old individuals was threefold higher than the prevalence in individuals over 60 years of age (American Psychiatric Association 2013; Kessler et al. 2003). Furthermore, women have an approximately twofold increased risk of major depression compared to men, but only during their child-bearing years (Kessler and Bromet 2013; Setse et al. 2009; Van de Velde et al. 2010).

Major depressive disorder may first appear at any age as a function of genetic and developmental predisposition as well as adverse life-events (Kessler et al. 2003; Kinyanda et al. 2013; Richards and Salamanca Sanabria 2014). An epidemiological study showed a 37 % genetic risk to develop depression (Bienvenu et al. 2011; Sullivan et al. 2000) which makes depression a highly heritable disorder. In addition, stressful life events such as job loss, marital difficulties, major health problems and loss of close personal relationships has been associated with the development of depression (Berchick et al. 2012; Fava and Kendler 2000; Galatzer-Levy and Bonanno 2012; Kessler 1997; Symoens et al. 2014; Tseng et al. 2014; Waaktaar et al. 2004). Furthermore, developmental stress such as adverse childhood experiences that include physical and sexual abuse, poor parent-child relationships and parental discord and divorce all increase the risk for development of depression in adulthood (Chapman et al. 2004; Fava and Kendler 2000; Larson and Halfon 2013; Lindert et al. 2014; Raudino et al. 2013). The genetic and early environmental risk factors are also mediated by personality of neuroticism and high levels have been shown to directly influence anxiety and depression by increasing the risk for development of depressive episodes in response to stressful experiences (Kendler and Gardner 2011).

Major depression is not limited to adult and elderly people since depression is often diagnosed in adolescents and children (Keith 2013; Klein et al. 1999a; Vogel 2012;

Weissman et al. 1999; Yorbik et al. 2004). Early-onset depression is a serious concern. It is associated with a longer duration of the major depressive episode, increased severity of symptoms, higher rates of recurrent depressive episodes, co-morbid psychiatric disorders and lifetime substance use disorders (Klein et al. 1999a; Klein et al. 1999b; Korten et al. 2012; Park et al. 2014; Sihvola et al. 2008; Zisook et al. 2007). Recovery is strongly dependent on the duration of the current depressive episode. Individuals who have been depressed for a short period of time can recover spontaneously (American Psychiatric Association 2013). This emphasizes the need for early treatment since remission is difficult for individuals who have a longer duration of untreated episodes or multiple recurrent depressive episodes (Fekadu et al. 2009; Ghio et al. 2014; Kurdyak and Cairney 2011). Other features associated with reduced recovery in patients with depression include symptom severity (Riedel et al. 2011; Riihimäki et al. 2014; Swindle, Jr. et al. 1998) and the co-morbid conditions (as described in section 1.1.2) such as psychotic features (Buoli et al. 2013), anxiety (Agosti 2014; Szádóczky et al. 2004) and personality disorders (Agosti et al. 2009; Agosti 2014; Holma et al. 2008).

1.1.4 Neuroanatomy

The heterogeneous nature of depression validates the proposal that the symptoms of depression are mediated by neural networks involving several brain regions. Indeed, neuroimaging data implicated a key role of the prefrontal cortex (PFC) and its connections to the amygdala, hippocampus, and ventral striatum, sites in the diencephalon such as the hypothalamus and brainstem in the pathophysiology of depression (Furman et al. 2011; Ongür and Price 2000; Salerian and Altar 2012).

The nucleus accumbens (NAc), as part of the ventral striatum, is associated with motivation, reward, motor activity and learning (Day and Carelli 2007; Shirayama and Chaki 2006). As illustrated in figure 1.2, the NAc receives afferent connections from various cortical and limbic structures such as the PFC (Brog et al. 1993) and amygdala (Brog et al. 1993; Wright et al. 1996), hippocampus (Brog et al. 1993; Groenewegen et al. 1987) and the ventral tegmental area of the midbrain (Zahm and Brog 1992). The ventral tegmental area sends dopamine projections to the NAc and other limbic areas such as the amygdala and hippocampus as well as the PFC (Kahn and Shohamy 2013; Pierce and Kumaresan 2006; Yetnikoff et al. 2014). These dopamine projections form the mesocorticolimbic pathway and are implicated in the pathogenesis and treatment of depression (Yadid and Friedman 2008). The NAc also receives noradrenergic and serotonergic input from the locus coeruleus and dorsal raphe nuclei, respectively (Ferrucci et al. 2013; Moret and Briley 2011; Shirayama and Chaki 2006; Tao and Ma 2012). The locus coeruleus neurons project from the brain stem to the thalamus, dorsal hypothalamus,

hippocampus, amygdala, cerebellum and cerebral cortex (Leonard 2003; Moret and Briley 2011). These brain areas are involved in emotion, cognition, as well as other functions affected in depression such as appetite, response to pain, levels of pleasure, sexual function and aggressive behaviour (Moret and Briley 2011). The serotonergic neurons in the dorsal raphe nuclei project to limbic structures such as the ventral hippocampus, amygdala, lateral septum and the striatum and cerebral cortex (Fukuyama et al. 2013; Lanzenberger et al. 2012). On the other hand, the median raphe nuclei project preferably to the temporal cortex, mammillary bodies, dorsal hippocampus and medial septum (Lanzenberger et al. 2012). Furthermore, the NAc receives glutamatergic afferents from the hippocampus, amygdala and PFC (Britt et al. 2012; Shirayama and Chaki 2006).

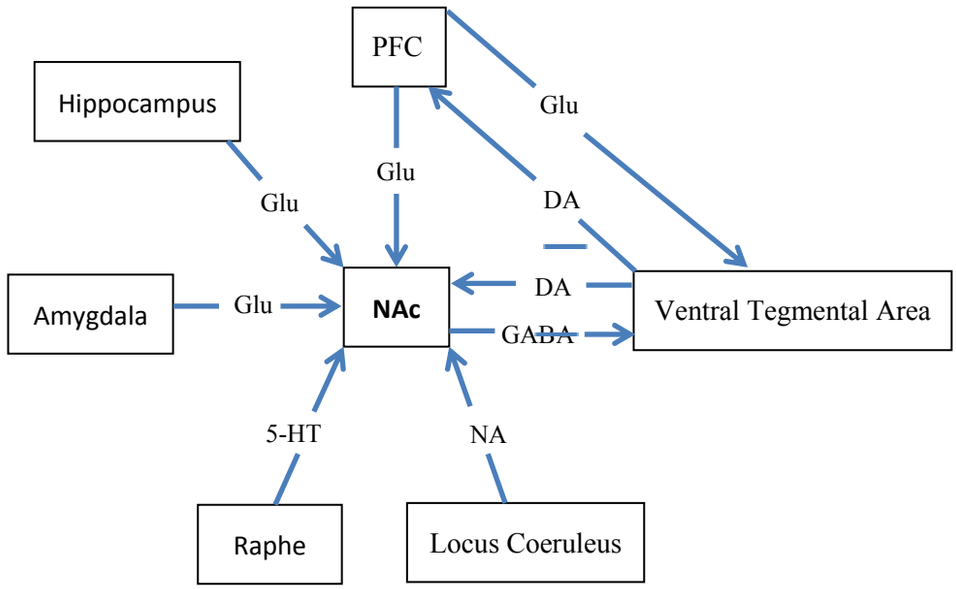


Figure 1.2: Connectivity of the NAc with other limbic brain areas associated with the pathophysiology of depression. Adapted from Shirayama and Chaki (2006).

The NAc contains two primary subregions, the central core and peripheral shell, with different anatomic organization and functional properties (Sturm et al. 2003). The NAc core (NAcC) is associated with extrapyramidal motor function and the NAc shell (NAcS) with the limbic system that has been associated with the pathophysiology of depression (Abosch and Cosgrove 2008; Sturm et al. 2003). The NAcC and NAcS receive different projections from the PFC and hippocampus as illustrated in figure 1.3 and possibly

relating to their different functional properties. The prelimbic area of the PFC projects throughout the NAcC and NAcS but is associated more with the NAcC whereas the infralimbic area projects selectively to the NAcS (Berendse et al. 1992; Chiba et al. 2001; Hayen et al. 2014; Vertes 2004). Furthermore, the NAcC receives afferent projections from the dorsal hippocampus whereas the NAcS receives afferent projections from the ventral hippocampus (Behrendt 2011) (Figure 1.3). Likewise, the efferent projections from the NAc core and shell are different. The NAcC projects to the pre-motor cortical areas through the motor thalamus whereas the NAcS projects to subcortical limbic regions such as the lateral hypothalamus, ventral pallidum and ventral tegmental area (Groenewegen and Trimble 2007; Zahm 1999). Also, direct connections between the NAcC and NAcS have been established indicating that these regions do not function completely separate from each other (Day and Carelli 2007; van Dongen et al. 2006). Therefore, both the NAcC and NAcS play a central role in connectivity between various limbic structures.

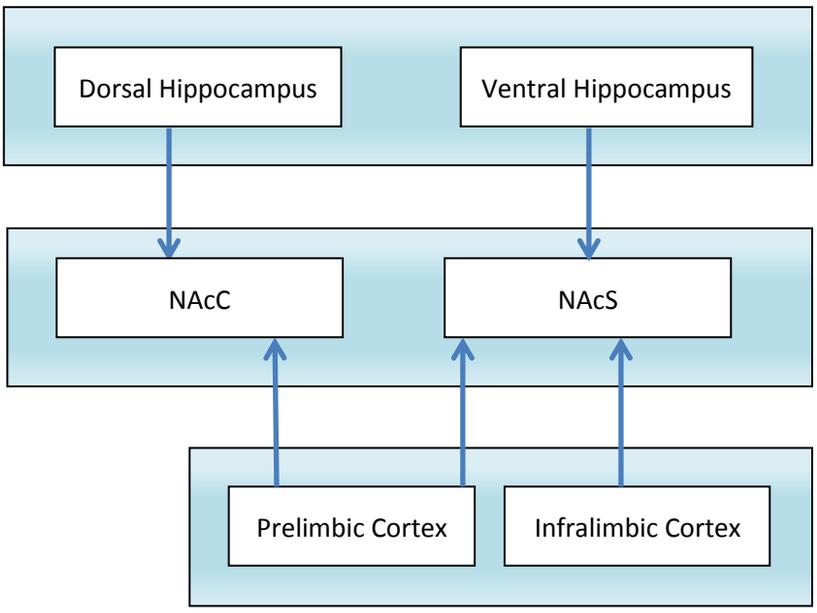


Figure 1.3: Connections between the NAcC and NAcS and subregions of the hippocampus and PFC. Adapted from Shirayama and Shigeyuki (2006).

Neuroimaging studies measuring blood flow and metabolism in patients with depression showed abnormalities in several brain areas such as the cortex and limbic regions (Salerian and Altar 2012). However, most of the studies were conducted in the cortical

and paralimbic areas and showed reduced blood flow and glucose metabolism in these areas of patients with depression (Fujimoto et al. 2008; Gonul et al. 2004; Kimbrell et al. 2002; Kohn et al. 2007; Martinot et al. 2011; Mayberg et al. 1994; Oda et al. 2003; Ota et al. 2014; Wang et al. 2014).

Further analysis was able to correlate the hypo-function of the PFC to the symptoms of depression as evidenced by studies showing that psychomotor impairment is associated with reduced blood flow in the PFC (Bennabi et al. 2013; Dolan et al. 1993; Narita et al. 2004; Videbech et al. 2002; Walther et al. 2012). Furthermore, treatment response to repetitive transcranial magnetic stimulation (rTMS) correlated with cerebral blood flow and may be a potential predictor of treatment response (Kito et al. 2012). However, studies showing evidence of abnormalities in limbic regions were less common. These studies showed increased metabolism or blood flow in the amygdala (Drevets et al. 2002; Martinot et al. 2011), basal ganglia and hippocampus (Monkul et al. 2012; Videbech et al. 2002) demonstrating that limbic and cortical brain function are differentially regulated.

Patients with depression also manifest structural abnormalities in brain areas, mostly in the hippocampus and PFC. Numerous studies have shown a decrease in volume of the hippocampus and PFC in patients with depression (Campbell et al. 2004; Frodl et al. 2002; Frodl et al. 2010; Kumar et al. 1998; Ming-Hong Hsieh et al. 2002; Ozalay et al. 2013; Sawyer et al. 2012; Shah et al. 1998; Sheline et al. 1996; Taylor et al. 2014). Furthermore, the duration or severity of depression correlated with the hippocampal volume loss (Sheline et al. 1996; Stratmann et al. 2014; Taylor et al. 2014), which appeared reversible following antidepressant drug treatment (Malykhin et al. 2010; Sheline et al. 2003). It is widely recognized that stress associated with the development of depression is implicated in the reduced structure of these brain areas. This was evidenced by clinical studies showing that childhood maltreatment, which contributed in the development of depression, decreased hippocampal and PFC volume (Frodl et al. 2010; van Harmelen et al. 2010).

Animal studies have similarly shown that prolonged stress and increased levels of glucocorticoids result in neuronal atrophy and decreased hippocampal neurogenesis (reviewed in Duman 2004; Duman 2014). Evidence for neuronal atrophy or decreased neurogenesis has been previously reported in rats exposed to various stressors such as exposure to a predator or its odor (Alani et al. 2013; Falconer and Galea 2003; Tanapat et al. 2001), chronic restraint stress (Grillo et al. 2014; Pham et al. 2003; Sliwowska et al. 2010), footshock stress (Kim and Seo 2013; Malberg and Duman 2003), chronic mild stress (Jayatissa et al. 2010; Jiang et al. 2014) and the stress of maternal separation (MS)

in early life (Baek et al. 2012; Hulshof et al. 2011). The stress response is regulated by the hypothalamic-pituitary-adrenal (HPA) axis that seeks to maintain homeostasis and therefore minimize the impact of threat on the brain (Herman 2013; McEwen 2007). Administration of adrenal glucocorticoids as well as excitatory neurotransmitters such as glutamate in rats decreased neurogenesis in the hippocampus, thereby mimicking the effects of stress and implicating the HPA axis functionality in depression (Cameron et al. 1998; Gould and Cameron 1996; McEwen 1999; Popoli et al. 2012). NMDA receptor blockade is also effective in preventing stress-induced dendritic atrophy in the PFC (Li et al. 2011; Martin and Wellman 2011).

Therefore, the role of the cortico-limbic brain areas together with their neurocircuitry is well recognized in the pathophysiology of depression although the precise role and interaction of these structures in depression is not well defined. The next section will focus on the neurochemistry in these brain areas related to depression.

1.1.5 Neurochemistry

1.1.5.1 Evidence that led to the monoamine hypothesis of depression

The monoamines noradrenaline, dopamine and serotonin are neurotransmitters that have been widely recognized to be involved in the pathophysiology of depression. The monoamine theory of depression was developed according to the known mechanisms of antidepressant drug action. Most of the antidepressant drugs available (e.g. tricyclic and selective serotonin reuptake inhibitors; section 1.4.1) block the reuptake of certain monoamines, thereby increasing the concentration of neurotransmitter at the synapse. On the other hand, drugs such as reserpine, that cause a depletion of brain monoamines, may induce depression (Elhwuegi 2004; Goodwin and Bunney 1971). The mechanism of action of reserpine and antidepressant drugs led to the monoamine hypothesis that simply states that depression is due to a deficiency of synaptic monoamines and is treated with antidepressant drugs that increase the monoamine activity (Baumeister et al. 2003; Schildkraut 1967). However, antidepressant drugs are expected to be effective in alleviating symptoms of depression after 2-4 weeks (Wells et al. 2003), suggesting that their effects are more complex than initially thought.

The noradrenergic system is involved in drive, motivation, reward and in rapid eye movement (REM) sleep (Leonard 2003) which are all aspects affected in depression. Various clinical studies have shown abnormalities in the noradrenergic system in postmortem tissue of patients that had depression. Previous studies indicate increased β-adrenergic receptor density in the temporal cortex and PFC of suicide victims that had

suffered from depression (Arango et al. 1990; Biegon and Israeli 1988; Mann et al. 1986; Rivero et al. 2014). In addition, postmortem and functional imaging studies of suicide victims showed increased density and expression of α2-adrenoceptors in the locus coeruleus and PFC (Escribá et al. 2004; Furczyk et al. 2013; Ordway et al. 2003; Rivero et al. 2014). Further support for a role for these noradrenergic receptors in the pathophysiology and treatment of depression, came from previous studies that showed that antidepressant drug treatment decreased the levels of α2-adrenoceptors in the occipital cortex and hippocampus of patients that had depression (De Paermentier et al. 1997) as well as blood platelets of patients with depression (García-Sevilla et al. 2004). Furthermore, a decrease in noradrenaline transporter binding (Klimek et al. 1997) and elevated levels of tyrosine hydroxylase (TH) (Ordway et al. 1994; Zhu et al. 1999) have been reported in the locus coeruleus of postmortem brain tissue.

Similarly, dopamine plays an important role in the pathophysiology of depression and is associated with motivation, psychomotor control, concentration and the ability to experience pleasure (Dunlop and Nemeroff 2007). The majority of clinical studies measuring the concentration of homovanillic acid, a dopamine metabolite, reports lower cerebrospinal fluid (CSF) and plasma concentrations in patients with depression compared with the controls (Engström et al. 1999; Mitani et al. 2006a; Reddy et al. 1992;Roy et al. 1989) therefore indicating reduced dopamine function. However, other studies failed to achieve similar results (Aklillu et al. 2009; Sullivan et al. 2006; Vestergaard et al. 1978). Additionally, pharmacological depletion or blocking synthesis of monoamines such dopamine can induce symptoms of depression in patients in remission or in healthy individuals with a family history of depression (Hasler et al. 2008; Pizzagalli 2014; Ruhé et al. 2007).

Serotonin (5-HT) modulates a variety of behavioural and neuropsychological processes such as mood, perception, reward, aggression, appetite, sleep, memory, sexuality and attention (Berger et al. 2009; Mück-Seler and Pivac 2011). Serotonergic activity is usually measured as the serotonin concentration and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in CSF and blood samples of patients with depression. Regardless of the monoamine hypothesis of depression, results are rather conflicting (Mück-Seler and Pivac 2011) indicating increased serotonin and 5-HIAA levels in the plasma (Barton et al. 2008; Mitani et al. 2006a) and decreased (Mann et al. 1996; Träskman-Bendz et al. 1984; Zhang et al. 2014) or no difference of 5-HIAA in the CSF or plasma of patients with depression (De Bellis et al. 1993; Engström et al. 1999). In addition, the 5-HT_{1A} and 5-HT_2 serotonin receptors have also been found to be disrupted in patients with depression. However, previous studies have also revealed contradictory results in both these receptors in blood

samples or postmortem brain tissue such as the PFC, hippocampus and amygdala of patients with depression (Arora and Meltzer 1989; Cheetham et al. 1988; Cheetham et al. 1990; Lowther et al. 1997; Matsubara et al. 1991; Stockmeier et al. 1997; Yates et al. 1990; Zhang et al. 2014). The reason for variability between clinical studies that may complicate comparison of serotonin activity may include methodological variability such as differences in region (brain tissue, plasma, CSF), severity of depression (e.g. suicide attempters vs. non-attempters), medication history of the patients (medicated vs. drug-free patients) as well as the diagnostic criteria used to diagnose depression. For example, the diagnoses in some studies that measured serotonin activity included patients with co-morbid conditions such as anxiety and schizoaffective disorders (Barton et al. 2008; Mann et al. 1996) and serotonin activity may therefore be affected by the subtype of depression (Mück-Seler et al. 1991). However, despite these variations between studies, the monoamine hypothesis is widely accepted. It has received support from studies that suggest hypo-activity of monoamines (Wainwright and Galea 2013; Yadid et al. 2000) and lowered mood in patients in remission following serotonin depletion (tryptophan depletion) (Hasler 2010; Ruhé et al. 2007). Furthermore, the development of antidepressant drugs has been based on their ability to increase monoamine neurotransmission.

However, the monoamine hypothesis has limitations: (1) not all patients with depression respond equally to the same antidepressant drug and (2) changes in the monoamine levels take place within hours following treatment with antidepressant drugs, but the therapeutic response requires repeated administration of the these drugs for weeks (Elhwuegi 2004). These limitations led to the more recent monoamine theory of depression which suggests that the acute increase in the levels of monoamines may only be an early step in a complex cascade of events that leads to a therapeutic effect (Pineyro and Blier 1999). For example, a recent study by Gaska et al. (2012) showed time-dependent changes in gene expression following administration of antidepressant drugs. Therefore, the monoamines are not the only role players in the pathophysiology of depression and the monoamine theory only partially explains the pathophysiology and the effects of antidepressant drugs in the treatment of depression.

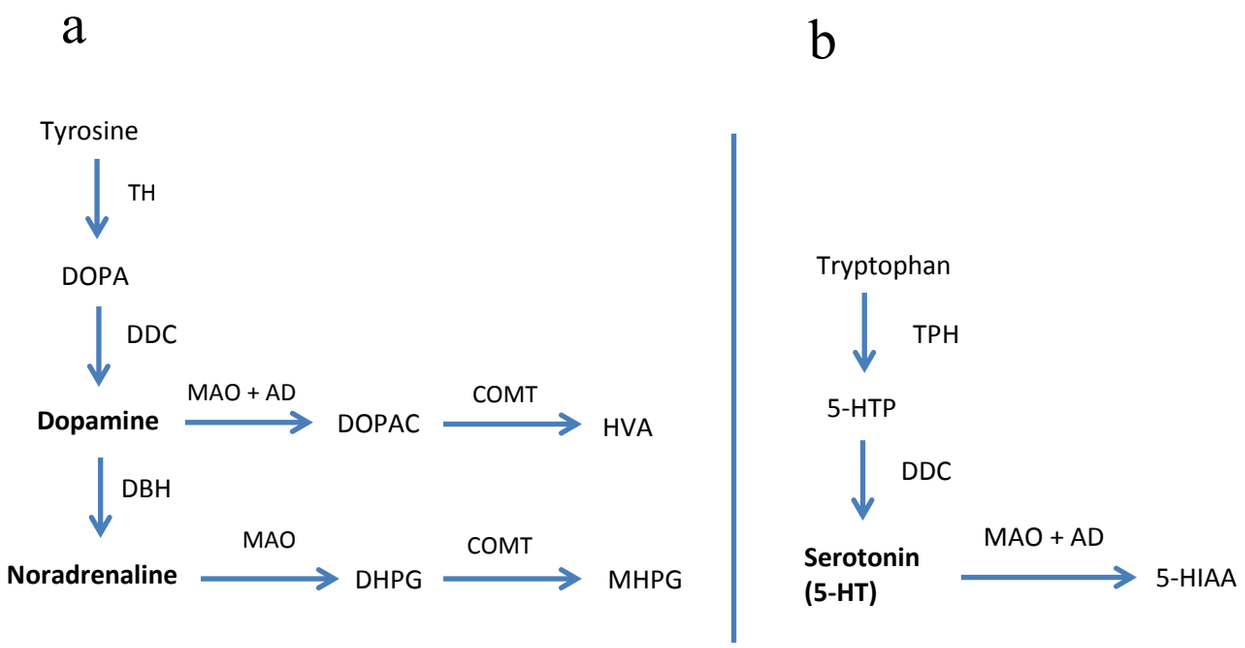


Figure 1.4: Pathways for the synthesis and metabolism of (a) dopamine and noradrenaline (in bold) and (b) serotonin (in bold). Noradrenaline is synthesized from the rate-limiting enzyme, tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and dopamine beta-hydroxylase (DBH) and metabolized to DHPG (3,4-dihydroxyphenyl glycol) and MHPG (3-hydroxy-4-methoxyphenyl glycol) by the enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT), respectively. Dopamine is synthesized from tyrosine by the enzyme TH and DDC and metabolized by MAO, AD (aldehyde dehydrogenase) and COMT to DOPAC (3,4-dihydroxyphenylacetic acid) and homovanillic acid (HVA). Serotonin is synthesized from the essential amino acid tryptophan by TPH (tryptophan hydroxylase) and DDC and metabolized to 5-HIAA (5-hydroxyindole acetic acid). DOPA = 3,4-dihydroxyphenylalanine; 5-HTP = 5-hydroxytryptophan. Adapted from Leonard (2003).

1.1.5.2 The role of glutamate in depression

According to neurophysiological studies, amino acids have been classified into two classes: (1) excitatory amino acids (glutamate and aspartate) which serve as neurotransmitters of excitatory synapses in the central nervous system, and (2) inhibitory amino acids (GABA and glycine) which serve as inhibitory neurotransmitters (Dickenson et al. 1997; Petroff 2002).

Glutamate is the main excitatory neurotransmitter in the central nervous system. In response to neuronal activity, the vesicles containing glutamate fuse with the synaptic membrane releasing glutamate into the synapse. Excitatory amino acid transporters

(EAAT) regulate the transport of glutamate at the synapse (Mitchell and Baker 2010). Glutamate also acts as a precursor to the major inhibitory neurotransmitter in the brain, GABA.

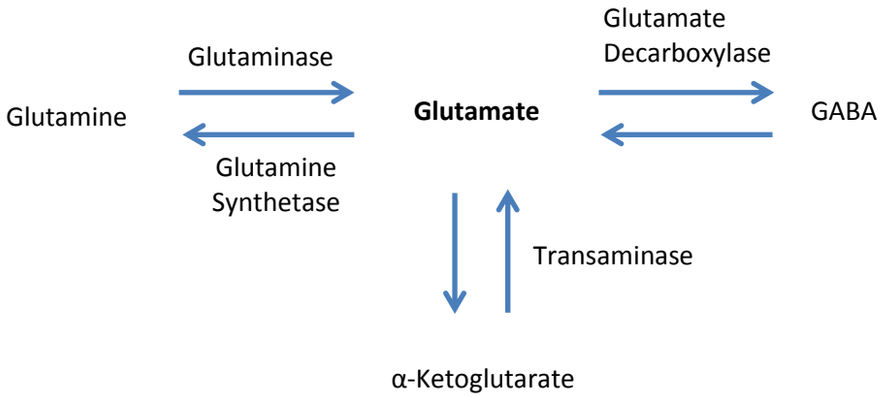


Figure 1.5: Metabolism of glutamate. Adapted from Mitchell and Baker (2010). Glutamate is derived from glucose and α-ketoglutarate and a small amount created from glutamine (Mitchell and Baker 2010). It is then taken up into secretory vesicles by the vesicular glutamate transporters (VGLUTs). (Takamori 2006). GABA = γ-Aminobutyric acid.

Glutamate functions by binding to its ionotropic or metabotropic receptors. There are three major classes of ionotropic receptors based on pharmacological, structural and functional criteria: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA) receptors (Niciu et al. 2014). NMDA receptors have the highest affinity for glutamate and three families of NMDA receptor subunits exist: (1) NR1, (2) NR2A-D and (3) NR3A-B (Niciu et al. 2014). AMPA receptor subunits are GluR1-4 (Niciu et al. 2014). AMPA/kainate receptor activation mediates the fast excitatory synaptic current (Na^+ influx) while NMDA receptor activation produces a delayed and prolonged current (Na^+ and Ca^{2+} influx) (Niciu et al. 2014). Calcium influx through the NMDA receptor regulates a number of intracellular kinases and phosphatases, thereby altering the characteristics of the synapse (Churn et al. 1995; Mitchell and Baker 2010; Zhou et al. 2013) and mediating important functions such as learning, memory and neural development (Shanmuga Sundaram et al. 2012; Vander et al. 1998). However, neuronal cell death results from excess influx of calcium through the NMDA receptor (Ankarcrona et al. 1995; Zhou et al. 2013). Therefore, glutamatergic signalling plays a role in both neuroplasticity and excitotoxicity if present in excess (Mitchell and Baker 2010). As the monoamines play a partial role in the pathophysiology

of depression, glutamate also contributes to the pathophysiology of depression (Niciu et al. 2014; Mitchell and Baker 2010).

Clinical studies reported abnormal levels of glutamate and its metabolites in depression (reviewed in Mitchell and Baker 2010). A variety of studies reported elevated glutamate levels in the plasma of patients with depression (Altamura et al. 1993; Küçükibrahimoglu et al. 2009; Mitani et al. 2006b). Using proton magnetic resonance spectroscopy it was shown that the occipital cortex, medial PFC and cingulate cortex had high glutamate levels (Grimm et al. 2012; McEwen et al. 2012; Sanacora et al. 2004). Postmortem studies of patients with depression showed that frontal cortex brain tissue had high levels of glutamate (Hashimoto et al. 2007). Furthermore, plasma glutamate levels correlated positively with the severity of depression (Mitani et al. 2006b). These studies were further supported by evidence showing decreased plasma glutamate levels in patients with depression following antidepressant treatment (Küçükibrahimoglu et al. 2009; Maes et al. 1998). However, other studies were unable to replicate these findings of increased glutamate in the plasma, cortex and cortical subareas in patients with depression (Altamura et al. 1995; Francis et al. 1989; Yüksel and Ongür 2010). Therefore, the above evidence indicates altered glutamate levels in depression but its correlation with symptoms of depression needs further elucidation.

In addition, NMDA receptor abnormalities have been observed in the PFC, amygdala and hippocampus in postmortem studies of patients with major depression. Reduced expression of the NR1, NR2A or NR2B subunit and NMDA receptor binding was found in the PFC of patients with major depression (Beneyto and Meador-Woodruff 2008; Feyissa et al. 2009). In other brain areas, NR2A and NR2C subunit expression was increased in the amygdala and locus coeruleus respectively (Karolewicz et al. 2005; Karolewicz et al. 2009) and NMDA receptor binding increased in the hippocampus of patients with major depression (Beneyto et al. 2007). Furthermore, several studies indicate the therapeutic role of NMDA receptor antagonists in depression (Javitt 2004; Lauterbach 2011; Niciu et al. 2014; Sultan et al. 2014). In addition, evidence from preclinical studies showed that chronic monoaminergic antidepressant drug treatment modulated the NMDA receptor and thereby affected monoamine turnover (Mayer et al. 2009; Popik et al. 2000; Skolnick 1999). The inhibitory effect of chronic treatment with various antidepressants disrupted the coupling between glycine and glutamate at the NMDA receptor binding site (Skolnick 1999). Therefore, adaptive changes to the NMDA receptor produced by monoaminergic antidepressants would be functionally equivalent to the effects produced by NMDA antagonists (Poleszak et al. 2011; Popik et al. 2000) and therefore supportive for a role of NMDA receptors in depression.

Glutamate neurotransmission has also been studied in animal models of stress/depression showing that glutamate injected into the NAc of rats decreased swimming in the forced swim test (FST) which is indicative of depression-like behaviour (Rada et al. 2003). Furthermore, the stress of swimming in the FST increased extracellular glutamate in the NAc as measured by microdialysis (Rada et al. 2003). In another study, rats bred to express learned helplessness behaviour, showed an increased glutamate/GABA ratio in the PFC and hippocampus as measured by magnetic resonance spectroscopy, an effect that was reversed by monoamine drug treatment and electroconvulsive shock therapy (Sartorius et al. 2007). Mice bred with reduced VGLUTs expression displayed increased depression- and anxiety-like behaviour in the FST, sucrose consumption test and light-dark exploration test (Garcia-Garcia et al. 2009; Tordera et al. 2007). Furthermore, knockout mice deficient of the NMDA receptor NR2A subunit displayed reduced depression- (FST and tail suspension test) and anxiety-like behaviour (EPM, light-dark exploration test and novel open field test) (Boyce-Rusta and Holmes 2006). A previous study by Ryan et al. (2009) showed that the NR1 subunit of the NMDA receptor is differentially modulated by factors that contribute to the development of depression-like behaviour. This was indicated by lower expression of the NR1 subunit at baseline in the genetically predisposed flinders sensitive line (FSL) rats whereas developmental stress such as MS of FSL rats increased the expression of the NR1 subunit compared to non-maternally separated FSL rats (Ryan et al. 2009).

1.1.5.3 The role of opioids in depression

The discovery of the function of endogenous opioids and their receptors came from the early use of opium in both medicinal and recreational practices (Dhawan et al. 1996; Lutz and Kieffer 2013). The opioid system is composed of three receptors: (1) κ-opioid receptor (KOR), (2) μ-opioid receptor (MOR) and (3) δ-opioid receptor (DOR) which interact with a family of endogenous opioid peptides known as β-endorphin, enkephalins, and dynorphins (Dhawan et al. 1996). The opioid receptors are expressed throughout the brain with the highest KOR densities observed in the NAc, claustrum, dorsal endopiriform nucleus and interpeduncular nucleus of rats (Dhawan et al. 1996). The highest density of MOR was observed in the caudate putamen, neocortex, thalamus, NAc, hippocampus and amygdala of rats (Dhawan et al. 1996). The opioid system has been implicated in a variety of biological functions such as drug dependence, pain sensitivity, learning and memory, immune function, thermoregulation, gastrointestinal motility, cardiovascular and respiratory function as well as endocrine function (Bodnar 2014). In addition, opioids are widely believed to play a role in depression.

Most clinical evidence indicated dysregulation of the receptors of the μ-opioid system in depression. One such study, using positron emission tomography, reported a reduction in MOR binding potential compared to healthy individuals in the posterior thalamus during neutral emotional states (Kennedy et al. 2006). Furthermore, a sustained sadness condition was associated with a decrease in MOR binding potential in the left inferior temporal cortex, anterior cingulate, rostral anterior cingulate, ventral pallidum and amygdala of patients with MDD (Kennedy et al. 2006; Zubieta et al. 2003). On the other hand, MOR agonists and KOR antagonists were effective in the treatment of refractory depression (Bodkin et al. 1995; Machado-Vieira and Zarate 2011; Nyhuis et al. 2008). In postmortem brains of depressed suicides, MOR density was found to be increased in the frontal cortex and caudate (Escribá et al. 2004; Gabilondo et al. 1995).

In preclinical studies, most evidence indicating an involvement of MOR and KOR in depression-like behaviour, came from studies measuring the behavioural effects of agonist and antagonists on these receptors. It was previously reported that administration of MOR agonists decreased depression- and anxiety-like behaviour in the learned helplessness paradigm, tail suspension test and FST in mice or rat models of depression (Berrocoso et al. 2013; Besson et al. 1996; Fichna et al. 2007; Tejedor-Real et al. 1995). These effects were blocked by various MOR antagonists (Berrocoso et al. 2013; Besson et al. 1996; Fichna et al. 2007; Ovsyukova et al. 2013; Tejedor-Real et al. 1995). Furthermore, the atypical antidepressant drug, tianeptine, has been reported to have agonist activity at the MOR (Gassaway et al. 2014). On the other hand, KOR activation mediates depression- and anxiety-like behaviour whereas KOR inhibition is a potential target of antidepressant drug treatment (Lutz and Kieffer 2013; Mague et al. 2003). This was evidenced by studies showing that various KOR antagonists caused increased time spent in the open arms of the elevated plus maze (EPM), decreased latency to enter the inner zone in the open field test (OFT), decreased immobility in the FST and decreased escape deficit in the learned helplessness paradigm indicative of anxiety- and depression-like behaviour in rat models of depression (Beardsley et al. 2005; Chartoff et al. 2012; Knoll et al. 2007; Mague et al. 2003; Rogala et al. 2012; Shirayama et al. 2004).

The opioid receptors have also been shown to be involved in the effect of stress induced by MS. Specifically, opioid receptors play a role in depression-like behavior in adult rats exposed to early developmental stress as evidenced by decreased MOR levels in the NAc (Dimatelis et al. 2012b). Evidence of opioid involvement in the effect of stress, induced by MS, is further strengthened by studies indicating that emotional distress-induced ultrasonic vocalizations (USVs) during MS were reduced by MOR agonists and reversed by MOR antagonism (Carden and Hofer 1990) whereas KOR stimulation provoked

calling during MS (Kehoe and Boylan 1994). Furthermore, activation of the MOR has been shown to be involved in infant attachment behaviour as evidenced by mice lacking the MOR gene showing a deficit in attachment behaviour (Moles et al. 2004). These data showed that MOR function is necessary for pups to develop normal attachment to the dam as shown by fewer distress vocalizations elicited upon MS (Moles et al. 2004). These results were also supported in a recent study, which showed that mice lacking the MOR gene and mice treated with a MOR antagonist displayed reduced interest in other mice and reduced approach behaviour towards their mother/nest bedding (Cinque et al. 2012).

Previous studies measuring endogenous opioid peptides such as dynorphins and enkephalins have indicated their involvement in stress-induced behaviours related to depression and anxiety (Bruchas et al. 2010; Dziedzicka-Wasylewska and Papp 1996; Shirayama et al. 2004). Different stress paradigms including immobilization, chronic mild stress, forced swim stress or learned helplessness increased dynorphin and decreased met-enkephalin levels in the hippocampus and NAc (Dziedzicka-Wasylewska and Papp 1996; Shirayama et al. 2004). Furthermore, it has been reported that chronic treatment with antidepressants increased enkephalin levels in the NAc and striatum (De Felipe et al. 1985). Therefore the role of the opioid system in depression is likely to be mediated by their endogenous proteins as well as their receptors.

1.1.5.4 Other neuropeptides associated with the intracellular signalling pathway

The delayed therapeutic onset of mood upliftment by currently available antidepressant treatment suggests that other mediators of depression are involved in the pathophysiology of depression. Other proteins involved in signal transduction pathways have been shown to play a role.

Brain-derived Neurotrophic Factor (BDNF)

BDNF, a member of the nerve growth factor (NGF) family, is densely expressed in the hippocampus (Yan et al. 1997) and suggested to be involved in the etiopathology of depression (Castrén and Rantamäki 2010; Duman and Monteggia 2006). In animals, BDNF is decreased in hippocampal tissue of rat models of depression (Elfving et al. 2010; Gersner et al. 2014; Roceri et al. 2002). Furthermore, it has been reported that chronic stress as a model of depression, decreased BDNF levels in the hippocampus and PFC of rodents (Ray et al. 2011; Xu et al. 2004; Xu et al. 2006) although several inconsistencies have been reported with some studies that showed an increase (Adlard et al. 2004; Larsen et al. 2010; Naert et al. 2011) or no effect on BDNF (Kuroda and McEwen 1998; Rosenbrock et al. 2005).

Glycogen synthase kinase (GSK)

GSK-3 plays a central role in many converging intracellular signalling pathways and is suggested to be involved in both neuroprotection and psychiatric disorders (Hunsberger et al. 2009; Rowe et al. 2007). GSK is generally pro-apoptotic as opposed to anti-apoptosis elicited through inhibition of GSK (Beurel and Jope 2006) and impairments in its regulation can have harmful effects on cell function, structure and survival leading to various psychiatric disorders (Jope and Johnson 2004). GSK3α and GSK3β are the two active GSK3 isoforms, with GSK3β the most extensively studied isoform mostly regulated by inhibitory control (Doble and Woodgett 2003). GSK3β inhibition can result from phosphorylation of the serine 9 at the N-terminal by several protein kinases such as protein kinase A (Fang et al. 2000), Akt (Cross et al. 1995) and protein kinase C (Goode et al. 1992). Besides phosphorylation, GSK3β can also be regulated by forming protein complexes through the Wingless (Wnt) signalling pathway (Kikuchi et al. 2007) or be directly/indirectly controlled by psychotropic drugs (Chen et al. 1999; Klein and Melton 1996; Roh et al. 2007). Direct inhibition of GSK3β by the mood-stabilizing drug, lithium, provided the first indication of the involvement of GSK3β in mood disorders including depression (Klein and Melton 1996; Stambolic et al. 1996). Since then, different neuromodulators involved in depression such as serotonin, dopamine and BDNF were found to regulate GSK3β via different downstream mechanisms (Foulstone et al. 1999; Li et al. 2004; Sutton and Rushlow 2011).

Clinical studies showed an increase in GSK3β enzyme activity and a decrease in phospho-GSK3β (p-GSK3β) (inactive form) in the postmortem PFC of patients that had depression and therefore implicating an increase in GSK3β function in depression (Karege et al. 2007; Karege et al. 2012). In further support, a previous study showed that a lower concentration of p- GSK3β in plasma of patients with depression was associated with a higher severity of depression (Pláteník et al. 2014).

Animal studies reported a decrease in p-GSK3β in the hippocampus and PFC of rats exposed to chronic stress-induced depression-like behaviour (14 days of FST stress) that was normalized following chronic antidepressant drug treatment (Chen et al. 2012; Liu et al. 2012). Furthermore, inhibition of GSK3β decreased depression-like behaviour in the FST in rats and mice (Liu et al. 2013a; Rosa et al. 2008). These studies therefore support a role for GSK3β in the pathophysiology of depression and highlight an important focus of research in understanding the mechanism of action of antidepressant drugs.

Mitogen Activated Protein Kinase (MAPK)/extracellular signal-regulated kinase (ERK)

The MAPK/ERK pathway is important for synaptic plasticity (Thomas and Huganir 2004) and has been shown to play a crucial role in depression and the action of antidepressant drugs (Fumagalli et al. 2005; Musazzi et al. 2010; Qi et al. 2009). Furthermore, ERK can be considered an integrator of neuroprotective pathways (Almeida et al. 2005; Bravo et al. 2009; Colucci-D'Amato et al. 2003). For example, it has been shown that GSK3β is regulated by activation of ERK1/2 which decreased GSK3β activity thereby mediating the neuroprotective effects of BDNF (Hetman et al. 2002). In turn, inhibition of GSK3 results in increased phosphorylation of ERK1/2 in cell lines (Wang et al. 2006).

1.2 Animal models of depression

Animal models are important tools to investigate the etiology and neurobiology of neuropsychiatric disorders as well as the mechanism of action of antidepressant drugs to screen for novel treatment options. However, animal models have some limitations as the model cannot fully exhibit the entire human condition. For example, feelings of worthlessness, guilt and suicidal ideation made by subjective self-report, are limited to patients with depression and can not easily be measured in animals (Nestler and Hyman 2010) . Although the animal model is not able to mimic the entire condition, previous criteria has been set out of which an animal model should comply (Willner 1984) and that can be used to improve current animal models or introduce new models that more closely mimic the human condition. However, since the neurobiology of neuropsychiatric disorders is largely unknown, these validation criteria are established on what is currently known about the etiology and neurobiology of depression and will be further discussed in detail for animal models of depression-like behaviour relevant to this study.

1.2.1 Validity

Willner (1984) proposed three different types of validity: face validity, construct validity and predictive validity to which an animal model should comply.

1.2.1.1 Face validity

Face validity refers to how well the behaviour of the animal resembles the symptoms of depression. An animal model that mimics multiple symptoms of depression is considered a valuable model of depression.

1.2.1.2 Construct validity

Construct validity is to assess the theoretical rationale in which the pathophysiological changes in patients with depression also manifest in the animal model. However, the pathophysiology of depression is not fully known and currently relies on our

understanding of the mechanism of antidepressants. Consensus on what a model is supposed to measure is also constantly changing as the theoretical understanding of depression evolves. Construct validity should also take into account principles of the etiology of a disorder (Pawlak et al. 2012). The models of depression link the pathophysiology of depression mostly with stress and/or genetic factors whereas the development of human depression can be caused by a combination of various psychological (adverse life events, adverse childhood experience and personality traits) and biological factors (genetic influences, illnesses and medication) (Fava and Kendler 2000; Saveanu and Nemeroff 2012). There is also a lack in understanding of how these etiological factors influence the theoretical basis underlining depression.

1.2.1.3 Predictive validity

Predictive validity is determined by the response of animal models of depression to antidepressants, at or near clinical doses, that is also effective in human depression. A valid model should be sensitive and specific in responding to effective antidepressants (true positive effects) and should be unresponsive to ineffective antidepressants (true negative effects) (Willner and Mitchell 2002).

1.2.2 Comparison of rat models of depression

Table 1.1: Comparison of rat models of depression in terms of validity. + positive results, - negative results, 0 no results

Rat models of depression	Face validity	Construct validity	Predictive validity	References
Environmental manipulations				
Stress in adulthood				
Learned helplessness	+	+	+	Reviewed in Vollmayr (2001) and Abelaira et al. (2013)
Chronic mild stress	+	+	+	Reviewed in Willner (1997)
Chronic Restraint stress	+	+	+	Reviewed in Buynitsky and

				Mostofsky (2009)
Social stress e.g. social defeat stress	+ / -	+ / -	+ / -	Reviewed in Yan et al. (2010)
Early life stress				
Maternal separation (MS)	+ / -	+ / -	+ / -	Reviewed in Vetulani (2013)
Lesions				
Olfactory bulbectomy	+	+	+	Reviewed in Kelly et al. (1997) and Yuan and Slotnick (2014)
Pharmacological				
Reserpine	0	0	+	Reviewed in O'Neil and Moore (2003)
Tryptophan	0	0	+	Reviewed in O'Neil and Moore (2003)
Genetic				
Selective breeding				
Wistar-Kyoto (WKY)	+ / -	+	+ / -	Reviewed in Crowley and Lucki (2005)
FSL	+ / -	+	+	Reviewed in Overstreet (1993)
High DPAT sensitivity animals	+ / -	+	+ / -	Reviewed in Overstreet and Steiner (1998)
Congenitally learned helplessness animals	+	+	+ / -	Reviewed in Willner and Mitchell (2002) and Henn and Vollmayr (2005)

Swim Low-Active animals	+	0	+ / -	(Weiss et al. 1998; West and Weiss 1998)
Transgenic				
Gene knockout	+	+	0	(Calabrese et al. 2015; Olivier et al. 2008; van der Marel et al. 2013)
Gene over-expression /under-expression	+	+	+	(Coelho et al. 2014; Kangussu et al. 2013)

There is no animal model yet available that can replicate the overall symptomology of depression. This could be due to the fact that depression is modeled on what is currently known of the theoretical basis of depression and also the complex classification of symptoms of depression that is unique for each patient and difficult to model in animals. The animal models of depression all differ in depression-related behaviour, neuropathology related to depression and treatment response to antidepressant drugs and although some models may be more valid than others in terms of the diversity of symptoms modeled, it may not necessarily be a useful model. For example, models based on adult stress-induced depression-like behaviour (learned helplessness, chronic mild stress, restraint stress, changing photoperiod) may have good face, construct and predictive validity but their usefulness as a model of depression is limited by the fact that depression-like behaviour develops over a long-term period. It was suggested that rather than creating an animal model that mimic the entire spectrum of disorders, it may be more useful to develop an animal model for a specific behavioural spectrum in order to identify specific neurobiological systems associated with these behavioural symptoms (Frazer and Morilak 2005). However, there is still a need to improve on the validity of current models of depression as our understanding of the neuropathology and etiology of depression evolves. For the purpose of this study, animals models listed in table 1.1 will be briefly referred to and only animal models of relevance will be further discussed in terms of face validity, predictive validity and construct validity.

1.2.3 Models of depression based on stress, lesions and pharmacological manipulations in adulthood

Some of the most important factors that induce depressive episodes are stressful life events (Kendler et al. 1999; Kessler 1997; Liu and Alloy 2010). During acute stress, systemic alterations strengthen the ability of an organism to maintain homeostasis and to minimize the impact of stress (Maccari and Morley-Fletcher 2007). However, when the stress is excessive, the adaptive responses to regulate the stress are lost. This could induce long lasting changes in neurotransmitter, neuroendocrine and hormonal systems that give rise to psychiatric disorders such as depression (Chrousos 1995; Tsigos and Chrousos 2002; Zhu et al. 2014). The activation of the hypothalamic-pituitary-adrenal (HPA) axis and the resulting release of glucocorticoids form the primary neuroendocrine response to stress and the basis on which most animal models of depression were and are still developed.

The stress hypothesis of mood disorders has led to the development of various animal models of depression (Table 1.1). The learned helplessness, chronic mild stress and restraint stress are the most widely used and best established animal models of stress in adulthood leading to depression-like behaviour in rodents. The learned helplessness was developed to model helplessness behavior that result from chronic or acute uncontrollable stress (Seligman and Beagley 1975). However, the usefulness of this model to mimic depression has been questioned as a measure that could also indicate learning to become inactive than helplessness per se. Furthermore, the learned helplessness rat model has a high predictive validity with response in their escape deficit to a variety of clinical effective antidepressants although some false positives (clinically ineffective antidepressant that is effective in learned helplessness) have been reported (Bourin et al. 2001a; Sherman et al. 1982; Yamada et al. 2014). However, several attempts have been made to increase the validity of the model that included selective breeding for learned helplessness (congenitally learned helplessness animals) (Henn and Vollmayr 2005; Vollmayr and Henn 2001). The chronic mild stress was developed to model depression-like behaviour such as anhedonia and considered to have good face validity, construct validity and predictive validity (Willner 1997). The usefulness of the model to mimic the depression state that develops over time is enhance by a chronic and more naturalistic unpredictable stress compared to the learned helplessness model (Yan et al. 2010). Restraint stress (see Stepanichev 2014) is considered a mild stressor with good validity and has been widely used to induce stress related behavioural, biochemical and physiological changes to study the neurobiology and treatment of depression (Dagnino-Subiabre et al. 2006; Lapmanee et al. 2013; Naert et al. 2011; O'Mahony et al. 2011).

Other adulthood stress related models of depression that have been frequently used is the resident-intruder test and the more recent photoperiod changing stress model. It has been suggested that models of social stress are more relevant to stress-induced neuropathology of depression in humans than environmental models, since they involve a social form of stress (Venzala et al. 2012). By introducing the male intruder to the territory of the resident animal, the intruders are attacked and defeated by the resident resulting in behavioural, physiological and neurobiological changes that can be reversed by chronic antidepressant treatment (Yan et al. 2010). However, due to the complex variety of behaviours induced by social defeat and the different actions of antidepressant drugs on behaviours such as anhedonia and social interaction this model could not be described as an animal model specific to depression (Chaouloff 2013; Venzala et al. 2012).

The olfactory bulbectomized model of depression has been extensively reviewed and established as a chronic rat model that meet most of the criteria of a valid model of depression (Kelly et al. 1997; Yuan and Slotnick 2014). The model is based on the lesion of the olfactory bulb, which disrupts the limbic-hypothalamic axis. However, the olfactory bulbectomized animal is characterized by an agitated type of depression which responded to chronic treatment with most antidepressants (Kelly et al. 1997; Lumia et al. 1992).

Pharmacological models of depression such as the reserpine and tryptophan models are used as tests to screen for antidepressant drugs with a particular pharmacological mechanism (O'Neil and Moore 2003). Reserpine treatment depletes the monoamines dopamine, serotonin and noradrenaline and induces a syndrome of reduced locomotor activity and body temperature. A similar method is used for identifying antidepressant drugs that selectively increase serotonin concentration by potentiating the serotonin syndrome induced by the administration of 5-hydroxytryptophan (5-HTP), the metabolic precursor of serotonin (Grahame-Smith 1971). The tryptophan model is therefore selective to antidepressant drugs that affect serotonin and antidepressant drugs active in reversal of reserpine-induced effects will not necessarily show an effect in this model (O'Neil and Moore 2003). However, these pharmacological models do not model the neurobiology of depression but rather are tests to screen for antidepressant drugs (O'Neil and Moore 2003).

1.2.4 Models of predisposition to depression

Most models of depression are described according to stress exposure in adulthood as a leading factor of depression. Although stress is a common risk factor for depression, not all stress responses are maladaptive since most people do not become depressed after

serious stressful events whereas some people become depressed after stressful events that are considered to be less stressful (Nestler et al. 2002). Therefore, to accurately model depression in animals, the inclusion of factors shaping an individual‟s response to stress to be more vulnerable to depression, is essential.

1.2.4.1 Genetic predisposition

Despite the role of stress in depression, previous studies showed a genetic predisposition to develop depression. Twin studies have estimated the heritability of depression to be in the range of 31 % - 42 % but suggested to be even higher for a reliable diagnosis or for subtypes of depression such as recurrent major depression (Bienvenu et al. 2011; Sullivan et al. 2000). Various genetic animals have been developed and provide the opportunity to study both the genetic determinants of depression and to more closely meet the clinical development of depression (Deussing 2007).

Low activity in the FST have been proposed to represent depression-like behaviour. This has led to the selective breeding of rats for high (swim high-active rats) and low immobility in the FST (swim low-active rats) in that such rats will prove useful in the study of depression (Weiss et al. 1998). These strains do not differ in general activity but display differences in activity in acute behavioural tests (FST, OFT and novel home cage) (Crowley and Lucki 2005; Weiss et al. 1998). However, their face validity is limited specifically to the reduced activity in the behavioural tests. Chronic antidepressant drugs were effective by increasing the activity in the FST but also revealed false negative (selective serotonin reuptake inhibitors were ineffective) and false positive results for a few antidepressant drugs (West and Weiss 1998). Evidence for the involvement of neurotransmitters is limited in this model (Overstreet and Wegener 2013). Therefore, the swim low-active rat model is mostly used as a screening model for antidepressant drugs.

Other rats were selectively bred according to altered cholinergic and serotonergic function. For example, the Flinders Sensitive Line (FSL) rats were developed according to their sensitivity to the anticholinesterase diisopropyl fluorophosphates (DFP) (Overstreet and Janowsky 1991). They showed a higher sensitivity to cholinergic agonists (Overstreet and Russell 1982) and increased muscarinic receptor density in the striatum and hippocampus compared to the Flinders Resistant Line (FRL) rats (Pepe et al. 1988). Furthermore, FSL rats showed more sensitivity to the hypothermic effects of the serotonergic agonist, 8-OH-DPAT and the hypothermic effects was correlated with their exaggerated immobility in the FST. Additionally, the FSL displayed other depression-like behaviours such as elevated REM sleep and reduced social interaction (Overstreet and Wegener 2013). Despite these similarities to depression, the FSL rats did not share all the

behaviours of depressed humans such as anhedonia (measured as reduced sucrose consumption). The FSL model of depression showed excellent predictive validity since all antidepressant drugs tested showed antidepressant-like effects on behaviour in the FST. Furthermore, the FSL rats were unresponsive to the effects of antidepressant drugs that were not expected to have these effects in the FST (Overstreet and Wegener 2013).

The High DPAT sensitive (HDS) model is another pharmacologically selected rat model of depression that is based on the high serotonin sensitivity of the FSL. This was achieved by selective breeding for high (High DPAT sensitive) and low (Low DPAT sensitive) sensitivity to the serotonin agonist, 8-OH-DPAT (Overstreet and Steiner 1998). The construct validity is still limited of this rat model since the cholinergic system has been the only system explored to date (Overstreet et al. 1994) and therefore not being able to make direct comparisons to patients with depression. The HDS rats were very similar to the FSL rats for almost all of the behaviours that have been studied (Overstreet and Steiner 1998) in addition to displaying a lack in anhedonia (increased sucrose consumption) showed in these rats compared to the Low DPAT sensitive rats (Overstreet et al. 1996). HDS rats responded to the antidepressant effect 8-OH-DPAT and desipramine as evidenced by a reduction in response rate and increased reinforcement rate in the differential reinforcement of low rate (DRL) 72-s operant schedule (Cousins et al. 2000). The DRL was proposed as a measurement to define an antidepressant-like effect (Cousins et al. 2000). However, fluoxetine was without effect in the DRL schedule (Cousins et al. 2000). Therefore, in comparison with FSL rats, behavioural and neurochemical comparison as well as response to a wider range of antidepressant drugs is incomplete for the HDS rats to be considered a valid model for depression.

Transgenic animal models have been developed to better understand the underlying neurochemical mechanisms leading to psychiatric disorders such as depression and to screen for antidepressant drugs (reviewed in Gardier et al. 2009). Although mutagenesis have been conducted mostly in mice, recent studies have also began to focus on rats (Calabrese et al. 2015; Coelho et al. 2014; Homberg et al. 2007; Kangussu et al. 2013; Olivier et al. 2008). As an example, genetic manipulation of the serotonergic system provided opportunities to study the role of 5-HT in the pathophysiology of depression. Knockout of the serotonin transporter gene in rats ($SERT^{-/-}$) affects serotonin homeostasis as evidenced by increased extracellular levels of 5-HT in the ventral hippocampus measured by microdialysis and consequently a reduction in several brain areas measured by high-performance liquid chromatography (HPLC)(Homberg et al. 2007; Neumann et al. 2011). Behavioural tests revealed that $SERT^{-/-}$ rats displayed depression-like behaviour as evidenced by increased immobility in the FST and reduced sucrose

consumption compared with $SERT^{+/+}$ rats (Olivier et al. 2008). Furthermore, they spent less time in the center part of the open field as well as on the open arm of the EPM, indicating enhanced anxiety-like behaviour (Olivier et al. 2008). However, the behavioural phenotype of SERT-/- mice is complex and dependent on genetic background (Domínguez-López et al. 2012; Haenisch and Bönisch 2011; Kalueff et al. 2006; Perona et al. 2008).

More recently, investigators have used transgenic rat models to explore other mechanisms involved in the pathophysiology of depression. For example, transgenic rats that expresses an anti-sense RNA against angiotensinogen to suppress its production in astrocytes (Kangussu et al. 2013) and over-expression of adenosine A_{2A} receptors in the brain (Coelho et al. 2014) were studied to measure their role in depression-/anxiety-like behaviour. Similar to $SERT^{-/-}$ rats, these rats showed depression- and anxiety-like behaviour in the FST, sucrose preference test and EPM, respectively (Coelho et al. 2014; Kangussu et al. 2013). Furthermore, administration of the selective serotonin reuptake inhibitor, fluoxetine, or angiotensin -(1-7), reversed the anxiety- and depression-like behaviour of transgenic rats with low brain angiotensinogen and implicating the role of angiotensin and serotonin in depression and anxiety (Kangussu et al. 2013).

Therefore, the ability to target specific genes of interest through knockout, under-expression or over-expression allows researchers to examine specific mechanisms associated with depression-/anxiety-like behaviour.

Wistar-Kyoto rat model

The inbred WKY rat strain was originally bred from the Wistar rat as a normotensive control strain for the spontaneously hypertensive rat (Okamoto and Aoki 1963). Although the WKY line was selected from Wistar rats with normal systolic blood pressure (Okamoto and Aoki 1963) several neuroendocrine, behavioural and neurochemical abnormalities have been reported (Lahmame and Armario 1996; Lahmame et al. 1997; Paré 2000; Solberg et al. 2001). This led to the suggestion of the WKY rat as a model of depression.

The large body of evidence indicated depression- and anxiety-like behaviour in WKY rats (Braw et al. 2006; Ivarsson et al. 2005; Jeannotte et al. 2009; Lahmame and Armario 1996; Lahmame et al. 1997; Lopez-Rubalcava and Lucki 2000; Malkesman et al. 2005; Malkesman et al. 2009; Nam et al. 2014; Paré 1992; Paré 2000; Tejani-Butt et al. 2003; Zafar et al. 1997). Depression-like behaviour was evidenced by increased immobility in the FST in WKY compared to Sprague-Dawley (SD), Wistar and Fischer-344 control rats

("despair" or learned helplessness) (Jeannotte et al. 2009; Lahmame and Armario 1996; Lopez-Rubalcava and Lucki 2000; Paré 1992). A previous study by Nam et al. (2014) showed that WKY rats were the only strain that spent more time immobile in the test swim of the FST compared to the pretest-swim and therefore an indication of learned helplessness. Similarly, a previous study also showed that WKY rats rapidly acquired the learned helplessness following exposure to inescapable shock (Paré 1994). Furthermore, WKY rats displayed a lack in general activity in the OFT and EPM (Malkesman et al. 2005; Nam et al. 2014; Tejani-Butt et al. 2003). Therefore, the WKY rats were less active in behavioural tests indicating psychomotor retardation. This was supported by evidence indicating that WKY rats did not exhibit motoric retardation since their number of wheel turning responses to avoid tail-shock were similar than Wistar and Fisher-344 control rats (Paré 1992). Anhedonic-like behaviour in the WKY rats was less consistent. Sucrose consumption (measure of anhedonia) was reduced in adolescent WKY rats but only during night time (Malkesman et al. 2005) whereas other studies found an increase (Nam et al. 2014) or no difference (Yamada et al. 2013) in sucrose consumption in adult WKY compared to SD and Wistar rats respectively. On the other hand, WKY rats showed less exploratory behaviour towards a conspecific rat (measure of anhedonia) (Nam et al. 2014; Pardon et al. 2002; Paré 2000). Another measure of depression-like behaviour is the time spent in the center zone of the EPM which indicates indecisiveness (Nosek et al. 2008). A previous study showed that the WKY rats spent more time spent in the center zone of the EPM (Nam et al. 2014) therefore displaying indecision in entering the arms of the EPM.

In addition, WKY rats showed anxiety-like behaviour in some of the behavioural tests as evidenced by increased freezing in the OFT in adolescent WKY rats (Braw et al. 2006;Malkesman et al. 2005) as well as a longer latency to feed in the novelty-suppressed feeding test in adult WKY rats compared to Wistar rats (Yamada et al. 2013). Furthermore, Carr and Lucki (2010) showed that WKY rats demonstrated decreased latency to begin burying and increased duration of burying in the conditioned defensive burying test. However, adolescent and adult WKY rats showed no anxiety-like behaviour in the EPM (Braw et al. 2006;Getachew et al. 2008; Nam et al. 2014; Pardon et al. 2002).

WKY rats also showed a heightened stress response that was revealed by measurement of their depression-like behaviour and susceptibility to stress-induced gastric ulcers. Previous studies showed that WKY rats that were exposed to chronic stress displayed a reduction in activity and longer response latencies to leave the start area in the OFT (Nosek et al. 2008; Zafar et al. 1997) as well as a reduction in investigatory behaviour towards a conspecific female rat (Paré 2000). Furthermore, WKY rats that were exposed

to water restraint stress showed a higher number and length of gastric ulcers compared to SD and Wistar rats (Paré 1992; Tejani-Butt et al. 2003).

The heightened stress response was further supported physiologically by elevated plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (De La Garza and Mahoney, III 2004; Pardon et al. 2002; Rittenhouse et al. 2002; Solberg et al. 2001). WKY rats showed prolonged diurnal plasma ACTH and corticosterone levels (Solberg et al. 2001) and elevated levels following stress that remained significant for an extended period after termination of the stress (Pardon et al. 2002; Rittenhouse et al. 2002; Solberg et al. 2001). Therefore, the WKY rats represent a useful model to assess the neurochemical basis of their depression-like behaviour in stressful environments.

Clinical depression has been associated with abnormal levels of central monoamines norepinephrine, serotonin and dopamine (Bonhomme and Esposito 1998; Delgado 2000). Similarly, WKY rats showed a dysfunction in monoaminergic neurotransmission compared to SD and Wistar rats (De La Garza and Mahoney, III 2004; Jeannotte et al. 2009; Novick et al. 2008; Pardon et al. 2002; Scholl et al. 2010; Yaroslavsky and Tejani-Butt 2010). A previous study indicated that basal levels of noradrenaline were reduced in several brain areas in the WKY rats that were dependent on the control strain being used since different noradrenaline levels were detected in SD and Wistar control rats (Scholl et al. 2010). This involved reduced noradrenaline levels in the ventral hippocampus, NAcS and dorsal raphe nuclei in WKY compared to SD rats and reduced noradrenaline in the lateral hypothalamus, NAcC and locus coeruleus compared to Wistar rats (Scholl et al. 2010). Furthermore, WKY rats showed reduced noradrenergic reactivity to acute stress as evidenced by reduced TH mRNA levels and noradrenaline release that was suggested to be the cause of their inability to cope with stressful situations (Pardon et al. 2002). In support of the noradrenaline deficiency, WKY rats have a higher density of noradrenaline transporter sites in limbic regions compared to control strains (Tejani-Butt et al. 1994).

In further support of the monoamine hypothesis, it was previously shown that WKY rats had lower serotonin levels and a higher serotonin turnover ratio (5-HIAA / 5-HT) in various brain areas such as the amygdala, hypothalamus, NAc, dorsal raphe nuclei, substantia nigra and locus coeruleus compared to either or both SD and Wistar rats (Scholl et al. 2010; Yamada et al. 2013). Similar to noradrenaline in WKY rats, strain differences in serotonin were dependent on the choice of control strain for comparison since SD and Wistar rats also revealed differences in serotonin levels (Scholl et al. 2010).

Given the role of dopamine in reward and drug addiction (Salamone et al. 2003) as well as evidence of dopamine in depression and antidepressant drug action (Bonhomme and

Esposito 1998), it is expected that dopamine may also play a role in the depression- and anxiety-like behaviour in WKY rats. Indeed, several lines of evidence showed a dysfunction in dopamine neurotransmission in WKY rats. These studies showed changes in levels of dopamine as well as its receptors (D1, D2, D3) and dopamine transporter (DAT) sites (Jiao et al. 2003; Novick et al. 2008; Scholl et al. 2010; Yaroslavsky et al. 2006). However, dopamine neurotransmission between strains were shown to be differentially regulated as evidenced by a decreased DAT binding in WKY rats in the NAc, amygdala and cell body areas (ventral tegmental area and substantia nigra) and increased DAT binding in the hypothalamus and hippocampus compared to either or both SD and Wistar rats (Jiao et al. 2003). The DAT differences across brain areas may possibly be due to compensatory mechanisms and may therefore affect the clearance of dopamine and resulting changes in dopamine receptor activity. In support of the monoamine hypothesis, WKY rats revealed lower levels of dopamine and its metabolite, DOPAC, in the PFC at baseline and in response to stress compared to Wistar and SD rats (De La Garza and Mahoney, III 2004). In addition, dopamine turnover was increased at baseline in WKY rats in the NAcS and striatum and decreased in the PFC and NAcS in response to acute stress compared to Wistar rats (De La Garza and Mahoney, III 2004). Another study was able to confirm lower DOPAC levels in the PFC and higher dopamine turnover in the NAcS in WKY rats compared to Wistar rats (Scholl et al. 2010). The increased dopamine turnover in the NAcS of WKY rats was suggested to be the results of lower dopamine transporter sites in this area, since reduced reuptake could increase extracellular dopamine and therefore dopamine turnover (Scholl et al. 2010). The dopamine deficiency was further supported by evidence of reduced post-synaptic D1 receptor binding at baseline in WKY rats in the striatum and NAcC compared to Wistar rats (Novick et al. 2008). The increased D2 receptor density in the ventral tegmental area and NAcS and increased D3 receptor density in the NAcS, NAcC and striatum was explained in term of the dopamine deficiency that resulted in compensatory up-regulation of these receptors (Yaroslavsky et al. 2006). However, the same argument could not be applied to other brain areas such as the NAcC, striatum and hypothalamus, which showed decreased D2 receptor binding in WKY compared to Wistar rats. Since dopamine transporter sites were reduced in the hypothalamus, it was suggested that the reduced D2 in WKY rats may be due to elevated levels of DA in this area. WKY rats had deficient dopamine transmission at basal levels as well as in response to stress and may provide an important animal model to study the neuropathology of depression. However, the dopamine deficiency hypothesis for previous studies was only consistent in some brain areas such as the NAcS and PFC and therefore indicates that dopamine is differently regulated in different brain areas that are associated with depression. However, the

overall activity of dopamine in WKY rats is complicated by studies that measured DAT in different brain areas than studies that measured levels of dopamine metabolites or dopamine receptors. Furthermore, different control strains were used for comparison with WKY rats.

Glutamate and GABA neurotransmitters have been shown to be involved in the pathology of depression and mechanism of antidepressant drugs (Luscher et al. 2011; Sanacora et al. 2012). Similarly, previous studies reported a dysfunction in glutamate and GABA receptor densities in WKY compared to Wistar rats (Lei and Tejani-Butt 2010; Lei et al. 2009; Tizabi et al. 2012). This was evidenced by a lower density of NMDA receptor in the substantia nigra, striatum, NAc, hippocampus (CA1 region) and cingulate cortex and a higher density of $GABA_A$ receptor in the substantia nigra, striatum, amygdala, hippocampus (CA2, CA3 and dentate gyrus regions) and in the periaqueductal grey area of WKY rats compared to Wistar rats (Lei et al. 2009). Another study was able to confirm a lower density of NMDA receptor at basal levels in the striatum, NAc, hippocampus and PFC of WKY rats compared to Wistar rats (Lei and Tejani-Butt 2010). It was suggested that the lower density of NMDA receptors reflected a deficiency in the NMDA receptor complex or a higher level of glutamate neurotransmission whereas the higher $GABA_A$ receptor density reflected a lower level of GABA neurotransmission (Lei and Tejani-Butt 2010; Lei et al. 2009). Furthermore, chronic stress was able to increase NMDA receptor density in the PFC, striatum and NAcC of WKY rats but not in Wistar rats (Lei and Tejani-Butt 2010). However, no difference has been shown previously in NMDA receptor density in the hippocampus in female WKY compared to Wistar rats (Tizabi et al. 2012).

The FST is widely used as a screening test for antidepressant drugs (Slattery and Cryan 2012). It has previously been shown that WKY rats displayed a selective antidepressant drug response in the FST. For example, immobility behaviour in WKY rats in the FST were reduced by chronic (7 days and 12 days) or sub-chronic (3 injections) treatment of tricyclic antidepressant drugs (desipramine, imipramine) and noradrenaline and dopamine uptake blockers (nomifensine) but unaffected by serotonin uptake blockers and agonists (fluoxetine, paroxetine and 8-OH-DPAT) (Lahmame and Armario 1996; Lahmame et al. 1997; Lopez-Rubalcava and Lucki 2000; Paré 1992; Tejani-Butt et al. 2003). Furthermore, differences in experimental protocols between studies had an influence on drug response in the WKY rats. For example, desipramine, the most widely tested antidepressant drug in WKY rats has been shown previously to have no effect on immobility in a single FST session at a sub-chronic dose (5 mg/kg – 15 mg/kg) but decreased immobility in the FST at a higher dose of 25 mg/kg (Lahmame and Armario

1996). However, when WKY rats were chronically treated, the immobility levels of WKY rats in a single FST were reduced with a lower dose of desipramine (10 mg/kg and 20mg/kg) (Paré 1992). On the other hand, sub-chronic and chronic treatment with desipramine was effective in reversing the immobility in the FST in WKY rats that were previously habituated to a pretest-swim in the FST at a dose of 5 mg/kg - 20 mg/kg (Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003). Therefore, the response to antidepressant drug treatment seemed to be dependent on the experimental protocol such as the dose of the antidepressant drug, chronic or sub-chronic treatment as well as the inclusion of a pretest or both a pretest and test swim session in FST.

Since WKY rats have been suggested to be a unique model of treatment-resistant depression, they have provided an opportunity to study novel drug targets to improve on the shortfalls of currently available antidepressant drugs. The NMDA receptor antagonist, ketamine, has proven to be such an antidepressant drug and could provide better insight in studying the neuropathology of depression. It has previously been shown that acute (2.5 mg/kg or 5 mg/kg) and chronic treatment (0.5 mg/kg or 2.5 mg/kg for 10 days) of ketamine in female WKY and chronic ketamine treatment (0.5 mg/kg for 10 days) in male WKY rats reduced their immobility in the FST, indicative of an antidepressant-like effect (Tizabi et al. 2012). Furthermore, the lasting effect of an acute dose of ketamine was evidenced by a decreased immobility in the FST at 1 week after the injection in female WKY rats (Tizabi et al. 2012). Molecular targets for ketamine treatment may therefore have clinical relevance and by studying the neurobiology in animals could reveal important mechanisms involved in treatment-resistant depression.

In summary, WKY rats displayed face validity, construct validity and predictive validity although some behavioural inconsistencies have been reported, especially the sucrose consumption test as measure of anhedonia. In addition, results for anxiety-like behaviour in the WKY are inconsistent with positive results obtained only in some of the tests whereas no anxiety-like behaviour was reported in the EPM. The WKY rats were hyper-responsive to stressful situations that affected the neuroendocrine system as well as leading to neurochemical changes. The neurochemistry in particular seemed to be distinct in WKY rats and could be partly explained according to the monoamine theory of depression at least in some limbic areas of the brain. However, since differences were reported in noradrenaline, serotonin and dopamine neurotransmission between the control Wistar and SD rats, studies comparing WKY with different controls will reveal inconsistent results. WKY rats were selective in their response to antidepressant drugs which may be due to their unique neurochemistry.

1.2.4.2 Developmental stress

Early life adversities (e.g. childhood physical, emotional, sexual abuse and parental loss) have been associated with the development of various neuropsychiatric disorders in adulthood such as depression and anxiety (Heim and Nemeroff 2001; Lindert et al. 2014; Otowa et al. 2014; Slavich et al. 2011; Springer et al. 2007). Furthermore, the type of aversive experience in early life was specific to the clinical spectrum of depression (e.g. depression with co-morbid conditions) affected in later life (Gibb et al. 2007; Harkness and Wildes 2002). For example, Gibb et al. (2007) reported that sexual abuse and emotional abuse were more associated with co-morbid anxiety whereas physical abuse was associated with co-morbid dysthymia.

Clinical evidence indicated that early adverse experiences that contributed to neuropsychiatric disorders later in life, were associated with alterations in the neuroendocrine system (Carvalho Fernando et al. 2012; Ehlert et al. 2001). Heim et al. (2008) reported that plasma adrenocorticotropin hormone (ACTH) and cortisol were elevated in response to psychosocial stress in humans with depression and a history of childhood abuse (Heim et al. 2000; Heim et al. 2008). This was further supported by the correlation of childhood abuse with morphological changes in the hippocampus that resulted in a smaller hippocampal volume than depressed individuals without a history of childhood abuse (Heim et al. 2008; Vythilingam et al. 2002). However, such research fails to establish a relationship between the type of trauma at different developmental stages and development of depression, which can be clearly manipulated in an animal experiment.

Several animal models of manipulation of the early environment have been used to study the predisposing factors contributing in the development of depression such as prenatal stress, postnatal handling and MS. These early life stressors cause changes in the behaviour and neuroendocrine system that persist into adulthood making them more sensitive to subsequent stress (Sánchez et al. 2001; Uchida et al. 2010; Zhang et al. 2013). Most of the recent animal models of depression are based on early life environmental manipulation that is considered a better model of predisposition of depression than the depression models based on a precipitating event (Willner and Mitchell 2002).

Maternal separation

MS is a well-established and widely accepted model for predisposition to depression. Typically, pups are separated from the dam during the first 2 weeks after birth which is a critical stage of brain development (Rice 2000). This period is characterized as the stress hyporesponsive period (SHRP) and protects the brain from high levels of glucocorticoids

during neuronal development in stress sensitive brain regions (Sapolsky and Meaney 1986).

However, early MS activates the HPA axis during the SHRP in MS rat pups that remained apparent into adulthood. This was evidenced by studies showing that repeated MS in early life leads to elevated ACTH and/or corticosterone levels during and after MS (Daniels et al. 2009; Jahng et al. 2010; McCormick et al. 1998) that persisted into adulthood (Daniels et al. 2004; Nishi et al. 2014) and potentiates the corticosterone and ACTH response to mild stressors (Francis et al. 2002; Lippmann et al. 2007; Roque et al. 2014). The above evidence therefore indicated that MS had immediate effects as well as effects in adulthood and suggested that early life stress alters the HPA system throughout life in rats. However, inconsistencies have also been reported with no change in basal levels of corticosterone following acute stress during 2 weeks of MS (Lajud et al. 2012) or in response to mild stressors in adulthood of previously MS rats (Hulshof et al. 2011; Lariviere et al. 2006).

Several studies reported that MS induced behavioural changes resembling depression and anxiety in humans (Daniels et al. 2004; Dimatelis et al. 2012b; Lambás-Señas et al. 2009; Lee et al. 2007; Li et al. 2013; Makena et al. 2012; Ryu et al. 2009). These studies showed increased depression-like behaviour as evidenced by increased immobility in the FST (Dimatelis et al. 2012b; Lambás-Señas et al. 2009; Lee et al. 2007; Ryu et al. 2009), reduced sucrose consumption (Aisa et al. 2007; Huot et al. 2001), reduced ambulatory activity (Lee et al. 2007), and cognitive deficits (Aisa et al. 2007; Couto et al. 2012). In addition, anxiety-like behaviours were measured in the EPM and showed that MS rats spent decreased time in the open arms and/or increased time in the closed arms indicative of anxiety-like behaviour (Daniels et al. 2004; Huot et al. 2001; Li et al. 2013; Ryu et al. 2009). Inconsistencies have also been reported with no change in sucrose consumption (Matthews et al. 1996; Shalev and Kafkafi 2002) and increased (Lambás-Señas et al. 2009) or no activity in the OFT (Shalev and Kafkafi 2002). Body weight was also less consistent with reduced (Huot et al. 2001) or increased (Lee et al. 2007; Ryu et al. 2009) measured body weight in MS rats. Although, most studies reported that MS increased immobility in the FST it was also previously reported that early life MS in SD rats at 3 h/day for 2 weeks, only increased immobility with exposure to an additional stressor such as chronic restraint stress (Marais et al. 2008). Previous studies also found disrupted prepulse inhibition in adolescent and adult rats that had been exposed to MS in early life after a single 24 h exposure (P3, P6, P9 and not P13) or repeated MS (P1 - P21) (Ellenbroek et al. 1998; Ellenbroek and Cools 2002; Li et al. 2013). Since anxiety-like behaviour was found in MS rats as well as disruption in prepulse inhibition and cognition,

other psychiatric disorders such as schizophrenia and diseases such as Alzheimer's disease were also studied (Garner et al. 2007; Klug and van den Buuse 2012; Li et al. 2013; Martisova et al. 2013). It was therefore concluded that MS is a valid rat model for psychiatric disorders (Li et al. 2013) and therefore not limited specifically to depression.

Early MS in rats also has distinct neurochemical consequences in adulthood. For instance, basal dopamine levels were increased in the striatum and dopamine turnover were reduced in the limbic areas such as the medial PFC of adult male rats previously exposed to MS (Matthews et al. 2001). Furthermore, dopamine-2 receptor expression was reduced in the hippocampus and NAc of MS adolescent rats (Li et al. 2013). In support of the dopamine hypothesis of depression, a previous study showed evidence of stress-induced increase in dopamine levels in the NAc, ventral tegmental area and substantia nigra only in non-maternally separated rats (Jahng et al. 2010). This provided evidence of a blunted dopamine response to the stress of MS (Jahng et al. 2010). Furthermore, reduced dopamine transporter densities were found in the NAcC and striatum of rats previously exposed to MS (P1 – P14 for 3h/day) (Brake et al. 2004).

Several studies have been conducted on the long-term effect of MS on serotonin neurotransmission. In support of the serotonin hypothesis of depression, serotonin levels were reduced in the hippocampus, medial PFC, raphe nucleus and NAc of adult MS rats (Jahng et al. 2010; Lee et al. 2007; Matthews et al. 2001). Furthermore, $5HT_{1A}$ serotonin receptors are involved in the pathophysiology of depression and mechanism of antidepressant drugs (Drevets et al. 1999; Haddjeri et al. 1998) and was shown to be altered in MS rats. For example, it has been reported that MS decreased 5-HT_{1A} expression in the hippocampus and medial PFC of adolescent rats (Leventopoulos et al. 2009; Li et al. 2013). However, other studies were unable to find differences in 5-HT_{1A} expression or serotonin transporter expression in various brain areas studied in MS rats (Arborelius et al. 2004; Vicentic et al. 2006).

Other pathways relevant to the pathophysiology of depression include the opioid system and were previously found to be regulated by MS (Dimatelis et al. 2012b; Michaels and Holtzman 2008). The stress of MS reduced the MOR density in the NAc in adult rats (Dimatelis et al. 2012b) which indicated a depression phenotype since activation of MOR leads to antidepression-like behaviour in the FST and learned helplessness rat model (Fichna et al. 2007; Rojas-Corrales et al. 2002). Also, it was reported that MS rats had a greater place preference for a MOR agonist and a greater aversion to a KOR agonist compared to non-maternally separated rats (Michaels and Holtzman 2008). This indicated that MS stress influence the rewarding or aversive effect of MOR and KOR agonists

(Michaels and Holtzman 2008). Further supporting evidence of the importance of the opioid system in mother-pup interaction, is studies showing a deficit in attachment behaviour in mice lacking the MOR gene (Moles et al. 2004; Wöhr et al. 2011). Furthermore, USVs in separated rat pups as an indication of mother-pup interaction (Portfors 2007), were reduced by MOR and delta opioid receptor agonists whereas a KOR agonist had the opposite effect (Carden et al. 1991). However, evidence for the long-term effects of early MS on opioid receptors is still limited.

MS may also affect brain function by affecting proliferation and apoptosis (Chocyk et al. 2011; Irles et al. 2014; Lee et al. 2001). BDNF has a neuroprotective role through the MAPK/ERK pathway (Peng et al. 2008). A decrease in BDNF has been suggested to be involved in mediating depression-like behaviour (reviewed in Duman and Monteggia 2006) and previously shown to be altered by the stress of MS in selected brain areas (Lippmann et al. 2007; Pinheiro et al. 2014; Roceri et al. 2002; Xue et al. 2013). This was evidenced by studies that showed reduced BDNF levels in the hippocampus and striatum, but an increase in the ventral tegmental area in the adult rats previously exposed to 3 h of daily MS (P1 - P14) (Lippmann et al. 2007; Pinheiro et al. 2014). Furthermore, intra-ventral tegmental infusions of BDNF resulted in a decreased latency to immobility in the FST in rats indicating depression-like behaviour following enhanced signalling of BDNF (Eisch et al. 2003). Furthermore, inhibition of the BDNF pathway in rats through intra-NAc injections of a virus expressing the truncated BDNF receptor, TrkB-T1, increased the latency to immobility in the FST suggesting antidepressant-like behaviour (Eisch et al. 2003). It therefore appeared that BDNF in brain areas associated with depression are differently regulated by stress. However, findings in brain areas such as the hippocampus did not always reveal consistent results, since previous studies also showed an increase (Greisen et al. 2005) or no change (Roceri et al. 2004) in BDNF in adult rats exposed to early-life MS for 3 h daily (P2 - P14).

MS rats also responded to antidepressant drugs such as tricyclic antidepressants (tianeptine), serotonin-norepinephrine reuptake inhibitors (venlafaxine) and selective serotonin reuptake inhibitors (fluoxetine, paroxetine and escitalopram) that attenuated the depression- and anxiety-like behaviours and enhanced HPA axis activity (Couto et al. 2012; El Khoury et al. 2006; Huot et al. 2001; Leventopoulos et al. 2009; Martisova et al. 2012; Trujillo et al. 2009). Studies showed that the antidepressant drugs reduced the elevated ACTH and corticosterone levels (Huot et al. 2001; Martisova et al. 2012), reduced immobility in the FST (Couto et al. 2012; El Khoury et al. 2006; Martisova et al. 2012), restored preference for sucrose in the sucrose consumption test (Huot et al. 2001; Leventopoulos et al. 2009) and improved memory deficits as measured by increased time

spent in the platform quadrant in the Morris Water Maze test (Couto et al. 2012). In contrast, a previous study found no effect of the selective serotonin reuptake inhibitor, escitalopram, on the depression-like behaviour of MS rats (Dimatelis et al. 2012b). Furthermore, the tricyclic antidepressant and selective serotonin reuptake inhibitors showed anxiolytic effects in MS rats as evidenced by increased time spent in the open arms of the EPM (Huot et al. 2001; Trujillo et al. 2009). In the MS rat model, the selective serotonin reuptake inhibitor is the most common antidepressant drug studied and further screening of a wider variety of effective antidepressant drugs is required to further establish the model‟s predictive validity.

Therefore, according to the evidence, the MS model of depression showed face validity, construct validity and predictive validity in a variety of behavioral, neurochemical and neuroendocrine changes induced by early life stress of MS that is associated with depression-/anxiety-like behaviour (Pryce et al. 2005). However, several behavioural and neurobiological inconsistencies have been reported, whereas the neuroendocrine changes seemed to be more consistent between studies. It should be noted that patients with depression my exhibit psychomotor retardation or agitation and weight loss or weight gain (American Psychiatric Association 2013) and are therefore not a reliable measure to model in animal models of depression . The reason for the inconsistencies between studies is often associated with different experimental protocols such as single or litter-wise separations, difference in frequency and timing of separation, gender differences and choice of control (Vetulani 2013). The use of single or litter-wise separation and male or female rats has considerable effects on brain function. For example, only female rats that were MS in a group for 6 h from P1 to P21 had a higher expression of $5HT_3$ receptor gene in the brain stem compared to female rats which were isolated from each other during MS (Oreland et al. 2009). Furthermore, the activity of the HPA axis and emotional behaviour is dependent on the gender of the rats (Renard et al. 2007). Lastly, behavioural, neuroendocrine and neurobiological comparison between MS and different controls (exposed or without environmental manipulations) may have contributed to the variability between studies. For example, studies often compare MS rats against controls that have been briefly handled, non-handled or alternatively, pups are kept under animal rearing facility conditions with their cages and bedding changed twice or three times per week. However, previous studies showed behavioural and neurochemical differences between these controls (Brake et al. 2004; Oreland et al. 2009). According to the aforementioned variability, it is evident that a more robust model of early developmental stress is needed.

1.2.4.3 Genetic predisposition and developmental stress

Various studies have shown that exposure to early life stress increased the risk for depression and that the experience of stressful events interacts with genetic risk factors (Aslund et al. 2009; Caspi et al. 2003; Kaufman et al. 2006; Myers et al. 2014). For example, patients with depression with a polymorphic change in the serotonin transporter gene (carriers of the S-allele) or oxytocin receptor gene had a higher risk to develop symptoms of depression during stressful events and childhood trauma (Caspi et al. 2003; Myers et al. 2014).

Therefore, instead of focusing on stress or genetic factors in contributing to a depression phenotype in rats, the focus has shifted in recent years to the combination of these factors in animal models of depression in order to further enhance construct validity (El Khoury et al. 2006; Musazzi et al. 2010; Nam et al. 2014; Neumann et al. 2005; Petersén et al. 2008; Ryan et al. 2009; Wörtwein et al. 2006). These studies aimed to determine how the genetic predisposition modifies the behaviour and neurochemical effects of early life trauma (stress of MS) and if early treatment affected the outcome. However, most of these studies studied the effect of MS on the FSL rat model of depression possibly because of its well characterized neurobiology. It has been shown previously that MS had marked effects on the swimming duration in the FST in FSL rats but was without effect in the FRL control rats (El Khoury et al. 2006; Petersén et al. 2008). Furthermore, only the FSL rats responded to the selective serotonin reuptake inhibitor, escitalopram, by increasing their swimming behaviour in the FSL (El Khoury et al. 2006; Petersén et al. 2008). Those studies also showed that the effect was only produced or more pronounced in the MS FSL compared to the non-maternally separated FSL rats (El Khoury et al. 2006; Petersén et al. 2008) . However, another study was unable to replicate these findings in MS FSL rats in the FST possibly because they measured immobility instead of swimming behaviour (Musazzi et al. 2010). In contrast to the former studies, Musazzi et al. (2010) found that MS had an effect only in the FRL control rats by increasing the immobility in the FST. Furthermore, although escitalopram treatment similarly decreased the immobility only in the FSL rats, the effect in the non-maternally separated FSL was more pronounced than in the MS FSL rats (Musazzi et al. 2010). Since the studies by El Khoury et al. (2006), Petersén et al. (2008) and Musazzi et al. (2010) used the same MS and treatment protocol in FSL and FRL rats, it therefore appeared that MS affected the immobility in the FST in FSL and FRL rats differently than the swimming behaviour. It has also been previously shown that anxiety-like behavioural effects of MS were highly dependent on the genetic background (Neumann et al. 2005). More specifically, rats that were bred for high anxiety-like behaviour (decreased time spent in the open arms of the

EPM, HAB rats), showed signs of reduced anxiety in the modified holeboard test following repeated MS (Neumann et al. 2005). In contrast, rats that were bred for reduced anxiety-like behaviour (increased time spent in the open arms of the EPM, LAB rats), showed signs of increased anxiety in the EPM following MS (Neumann et al. 2005). As a result of MS, the anxiety-like behaviour in the modified holeboard test did not differ between HAB and LAB rats (Neumann et al. 2005). Therefore, according to above studies, the neurobiological outcome and ultimately the psychopathology of developmental stress will depend on the genetic vulnerability of the animal/individual.

Only a few studies measured the anxiety-/depression-like behavioural effects of MS in WKY rats (Nam et al. 2014; Sterley et al. 2011; Womersley et al. 2011). In adolescent and adult WKY rats, MS had no overall effect on anxiety-like behaviour and depression-like behaviour as evidenced by the time spent in the open arms of the EPM and immobility in the FST, respectively (Nam et al. 2014; Sterley et al. 2011; Womersley et al. 2011). However, evidence showed that adolescent MS WKY rats spent more time in the closed arms of the EPM during the first minute of testing indicative of some anxiety-like behaviour (Womersley et al. 2011). Furthermore, when measuring anhedonia behaviour, MS WKY rats spent the same amount of time as non-maternally separated WKY rats in the avoidance zone in response to the stimulus of a female and male rat (Nam et al. 2014). It was previously suggested that that lack of environmental influences may be due to the strong influence of their genetic profile in shaping their depression-like behaviour (Nam et al. 2014). However, this requires further investigation and the need to study the effects of early life stress on the antidepressant response in WKY rats.

1.3 Tests for depression

In contrast to an animal model of depression, tests for depression provides a behavioural or physiological endpoint measure and are designed to assess the effect of a genetic, pharmacological or environmental manipulation (Yan et al. 2010). Behavioural tests will be further discussed based on its relevance to this study.

Table 1.2: Classification of some of the behavioural assays used to measure depression- and anxiety-related behaviour in rodents. Adapted from supplementary information (Nestler and Hyman 2010).

Symptom Cluster	Assay	Validity
Treatment-based screens	FST, tail suspension test, learned helplessness	Predictive
Mood	Tests of anhedonia: sucrose preference, investigatory behaviour, sexual behaviour Intracranial self-stimulation	Face
	USV ?	?
Neurovegatative (or homeostatic)	Changes in weight, sleep pattern, circadian rhythms	Face
Depression-associated emotional symptoms: anxiety	EPM, OFT, USV and novelty suppressed feeding	Predictive, face

1.3.1 Treatment-based screens

1.3.1.1 Forced swim test

The FST, also known as the behavioural despair test or the Porsolt test was developed by Porsolt and colleagues in rats (Porsolt et al. 1977; Porsolt et al. 1978). The FST is the most widely used tool for assessing antidepressant-like behaviour. It is based on the observation that rats, following initial escape-orientated activities such as swimming and struggling, developed immobility when placed in an inescapable cylinder of water (Cryan et al. 2002). On return in the cylinder of water 24 h later, they resumed to be immobile more rapidly (Cryan et al. 2002). The original view by Porsolt is that since the rat was more immobile in the second immersion; it indicated a state of "despair" because the rat had learn that escape is impossible (learned helplessness) (Porsolt et al. 1977). However, describing activity in the FST as depression-related has been previously questioned (Borsini and Meli 1988) and therefore believed to rather reflect learned immobility (Parra et al. 1999) or described as the development of passive behaviour that disengages the rat

from active behaviour of coping with stress (Cryan et al. 2002). Nonetheless, the FST has received considerable recognition as a screening tool for antidepressant drugs with strong predictive validity (Cryan et al. 2005; Detke et al. 1997).

The FST is sensitive to a variety of antidepressant drugs including all of the major classes of tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, atypical antidepressants and electroconvulsive shock treatment (Borsini and Meli 1988). Treatment with antidepressant drugs (typically 3 injections) between the pretest-swim and test swim reduced the amount of time spent immobile and increased the time spent in active behaviours (swimming and climbing) during the test swim session (Detke et al. 1995; Slattery and Cryan 2012). However, stimulants, sedatives or motor-impairing compounds showed false positive results in the test (Borsini and Meli 1988). Therefore, it is necessary to determine if the tested drug alters locomotor activity (see OFT in section 1.3.2.3 below). For example, a stimulant may decrease immobility in the FST but may also increase locomotor activity, thereby confounding the FST results. Furthermore, the FST is unique in distinguishing between serotonergic and noradrenergic antidepressant drugs based on different active behaviours (Detke et al. 1995; Detke and Lucki 1995). For example, in addition to decreasing the immobility, the selective noradrenaline reuptake inhibitors (desipramine, maprotiline) selectively enhanced climbing behaviour and selective serotonin reuptake inhibitors (paroxetine, sertraline, fluoxetine) selectively enhanced swimming behaviour in the FST (Detke et al. 1995). The major drawback of the FST is that short-term antidepressant treatment reduced immobility in the FST whereas in patients it can take weeks for a therapeutic effect. However, it has been demonstrated that doses of antidepressant drugs that were inactive acutely, reduced the immobility when administered chronically which further validates the FST (Detke et al. 1997).

The FST has also been used to establish that greater immobility is produced following exposure to chronic stress (Chiba et al. 2012; Liu et al. 2013b; Morley-Fletcher et al. 2003) or other conditions that provoked depression-like behaviour such as acute drug withdrawal (Cryan et al. 2003), developmental stress of MS (Lambás-Señas et al. 2009; Sung et al. 2010) or selective breeding (Overstreet 1993; Tejani-Butt et al. 2003; Weiss et al. 1998). The FST procedure has been adapted for rats selectively bred for depression-like behaviour (Lahmame et al. 1997; Malkesman et al. 2009; Overstreet 2002). It has been suggested that since these rats are characteristically more immobile in the FST, no pretest-swim was necessary (Overstreet et al. 2005). However, this adapted FST protocol has not been performed consistently since other studies using established genetic rat

models of depression included both a pretest-swim and test swim in the FST (Lopez-Rubalcava and Lucki 2000; Nam et al. 2014; Tejani-Butt et al. 2003).

Therefore, the fact that the FST gives bi-directional results (pro-depression- and antidepressant-like effects) to common manipulations gives the test high predictive validity and behavioural dimension that is relevant to depression (Cryan et al. 2005; Slattery and Cryan 2012).

1.3.2 Mood / anxiety

1.3.2.1 Ultrasonic vocalizations

Rodents produce and perceive calls in the ultrasonic range, referred to as USVs. An affective hypothesis and social signalling hypothesis was proposed (Knutson et al. 2002) based on evidence that USVs have a communicative function (to direct specific behaviour) (Farrell and Alberts 2002; Takahashi et al. 1983) as well as providing information on the emotional state (Knutson et al. 2002; Seyfarth and Cheney 2003). However, the emotional and communicational functions of USVs are not necessarily separable (Brudzynski and Pniak 2002).

Three types of USVs can be distinguished in rats that are dependent on the stage of development, affective state and social context. For example, infant rats emit isolation-induced 40-kHz USVs when separated from their mother and littermates indicating anxiety-like behaviour (Treit et al. 2010). Adolescent and adult rats are able to emit two primary types of USVs: 22-kHz and 50-kHz (Knutson et al. 2002; Natusch and Schwarting 2010; Portfors 2007). The 22-kHz calls are emitted in various aversive situations such as exposure to predators (Blanchard et al. 1991), inescapable footshock (Sánchez 2003; Wöhr et al. 2005) and inter-male aggression (Thomas et al. 1983) and therefore indicating a negative emotional state. The affective hypothesis of 22-kHz USVs is further supported by evidence showing that rats that displayed high levels of anxiety-like behaviour in the EPM (decreased time spent in open arms of EPM) and freezing behaviour, vocalized more than the rats that displayed low levels of anxiety-like behaviour and freezing behaviour (Borta et al. 2006). Therefore, not only the aversiveness of the context but also the individual disposition affects the 22-kHz USVs. Consistent with the social signalling hypothesis, 22-kHz USVs may also serve as alarm cries. It has been previously shown that 22-kHz USVs in response to a predator (cat) is dependent on the presence of other rats since 22-kHz USVs were absent when a rat was exposed to a cat without the presence of other rats (Blanchard et al. 1991; Wöhr and Schwarting 2010). This was further supported by studies showing that playback of 22-

kHz calls induce long-lasting behavioural inhibition as measured by general activity (Brudzynski and Chiu 1995; Wöhr and Schwarting 2007).

On the other hand, 50-kHz vocalizations are normally emitted in appetitive situations such as play (rough-and-tumble play and tickling), mating, anticipation of food reward, electrical brain stimulation and administration of drugs of abuse such as amphetamine (Burgdorf et al. 2000; Burgdorf et al. 2008; Knutson et al. 1998; Panksepp and Burgdorf 2000; Thompson et al. 2006) therefore indicating a positive emotional state. This was further supported by evidence showing that aversive stimuli such as cat odor or bright light reduced the emission of playful tickling-induced 50-kHz USV (Panksepp and Burfdorf 1999). Furthermore, rats selected with lower positive affect at an early age, indicated by lower 50-kHz response to tickling, elicited increased levels of 22-kHz USVs and depression-like behaviour in adulthood such as lower sucrose intake and preference, higher levels of immobility in the FST and spending less time in contact with other rats (Burgdorf et al. 2009; Mällo et al. 2009). This suggests that rats with low positive emotional state indicated by reduced 50-kHz USVs are predisposed to affective disorders such as depression-like behaviour (Mällo et al. 2009). In addition, hippocampal cell proliferation, that has been associated with depression, positively correlated with 50-kHz USV and negatively correlated with 22-kHz USVs during tickling (Wöhr et al. 2009). Furthermore, rats that experienced tickling as appetitive, as indicated by high number of 50-kHz USV, had increased hippocampal cell proliferation levels as compared to non-tickled control rats (Wöhr et al. 2009).

However, 50-kHz calls have not always been found in situations generally seen as rewarding. For example, 50-kHz USVs occurred in response to separation from conspecifics (Wohr et al. 2008). It was shown that the rat that remained alone in the home cage following separation from the cage mates produced a high number of 50-kHz USVs (Wohr et al. 2008). This was suggested to indicate the need for social contact and therefore function as social contact calls (Wohr et al. 2008). In further support of their communicative function, playback of 50-kHz USVs induced social exploratory behaviour directed towards the sound source (Wöhr and Schwarting 2007). This was evidenced by the increased entries of recipient rats in the three proximal arms of a radial maze in front of a loudspeaker compared to fewer entries in the distal arms (Wöhr and Schwarting 2007). Other situations seen as non-rewarding showed 50-kHz calls during morphine withdrawal (Vivian and Miczek 1991) and aggressive encounters with resident-intruder tests that were reduced by anxiolytic drugs (Miczek et al. 1995; Tornatzky and Miczek 1995).

The 50-kHz vocalizations have been further characterized according to the affective and social signalling hypothesis into flat and frequency modulated (FM) calls. The flat 50-kHz calls are emitted during anticipation of social contact, as opposed to FM calls which are indicative of the emotional state (Brudzynski and Pniak 2002; Wohr et al. 2008). For example, Burdorf and Panksepp (2006) divided 50-kHz of tickled responders and tickled non-responders into flat and FM calls and showed that tickle responder rats exhibited more of the FM calls indicative of positive emotion (Burgdorf and Panksepp 2006). This was further supported by evidence showing positive correlation between FM 50-kHz USV and appetitive behaviour such as rough-and-tumble-play behaviour and mating (Burgdorf et al. 2008). On the other hand, a high number of flat USV calls occurred in the rat that remained alone in the home cage following separation from the cage mate indicating its relevance in a social-coordinating function (Wohr et al. 2008).

1.3.2.2 Elevated plus maze

The EPM has become one of the most important behavioural tests to evaluate anxiety-like behaviour in rodents and the effect of anxiolytic and anxiogenic drugs (Braun et al. 2011; Hui et al. 2014; Nosek et al. 2008; Pellow and File 1986). The EPM is a plus-shaped maze composed of two open arms and two closed arms that is connected by a central area. The test is based on the fact that rats under normal conditions show preference for closed alleys (closed arms) compared to open alleys (open arms). Open arm avoidance is exaggerated by drugs with anxiogenic effects and reversed by treatment with standard anxiolytics (Pellow and File 1986). Furthermore, the EPM is sensitive in showing anxiety-like behaviour in rat models predisposed to depression-/anxiety- like behaviour (Braw et al. 2006; Daniels et al. 2004; Landgraf and Wigger 2002). Another behaviour infrequently measured, is the time spent in the center of the EPM and suggested to indicate indecision or ambivalence that may be related to approach/avoid conflict (Bourin et al. 2001b; Nosek et al. 2008; Rodgers and Johnson 1995). Therefore, the EPM has been suggested to have good face and predictive validity (Pawlak et al. 2012; Walf and Frye 2007).

1.3.2.3 Open field test

The OFT is another test commonly used to assess activity and anxiety-like behaviour in rodents (reviewed in Prut and Belzung 2003). The test is performed by placing the animal close to the walls or center of the apparatus and the following behaviour recorded: horizontal locomotion, frequency of rearing or leaning and grooming. When exposed to an open field, rodents spontaneously prefer to stay close to the walls of the apparatus and spent less time in the central part of the open field. Increased time spent in the central part

or a decrease latency to enter the central part of the open field is an indication of reduced anxiety-like behaviour that is induced by anxiolytic drugs. Anxiety-like behaviour in the OFT is triggered by two factors: individual testing (separated from social group) and agoraphobia (size of the arena relative to the animal‟s natural environment) (Prut and Belzung 2003). Therefore, anxiolytic treatments may not affect exploration of the animal, but the effects on the reaction of the animal to a stressful event (Prut and Belzung 2003). In addition, the OFT has been more routinely used in combination with the FST to assess locomotor activity in rats following antidepressant drug treatment (Akinfiresoye and Tizabi 2013; Overstreet and Griebel 2004; Tejani-Butt et al. 2003; Tizabi et al. 2012). Since the FST is a result of an activity measure it has been suggested that the results obtained in the FST should be confirmed by additional testing in a locomotor paradigm such as the OFT (Slattery and Cryan 2012).

1.4 Treatment

1.4.1 Monoaminergic antidepressant drugs

Since its introduction, nearly 50 years ago, the monoamine hypothesis (as discussed in section 1.1.5.1) provided the only neurobiological explanation according to the mechanism of antidepressant action. Antidepressants, currently available, are effective in many patients (Morilak and Frazer 2004). However, the antidepressants have a few shortcomings such as undesirable side effects, treatment resistance and the delayed onset of action. Remission is not often achieved and only 60% - 70% of patients with depression respond to antidepressant drug treatment while 10% - 30% of those patients remain resistant to treatment (Al-Harbi 2012). Furthermore, depression symptoms can even return over the course of chronic treatment (Solomon et al. 2005). A serious drawback of all antidepressants is the requirement of long-term treatment for 2-4 weeks before a therapeutic effect is achieved (Wells et al. 2003) because of the the long-term adaptive changes required for a change in mood/behaviour. Environmental factors and antidepressant drugs are able to modify the expression of genes (epigenetics) by altering DNA methylation and chromatin activation (Dalton et al. 2014). For example, overexpression of histone deacetylase 5 (HDAC5), an enzyme that decreases gene expression by decreasing histone acetylation prevented the antidepressant effects of imipramine in stressed animals (Tsankova et al. 2006).

The monoamine oxidase inhibitors and tricyclic antidepressants were the first antidepressant discovered (Baldessarini 2001). The discovery initiated the first investigations of the involvement of neurochemistry in depression. The acute mechanisms of these antidepressants were identified as:

1) Monoamine oxidase inhibitors (e.g. iproniazid, phenelzine, tranylcypromine): Inhibition of monoamine oxidase

2) Tricyclic antidepressants (e.g. imipramine, amitriptyline, desipramine): inhibition of serotonin or noradrenaline reuptake transporters

This led to the second generation of antidepressant drugs that were more selective for the monoamine neurotransmitters and therefore had fewer adverse effects. These antidepressants are widely used and include: selective serotonin reuptake inhibitors (SSRIs; e.g. fluoxetine, paroxetine), norepinephrine reuptake inhibitors (NRIs; e.g. atomoxetine), serotonin-norepinephrine reuptake inhibitors (SNRI; e.g. venlafaxine) noradrenergic and specific serotonergic antidepressants (NaSSAs; e.g. mirtazapine), serotonin antagonist and reuptake inhibitors (SARIs; e.g. trazodone) and norepinephrine-dopamine reuptake inhibitors (NDRIs; e.g. bupropion).

Despite the drawbacks of available antidepressants, they still provide tools to validate behavioural tests to study depression-like behaviour in animal models. Most importantly, they also provide tools to study brain function in depression in order to identify specific neurochemical abnormalities as well as to study novel targets for antidepressant drug treatment. Therefore, alternative drug targets for depression are being investigated to improve efficiency and onset of antidepressant drugs with fewer side effects.

1.4.2 Ketamine

Although the development of novel antidepressant drugs has proven to be extremely difficult because of the heterogeneous symptoms of depression, recent studies have found that previously known psychotropic drugs are capable of producing a rapid antidepressant response. The NMDA receptor antagonist ketamine has recently been used for the treatment of depression. When administered in subanaesthetic doses, ketamine has rapid and long lasting antidepressant effects in patients with major depression (Berman et al. 2000; Larkin and Beautrais 2011), bipolar disorder (Zarate et al. 2012) and treatment-resistant depression (Zarate et al. 2006).

In various rodent models of depression, ketamine showed rapid antidepressant-like effects on behaviour in the FST, learned helplessness paradigm and tail suspension test (Garcia et al. 2008; Koike et al. 2011; Li et al. 2010; Maeng et al. 2008; Tizabi et al. 2012). Ketamine treatment of mice exposed to chronic mild stress at a dose of 3 mg/kg, decreased duration of immobility in the FST, latency to feed in the OFT (measure of anxiety) and increased sucrose consumption (measure of anhedonia) (Autry et al. 2011). Ketamine treatment of rats at a dose of 10 mg/kg and 15 mg/kg has been shown to reduce

the duration of immobility in the FST induced by the 15-min pretest-swim, at 60 min after treatment (Garcia et al. 2008). Furthermore, ketamine treatment at a dose of 10 mg/kg has been shown to reduce the number of escape failures in the learned helplessness paradigm, increased sucrose consumption and reduced latency to feed in rats that have been exposed to chronic unpredictable stressors (Koike et al. 2011; Li et al. 2011). However, previous studies showed that at higher doses (80 mg/kg or 160 mg/kg), ketamine had no sustained antidepressant effect in the FST in rats at 24 h or 1 week after acute treatment (Li et al. 2010; Popik et al. 2008). Furthermore, repeated treatment (3 days, 7 days or 3 injections at 24, 5 and 1 h before test swim) of ketamine at a dose of 25 mg/kg had no immediate effect on immobility in the FST at 1 h after treatment in rats (Fraga et al. 2013; Gigliucci et al. 2013). The long-lasting behavioural effects of ketamine may lead to more insight into the molecular mechanisms that needs to be fully understood in order to develop more effective antidepressant drugs.

A previous study by Li et al. (2010) suggests that the immediate and long-lasting effects of ketamine are mediated by changes in the signalling pathway of the mammalian target of rapamycin (mTOR). Ketamine administration rapidly (30 min after injection) increased phosphorylated and activated levels of mTOR, eukaryotic initiation factor 4E and p70S6 kinase in synaptoneurosomes (Li et al. 2010). Furthermore, the levels of these phosphorylated factors returned to baseline after 2 h. On the other hand, PSD95, GluR1 and synapsin are upregulated at 2 h after ketamine administration and remained elevated for up to 72 h (Li et al. 2010). Activation of the mTOR pathway by ketamine increased BDNF levels in the hippocampus which was suggested to mediate the rapid antidepressant-like effects of ketamine (Autry et al. 2011; Garcia et al. 2008).

1.5 Conclusion

Major depression is a serious, heterogeneous disorder that is highly prevalent and activated by a complex interaction of genetic and environmental factors (Millan 2006). Accordingly, depression is associated with disability, increased medical co-morbidities and mortality that results in a significant social and economic burden (Lépine and Briley 2011). Despite the serious consequences of depression, there is still no diagnostically useful biological test to diagnose despression (Saveanu and Nemeroff 2012). This may be because the neurobiology underlying depression remains to be poorly understood. Clinical and preclinical evidence show abnormal monoamine neurotransmission of serotonin, noradrenaline and dopamine in relevant brain areas associated with depression or depression-like behaviour although several inconsistencies have been reported. These inconsistencies may be related to different methodology between studies as well as

differences in diagnostic features between patients. Furthermore, the mechanism of action of most antidepressant drugs, effective in many people, increases these monoamine neurotransmitters at the synapse. However, the currently available antidepressant drugs have several unmet clinical needs ranging from efficacy in treatment resistant patients, faster onset of action and decreased side effect profile, emphasizing the need for improved drug therapy targeting other neurochemical pathways. During the past decade, converging lines of evidence have led beyond the monoamine hypothesis of depression to improve antidepressant drug therapy. NMDA, opioid receptors and intracellular signalling pathways mediating neuroprotection via BDNF, ERK and GSK3β, have been shown to be involved in the pathophysiology of depression. However, evidence indicating how different risk factors of depression interact with these signalling pathways as well as the mechanism of action of monoaminergic antidepressant drugs on these pathways seems to be unresolved. There is no doubt that various neurotransmitter pathways are associated with the pathology of depression and likewise, several brain areas such as the limbic and PFC are associated with these pathways. Advances in brain imaging has allowed the identification of these brain regions and associated circuits in the pathophysiology of depression, more specifically, reduced activity in the frontal cortical areas and hyperactivity in the hippocampus and other limbic brain areas. Furthermore, the NAc plays a central role in the limbic system of the brain and regulates goal-directed behaviours by integrating information from limbic structures and the PFC (Goto and Grace 2008).

Animal models of depression provide important tools to unravel the neurobiological underpinnings of depression as well as providing development of novel therapeutic strategies. Various studies in the past have focused on animal models with a genetic predisposition or stress in adulthood. However, although these animal models demonstrated to have good validity, some are less reliable and useful considering that depression develops over a long-term period with multiple risk factors. In addition, few studies provided evidence for depression-/anxiety-like behaviour from the combination of these factors. The genetic WKY rat model of depression showed variability in results between studies in anxiety-like behaviour and depression-like behaviour such as measures of anhedonia as well as variability in response to antidepressant drug treatment. Also, previous studies reported several behavioural and neurobiological inconsistencies in the widely accepted MS model of depression.

Therefore, it is considered that MS will enhance the validity of WKY rats by superimposing the developmental stress of MS onto the genetic WKY model. As an alternative, it is considered that MS subjected to additional stress in adulthood will

enhance depression-/anxiety-like behaviour and provide novel insight in the molecular mechanisms involved in their depression-/anxiety-like behaviour. Therefore, by establishing models that will provide greater predictive validity and higher reliability, the rate at which novel drug therapies are developed can be increased.

1.6 Aims and Objectives

Study aims:

- To characterize the WKY rat model of depression-/anxiety-like behaviour.
- To establish a more robust WKY model of depression by subjecting WKY to MS.
- To measure depression-/anxiety-like behaviour and associated changes in relevant brain areas in the MS SD rat model that will be subjected to stress in adulthood and serve as a comparator model of depression.
- To determine the effect of ketamine in WKY and MS SD models of depression in order to provide further evidence for their predictive validity.

Study objectives:

- The WKY rat model will be characterized and appropriate substrain of WKY (WKY/NCrl and WKY/NHsd) selected based on its optimal depression- and anxiety-like behaviour in the FST, OFT and EPM compared to the Wistar control strain. This will also include the measurement of antidepressant response to desipramine in the FST to establish the optimal dose of desipramine and whether a pretest-swim is required. Furthermore, changes in opioid receptors and TH in response to desipramine treatment will be measured.
- Following selection of the appropriate substrain of WKY, depression- and anxiety-like behaviour and antidepressant response to desipramine will be measured in MS WKY rats in the FST, OFT, EPM and USVs recorded in order to establish a more robust model of depression. The function of USVs in WKY rats will be further explored in order to determine its usefulness as a marker of depression and provide further evidence for their depression phenotype. Furthermore, the effect of desipramine treatment on serotonin levels as well as p-GSK3β and p-ERK will be measured in the PFC and dopamine levels and opioid receptors in the NAc.
- The widely used MS SD rat model, as a comparator model, will be subjected to an additional stress in adulthood (restraint stress) to determine if restraint stress exaggerates the depression-/anxiety-like behaviour of MS SD rats. Furthermore,

the effect of MS and restraint stress on BDNF levels in the ventral hippocampus and protein profile in the PFC of SD rats, will be measured.

- The rapid and sustained antidepressant effects of ketamine on WKY and MS SD rat models of depression will be measured in the FST as well as in their response in USVs to removal of cage mate(s) as a marker for depression-like behaviour.

1.7 Study layout

The thesis comprises four studies that directly relate to the above aims and objectives and each will be presented sequentially as follow:

- Chapter 2 (WKY/NCrl, WKY/NHsd and Wistar rats) contains three experiments aimed at characterizing the WKY as a valid model of depression. This will include selecting the appropriate substrain of WKY (experiment 1), measuring the response to the optimal dose of desipramine (experiment 2) and the effect of desipramine on the neurochemistry of WKY rats (experiment 3).
- Chapter 3 will further proceed to study the depression-/anxiety-like behaviour, antidepressant effect of chronic treatment with desipramine on depression-/anxiety-like behaviour and the effect of desipramine treatment on the neurochemistry of the appropriate substrain of WKY that will be subjected to MS (WKY/NCrl, MS WKY/NCrl and Wistar control).
- As an alternative, the study in Chapter 4 (non-maternally separated SD, MS SD and MS SD subjected to restraint stress) will study depression-/anxiety-like behaviour and neurochemistry in the MS SD rats subjected to restraint stress.
- Chapter 5 (WKY/NCrl and Wistar; non-maternally separated SD and MS SD rats) contains three experiments aimed at determining the sustained (experiment 1) and rapid effects (experiment 2) of acute ketamine treatment at different subanaesthetic doses in WKY/NCrl rats. In addition, experiment 3 will determine the sustained effects of acute ketamine treatment in MS SD rats.

Chapter 2

Strain differences in depression-/anxiety-like behaviour and antidepressant response

2.1 Introduction

The WKY rat was developed from the outbred Wistar rat as a control strain for the spontaneous hypertensive rat (Okamoto and Aoki 1963) and has also been described as a genetic animal model of depression with hyper-responsiveness to stress (Paré 1992; Paré 2000; Tejani-Butt et al. 2003). The WKY's selective response to antidepressant drugs in the FST provides a unique model to study the neurochemical dysfunction responsible for the underlying depression-like behaviour.

The association of the opioid system with depression is well recognized (Chen and Lawrence 2004; Lutz and Kieffer 2013; Vilpoux et al. 2002). Recently, it has been shown that opioid receptors are altered in WKY rats (Carr et al. 2010). This was evidenced by higher KOR and dynorphine protein levels in the cortex and NAc as well as antidepressant-like behaviour to a KOR antagonist in the FST (Carr et al. 2010). However, the effect of antidepressant drugs such as desipramine, which reverses depression-like behaviour of WKY rats, on opioid receptors is unknown. The KOR as well as MOR are both expressed on dopamine terminals in the NAc where it has been shown to regulate neurotransmission such as dopamine release (Ebner et al. 2010; Mansour et al. 1995).

Previous studies found behavioural differences between WKY obtained from different vendors (Browne et al. 2015; Paré and Kluczynski 1997; Sagvolden et al. 2008) and therefore the first aim of this study was to compare the depression-/anxiety-like behaviour of inbred WKY rats obtained from Charles River Laboratories (WKY/NCrl) to WKY rats from Harlan (WKY/NHsd) and outbred Wistar rats in order to establish a robust animal model of depression. Following selection of the appropriate substrain of WKY to use as a model of depression, the response to different doses of the antidepressant drug, desipramine (8 mg/kg and 15 mg/kg) at the shortest treatment period, as well as the effect of a FST pretest-swim on the antidepressant response, was tested. The effect of the pretest-swim was tested by comparing a single FST session with the FST that comprised of a pretest-

swim and test swim since both FST protocols have been used previously on the WKY animal model of depression (Lahmame et al. 1997; Lopez-Rubalcava and Lucki 2000; Malkesman et al. 2009; Paré 1992; Tejani-Butt et al. 2003). To determine if the antidepressant response in the WKY is related to changes in opioid and dopamine neurotransmission, we measured the density of MOR, KOR and TH using polyacrylamide gel electrophoresis and western blotting.

2.2 Materials and Methods

2.2.1 Animals

A total number of 82 animals (52 WKY/NCrl rats from Charles River Laboratories (USA); 15 WKY/NHsd from Harlan (UK); 15 outbred Wistar rats from Charles River Laboratories (USA)) bred in the University of Cape Town Animal Unit, were used for this study. Rats were housed in plexiglass cages with woodchip bedding in a temperature-controlled room (23±1 °C) with food and water available ad libitum. The housing facility was maintained on a 12 h light/12 h dark cycle (lights on from 06h00 to 18h00).

The study was conducted in accordance with the guidelines of the South African National Standard: The care and use of animals for scientific purposes (2008) and approved by the University of Cape Town Faculty of Health Sciences Animal Ethics Committee (#010/036).

This study consisted of 3 behavioural groups for experiment 1: WKY/NHsd (n = 15), WKY/NCrl (n = 15) and Wistar (n = 15) rats. At approximately postnatal day 60 (P60), WKY/NHsd, WKY/NCrl and Wistar rats were tested in the EPM and immediately thereafter in the OFT. One day after the EPM and OFT (P61), rats were allowed to swim for 15 min (pretest-swim). After 24 h, rats were exposed to a second 5-min test swim session.

Experiment 2 and experiment 3 consisted of different groups of WKY/NCrl rats that were randomly divided into treatment groups for each experiment. Experiment 2 consisted of the following treatment groups: (a) WKY/NCrl treated with Saline (WKY Sal) for 4 days (n = 3), 9 days (n=4) or 14 days (n = 4) and (b) WKY/NCrl treated with 8 mg/kg desipramine (WKY DMI-8) for 4 days (n = 4), 9 days (n = 4) or 14 days (n = 8). Starting on P60, different groups of WKY/NCrl rats were treated for 4 days, 9 days or 14 days with 8 mg/kg desipramine or saline. At 19 h after the last desipramine/saline injection, a single 15-min FST was performed on P64 (for rats treated for 4 days), P69 (for rats treated for 9 days) and P74 (for rats treated for 14 days).

Experiment 3 consisted of the following treatment groups: (a) WKY/NCrl treated with Saline (WKY Sal, n = 5) and (b) WKY/NCrl treated with 15 mg/kg desipramine (WKY DMI-15, n = 5). Starting on P60, WKY/NCrl rats were treated for 26 days with 15 mg/kg desipramine or saline. At 19 h after the last desipramine/saline injection (P85), a pretest 15-min swim was performed. After 24 h, rats were exposed to a 5-min test swim session. On P87, rats were decapitated and brain areas rapidly dissected, snap frozen in liquid nitrogen and stored at -80°C until later analysis of the density of MOR, KOR and TH by polyacrylamide gel electrophoresis and western blotting. The same 5 rats/group used for measuring FST behaviour, were also selected for biochemical analysis.

2.2.2 Behaviour

Behavioural tests were chosen according to the aims of the study with minimal stress to the animals. Behavioural tests were conducted in the light phase (06h00-09h00). Adult male rats were transferred to the behavioural room on the morning of behavioural testing and allowed to habituate for 1 h to the behavioural room conditions.

2.2.2.1 Elevated plus maze (Experiment 1)

The EPM test is an established measure of anxiety-like behaviour in rodents (Walf and Frye 2007). The EPM consist of two opposing open arms 50×10 cm and two opposing closed arms with 40 cm high walls and elevated 50 cm above the floor. The arms are connected by a central 10×10 cm square. The EPM test was performed in experiment 1. At approximately postnatal day 60 (P60), the rat was placed in the central square facing the open arm and allowed to freely explore the open and closed arms of the EPM for 5 min. Between recordings, the arena was cleaned with 70% ethanol to prevent the scent of the previous rat in apparatus to influence other animals‟ behaviour. An aerial view of time spent in the open arms, closed arms and central square was recorded with a Sony Handycam, DCR-SX83E video camera for subsequent scoring with Noldus ethovision XT version 7 software (Noldus, Wageningen, Netherlands). Center-point detection was used by Noldus ethovision to track the movement of the rat in order to determine time spent in the arms and center zone of the EPM. The lighting in the room was 80 lux.

2.2.2.2 Open field test (Experiment 1)

The OFT was performed immediately after the EPM in the same room, with lighting at 80 lux. The open field (OF) consists of a 100×100 cm black arena with 50 cm high walls. The arena was cleaned with 70 % ethanol after each behavioural recording. The OFT was performed by placing the rat in one of the corners of the OF facing the open arena. The rat was allowed to explore the arena for 5 min. Behaviours was recorded with a video camera

(Sony Handycam, DCR-SX83E, Japan) and subsequently analyzed using Ethovision software (Noldus, Wageningen, Netherlands). Locomotor activity (total distance travelled) and anxiety-like behaviour (entries into the inner zone and time spent in the inner zone) was scored.

2.2.2.3 Forced swim test (Experiment 1-3)

The FST was carried out according to Porsolt et al. (1977) as modified by Detke and Lucki (1995). Rats in experiment 1 (1 day after the EPM and OFT at P61), rats in experiment 2 (P64, P69, P74) and rats in experiment 3 (P85) were placed in individual transparent cylinders (40×19 cm) containing 30 cm of water (23–25 °C) without their tails touching the bottom of the cylinder. Each rat was allowed to swim for 15 min, which facilitates development of immobility 24 h later in the 5-min test swim as well as being a requirement to differentiate between vehicle-treated and drug-treated rats in the test swim (Borsini et al. 1989; Slattery and Cryan 2012). The water was changed after each rat‟s swim session, to avoid the influence of alarm substances left behind by the previous rat (Abel and Bilitzke 1990). After 24 h, the rats in experiment 1 and experiment 3 were exposed to a second 5-min test swim session (19 h after desipramine/saline injection). Experiment 2 was performed with a single 15-min FST (19 h after desipramine/saline injection) with immobility measured in the first 5 min because a similar result for immobility was obtained for the 15-min pretest and 5-min test swims in experiment 1 and behavioural differences between WKY and Wistar rats were already apparent within the first 5 min of the 15 min pre-test swim. Similarly, previous studies also showed antidepressant effects in genetic rat models of depression in a single 5-min FST session (Lahmame et al. 1997; Malkesman and Weller 2009; Overstreet 1993; Yadid et al. 2000). The FST behaviour was recorded for both the 15-min pretest-swim and 5-min test swim on a video camera for later analysis. Total time immobile (making only movements necessary to keep its head above the water), swimming (when the rat moved around the tank in a non-vigorous manner) and struggling (diving, jumping, climbing or strongly moving all four limbs) was tracked by Ethovision.

2.2.3 Drug Treatment (Experiments 2-3)

Starting on P60, rats in experiment 2 and experiment 3 were either injected with 8 mg/kg desipramine hydrochloride (Sigma-Aldrich, St Louis, MO, USA), 15 mg/kg desipramine hydrochloride or an equivalent volume of 0.9 % sterile saline (n=5) between 11h00 and 12h00. Desipramine/saline was administered for 4 days, 9 days or 14 days in experiment 2 (and 26 days in experiment 3). The dose 8 mg/kg and 15 mg/kg was based on previous

studies (Armario et al. 1988; Zhao et al. 2008) and the treatment period for experiment 2 was selected according to the range from previous studies (Deupree et al. 2007; Paré 1992; Tejani-Butt et al. 2003). Desipramine and saline were administered intraperitoneally in a volume equivalent to 2.5 ml/kg. Desipramine was freshly prepared each morning by dissolving it first in deionized H_2O to enhance solubility and then making it up to the required volume with 0.9 % sterile saline (50 % v/v; H_2O/saline). On P87 for experiment 3, rats were decapitated and brain areas rapidly dissected, snap frozen in liquid nitrogen and stored at -80°C until later biochemical analysis.

2.2.4 Biochemistry (Experiment 3)

2.2.4.1 Western blotting with chromogenic detection

The NAcC, NAcS and PFC brain tissue samples were sonicated for 25 s each in radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 1 % Triton X-100, 0.1 % sodium dodecyl sulfate, 20 mM Tris base (pH 7.5), 1 % deoxycholate) and protease inhibitor cocktail (2 µl/1ml). Chemicals were supplied by Merck (NaCl, TritonX-100, Tris base), Sigma-Aldrich (deoxycholate) and Thermo Scientific (protease inhibitor cocktail). Following sonication, the samples were mixed on a vortex mixer and centrifuged at 17200 x g for 30 min at 4 °C. The supernatants were collected and protein concentrations determined with the Pierce BCA Protein Assay kit (Thermo Scientific, Rockford, USA). Samples were read at 562 nm on a Kayto RT-2100C microplate reader. After the protein determination, samples were boiled at 95°C for 4 min containing added 10 mM dithiothreitol (DTT) and 6x loading dye (4 % sodium dodecyl sulfate, 20 % glycerol, 10% 2-mercaptoethanol, 0.125 M Tris base (pH 6.8), 0.03 % bromophenol blue). Twenty micrograms of total protein was loaded onto 12% SDS-polyacrylamide gels and electrophoresed at 150 V for 1.25 h. Electrophoresed proteins were transferred onto nitrocellulose membranes (Amersham Hybond-ECL, GE Healthcare, USA) at 100 V for 1 h. The membranes were blocked with 5 % bovine serum albumin (BSA) in phosphate-buffered saline (PBS) containing 1 % Tween (PBS-Tween) solution for 1 h and then immunolabeled with rabbit polyclonal primary antibody against the µ-opioid receptor-1 (MOR-1, C-20, sc-7488-R; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:1000) or blocked with 5 % powdered milk (Clover SA, Roodepoort, South Africa) in PBS-Tween buffer for 1 h and then immunolabeled with rabbit polyclonal antibody against the κ-opioid receptor (KOR-1, H70, sc-9112; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:2000) or mouse monoclonal antibody against TH (TH-16, T2928; Sigma-Aldrich, St. Louis, MO, USA) (1:8000). Membranes were incubated with their respective antibodies overnight at 4 °C. This was followed by incubation with the appropriate secondary HRP-

conjugated antibodies for 2 h. Blots were developed using a metal intensified 3,3‟-Diaminobenzidine (DAB) protocol based on Adams (1981) and optimized for western blot detection. Following secondary antibody incubation, the membranes were washed with PBS buffer and developing medium added. The developing medium consisted of 5 mg/ml DAB, 10% $NiNH_4SO_4$ and 30% $CoCl_2$ which was filtered and 50% H_2O_2 added before addition to the membrane. The membrane was left to develop between 1-5 min and the reaction was stopped by rinsing the membrane in PBS when the bands appeared. Blots were reprobed with rabbit polyclonal antibody against p38 MAP kinase (P38, Sigma-Aldrich, St. Louis, MO, USA) to confirm equal loading of protein. Densitometric analysis was performed using Un-Scan-It gel analysis software (Silk Scientific, Inc, USA). The density of each band was determined and expressed as a percentage of the mean density of all the bands that represented that specific protein on the gel.

2.2.5 Statistical Analysis

Data were normally distributed (Shapiro-Wilk test). Data for experiment 1 were analyzed by means of analysis of variance (ANOVA), followed by Tukey‟s post-hoc test with correction for multiple comparisons. Data for experiment 2 and experiment 3 were analyzed by using the unpaired t-test. Data are presented as mean ± SEM.

2.3 Results

2.3.1 Behaviour

2.3.1.1 Experiment 1: Strain difference in depression-/anxiety-like behaviour

Elevated plus maze

One-way ANOVA showed a significant effect of rat strain on time spent in the center area of the EPM ($F_{(2, 42)} = 8.11$, $p < 0.01$) with no effect on the amount of time spent in the open arms (Fig. 2.1a) or closed arms (Fig. 2.1b). Post-hoc comparisons showed that the WKY/NCrl and WKY/NHsd rats spent significantly more time in the center area of the EPM than Wistar rats ($p < 0.01$; Fig. 2.1c).

Open field test

One-way ANOVA revealed a significant effect of strain on distance travelled ($F_{(2, 42)} = 39.52$, $p < 0.001$), entries into the inner zone ($F_{(2, 42)} = 19.73$, $p < 0.001$) and time spent in the inner zone ($F_{(2, 42)} = 6.84$, $p < 0.01$) of the OFT. Post-hoc comparisons revealed that both WKY/NHsd and WKY/NCrl rats travelled a shorter distance than Wistar rats ($p <$

0.001; Fig. 2.2). WKY/NCrl rats travelled a significantly shorter distance than WKY/NHsd rats ($p < 0.05$). WKY/NHsd and WKY/NCrl entered the inner zone less frequently than Wistar rats ($p < 0.001$). Furthermore, WKY/NHsd ($p < 0.05$) and WKY/NCrl ($p < 0.01$) spent less time in the inner zone than Wistar rats.

Forced swim test

One-way ANOVA revealed a significant effect of strain on immobility ($F_{(2, 42)} = 22.05$, $p < 0.001$), swimming ($F_{(2, 42)} = 14.19$, $p < 0.001$) and struggling ($F_{(2, 42)} = 22.97$, $p < 0.001$) in the FST. Post-hoc comparisons showed that WKY/NCrl ($p < 0.001$) and WKY/NHsd ($p < 0.01$) spent significantly more time immobile than Wistar rats (Fig. 2.3a). WKY/NCrl spent significantly more time immobile than WKY/NHsd rats ($p < 0.01$). The WKY/NCrl spent less time actively swimming than WKY/NHsd ($p < 0.01$) and Wistar rats ($p < 0.001$; Fig. 2.3b). The WKY/NHsd and WKY/NCrl spent significantly less time struggling than Wistar rats ($p < 0.001$; Fig. 2.3c). WKY/NCrl spent significantly less time struggling than WKY/NHsd ($p < 0.05$).

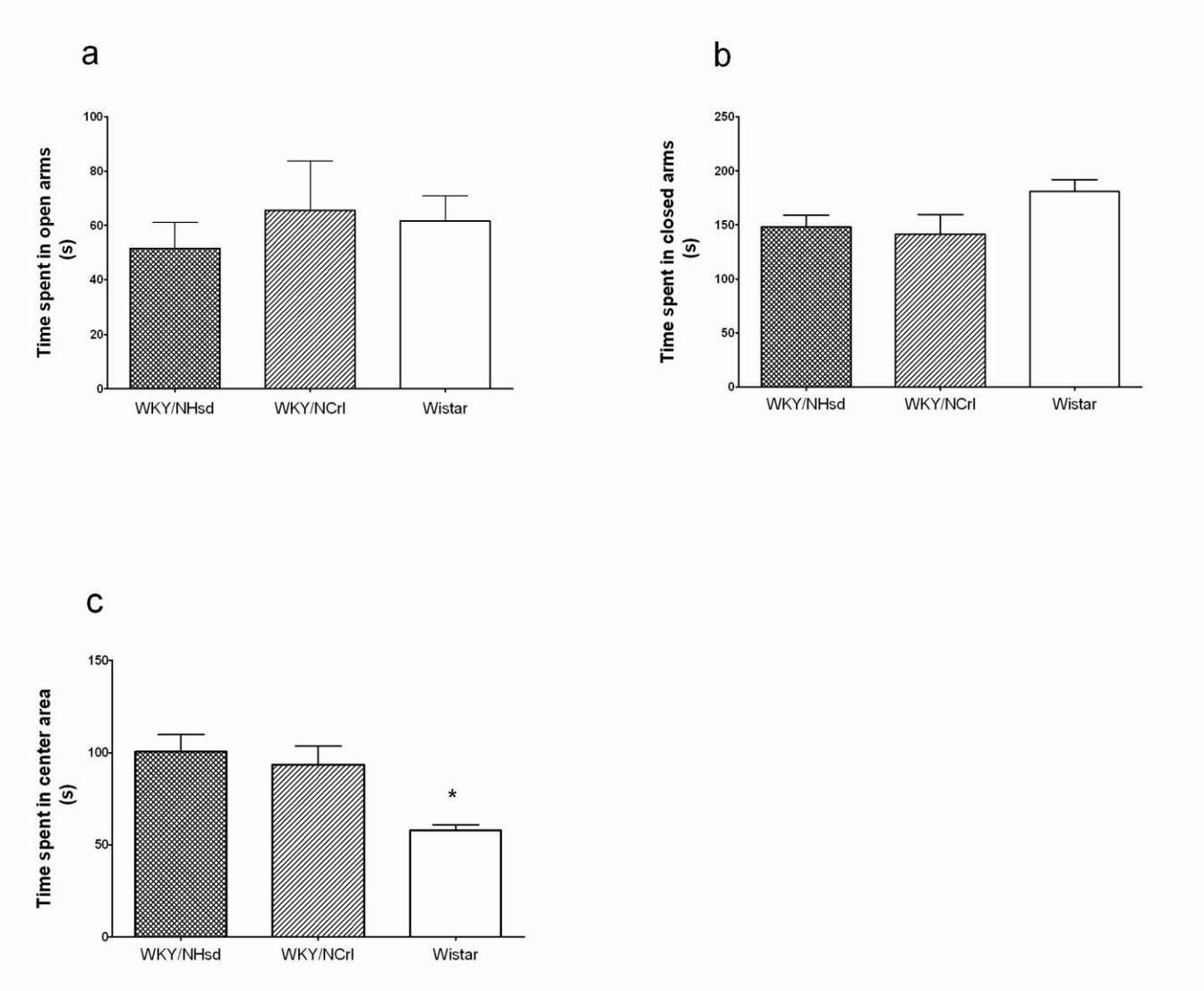


Figure 2.1: Time spent in the open arms, closed arms and center area by WKY/NHsd, WKY/NCrl and Wistar rats in the EPM. The EPM revealed no difference between WKY/NHsd, WKY/NCrl and Wistar rats in time spent in the open arms (a) and closed arms (b). The WKY/ NHsd and WKY/NCrl rats spent more time in the center zone (c) than Wistar rats. * Wistar significantly different from WKY/NHsd andWKY/NCrl rats, $p < 0.05$; Tukey post-hoc test (n = 15/group). Data presented as mean ± SEM.

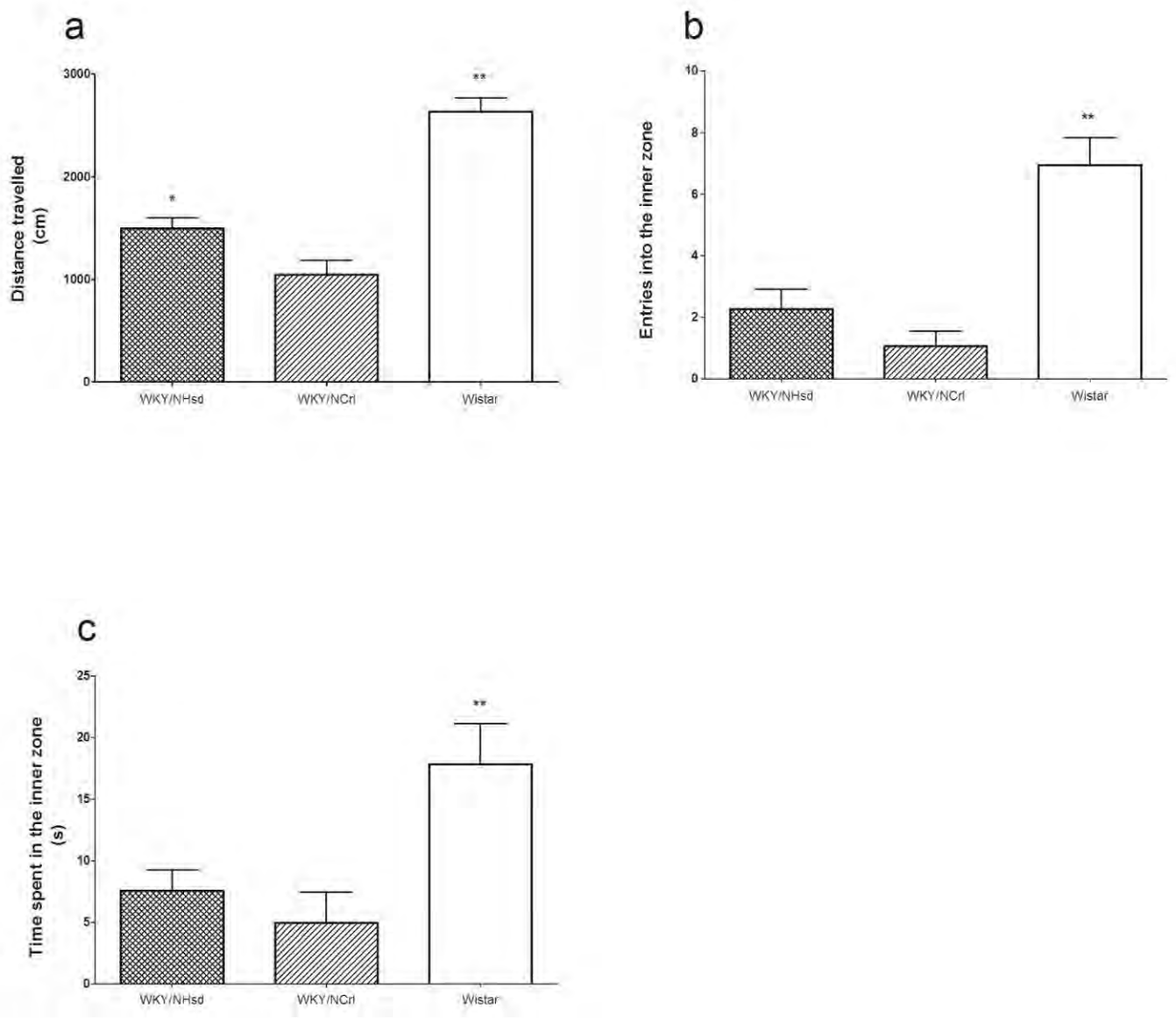


Figure 2.2: Distance travelled, entries into the inner zone and time spent in the inner zone by WKY/NHsd, WKY/NCrl and Wistar rats in the OFT. The WKY/NCrl were less active than WKY/NHsd rats in the OFT (a) Both WKY/NCrl and WKY/NHsd travelled a shorter distance than Wistar rats in the OFT. The WKY/NCrl and WKY/NHsd entered the inner zone less frequently (b) and spent less time in the inner zone (c) than Wistar rats. *WKY/NHsd significantly different from WKY/NCrl, $p < 0.05$; ** Wistar significantly different from WKY/NHsd and WKY/NCrl rats, $p < 0.001$; Tukey‟s post-hoc test (n = 15/group). Data presented as mean ± SEM.

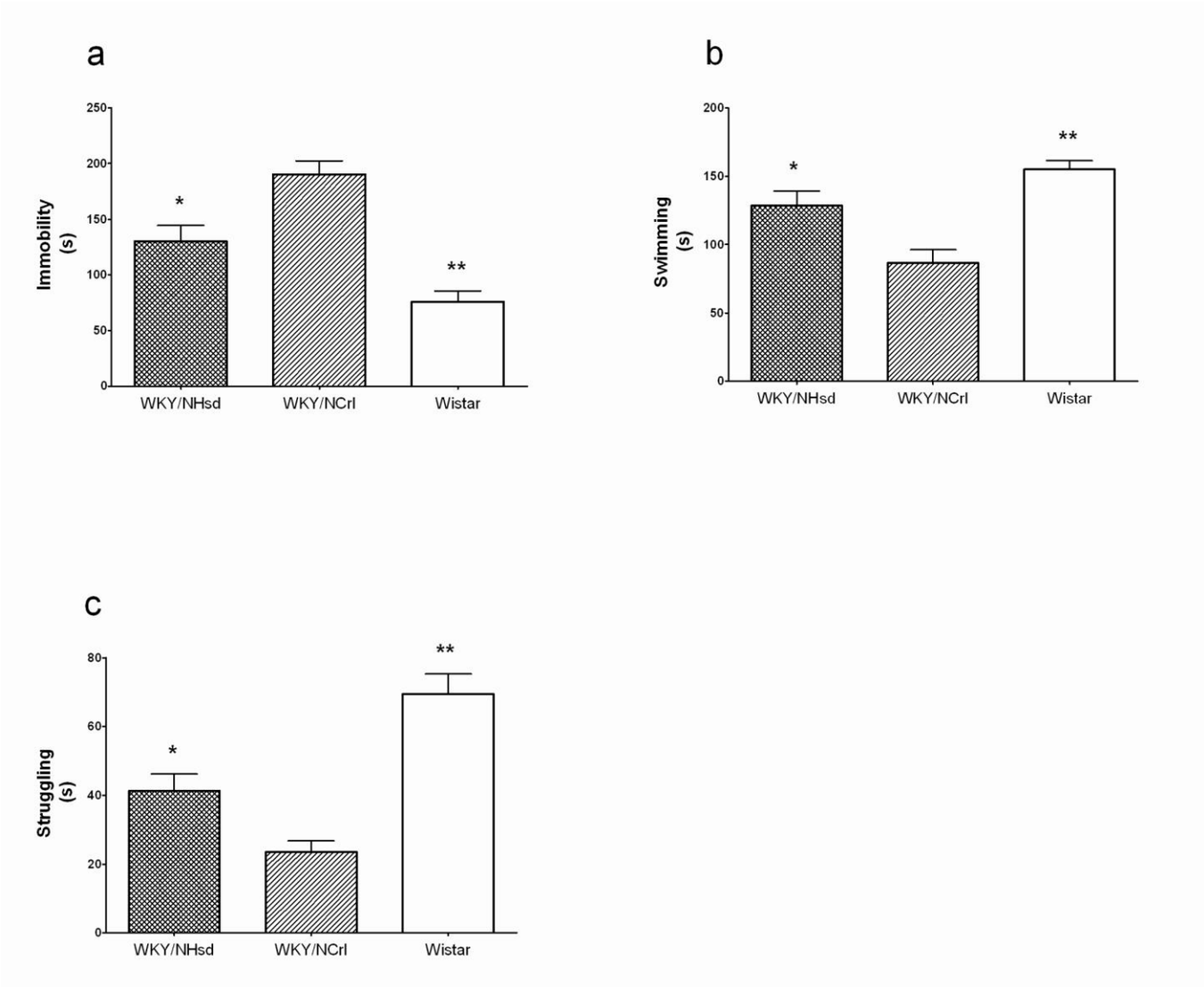


Figure 2.3: Immobility, swimming and struggling behaviours of WKY/NHsd, WKY/NCrl and Wistar rats in the FST. The WKY/NCrl displayed more immobility (a) and less active swimming (b) and struggling (c) behaviours than WKY/NHsd rats and both displayed less activity than Wistar rats in the FST. * WKY/NHsd significantly different from WKY/NCrl, $p < 0.05$; ** Wistar significantly different from WKY/NCrl andWKY/NHsd rats, $p < 0.01$; Tukey‟s post-hoc test (n = 15/group). Data presented as mean ± SEM.

2.3.1.2 Experiment 2: Effect of 8 mg/kg desipramine treatment on a single forced swim test session of WKY/NCrl

Forced swim test

Unpaired t-test indicated no significant difference between WKY DMI-8 and WKY Sal rats in immobility (Fig. 2.4a), swimming (Fig. 2.4b) and struggling (Fig. 2.4c) behaviours in the FST after 4 days, 9 days or 14 days of treatment.

2.3.1.3 Experiment 3: Effect of 15 mg/kg desipramine on WKY/NCrl in the pretest-swim and test swim session

Forced swim test

Unpaired t-test revealed no significant difference between WKY Sal and WKY DMI-15 rats in immobility in the pretest-swim of the FST (Fig. 2.5). Unpaired t-test indicated that WKY Sal rats spent more time immobile than WKY DMI-15 rats ($t_{(8)} = 3$, $p < 0.05$; Fig. 2.6a) and WKY DMI-15 rats spent more time struggling than WKY Sal rats ($t_{(8)} = 4.28$, $p < 0.01$; Fig 2.6c) with no significant effect on swimming in the 5-min test session of the FST (Fig. 2.6b).

2.3.2 Biochemistry

2.3.2.1 Experiment 3: Density of tyrosine hydroxylase and opioid receptor protein in the nucleus accumbens and prefrontal cortex

Unpaired t test revealed no significant difference between WKY Sal and WKY DMI-15 rats in density of KOR in the PFC (Fig. 2.7a), NAcC (Fig. 2.8a) and NAcS (Fig. 2.9a), MOR in the PFC (Fig. 2.7b), NAcC (Fig. 2.8b) and NAcS (Fig. 2.9b) and TH in the NAcC (Fig. 2.8c) and NAcS (Fig. 2.9c).

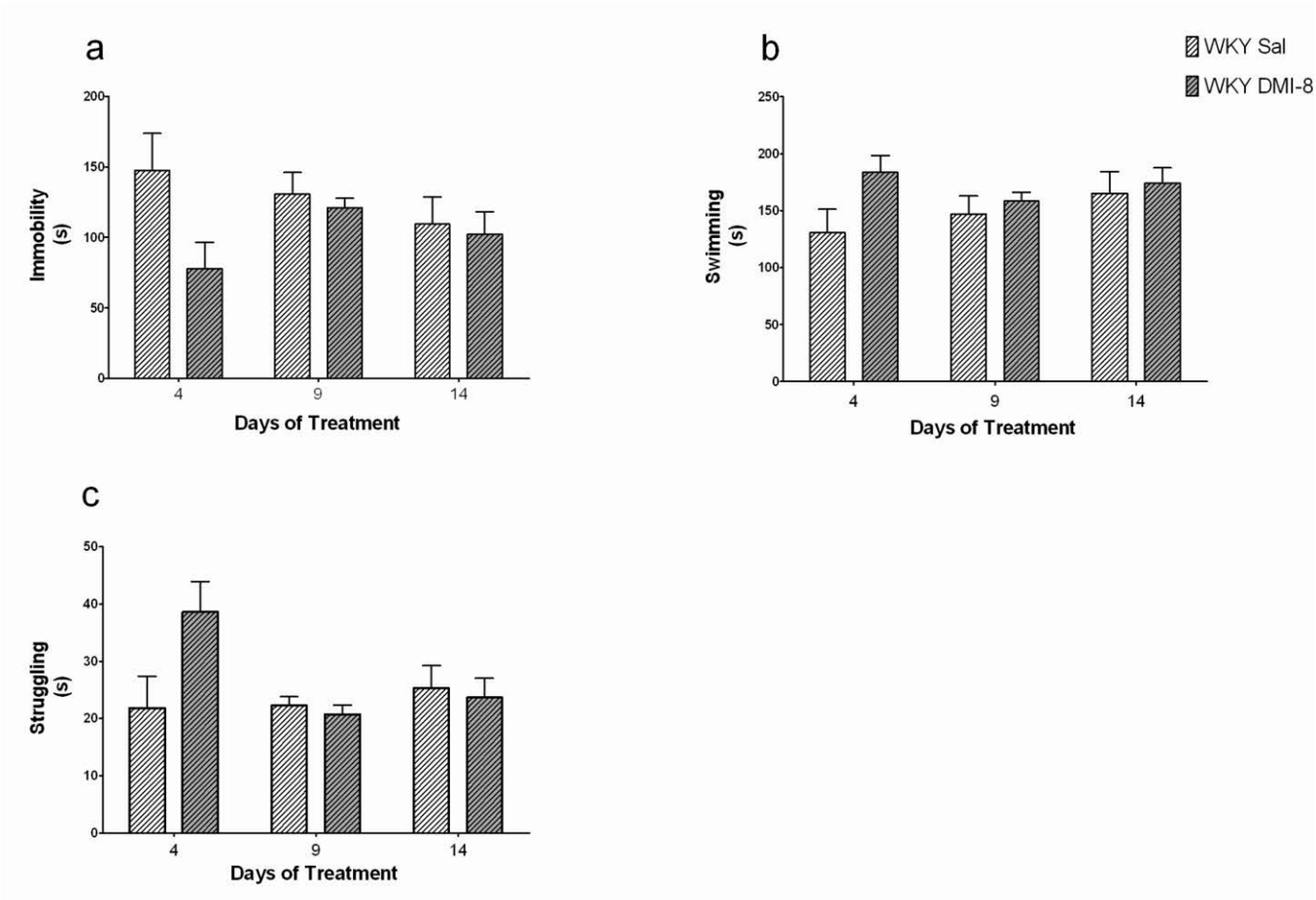


Figure 2.4: Immobility, swimming and struggling behaviours of WKY/NCrl rats in the FST following treatment with saline or 8 mg/kg desipramine. The FST revealed no significant difference between WKY DMI-8 and WKY Sal on day 5, day 10 and day 15 in immobility (a), swimming (b) and struggling (c) behaviours (n = 3-8/group). Data presented as mean ± SEM.

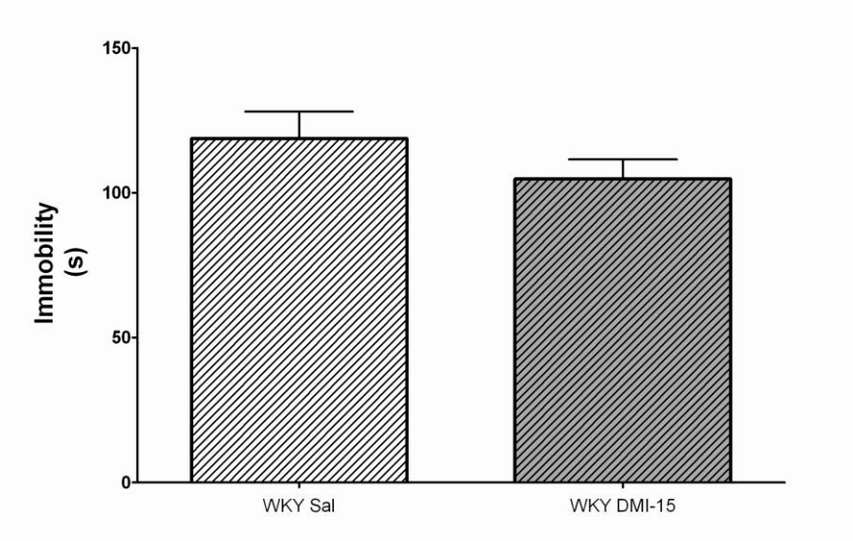


Figure 2.5: Immobility of WKY/NCrl rats in the FST (pretest-swim) following treatment with saline or 15 mg/kg desipramine. The FST revealed no significant difference between WKY Sal and WKY DMI-15 rat groups in the first 5 min of a pretest-swim session following 15 mg/kg desipramine or saline treatment (n = 5/group). Data presented as mean ± SEM.

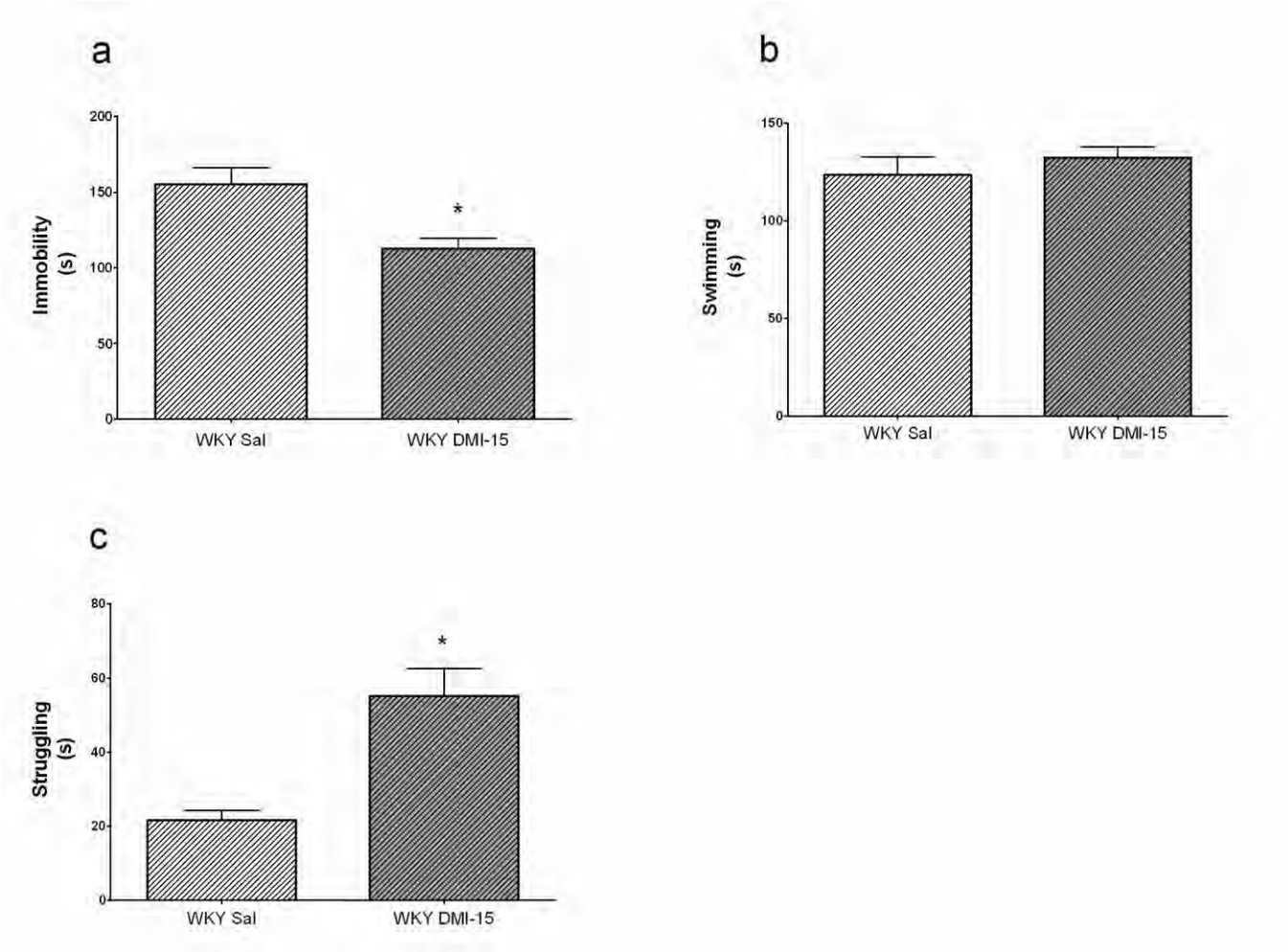


Figure 2.6: Immobility, swimming and struggling behaviours of WKY/NCrl rats in the FST (test swim) following treatment with saline or 15 mg/kg desipramine. The WKY DMI-15 displayed less immobility (a) and more active struggling (c) behaviour than WKY Sal rats. * WKY DMI-15 significantly different from WKY Sal, $p < 0.05$; Unpaired t test (n = 5/group). Data presented as mean ± SEM.

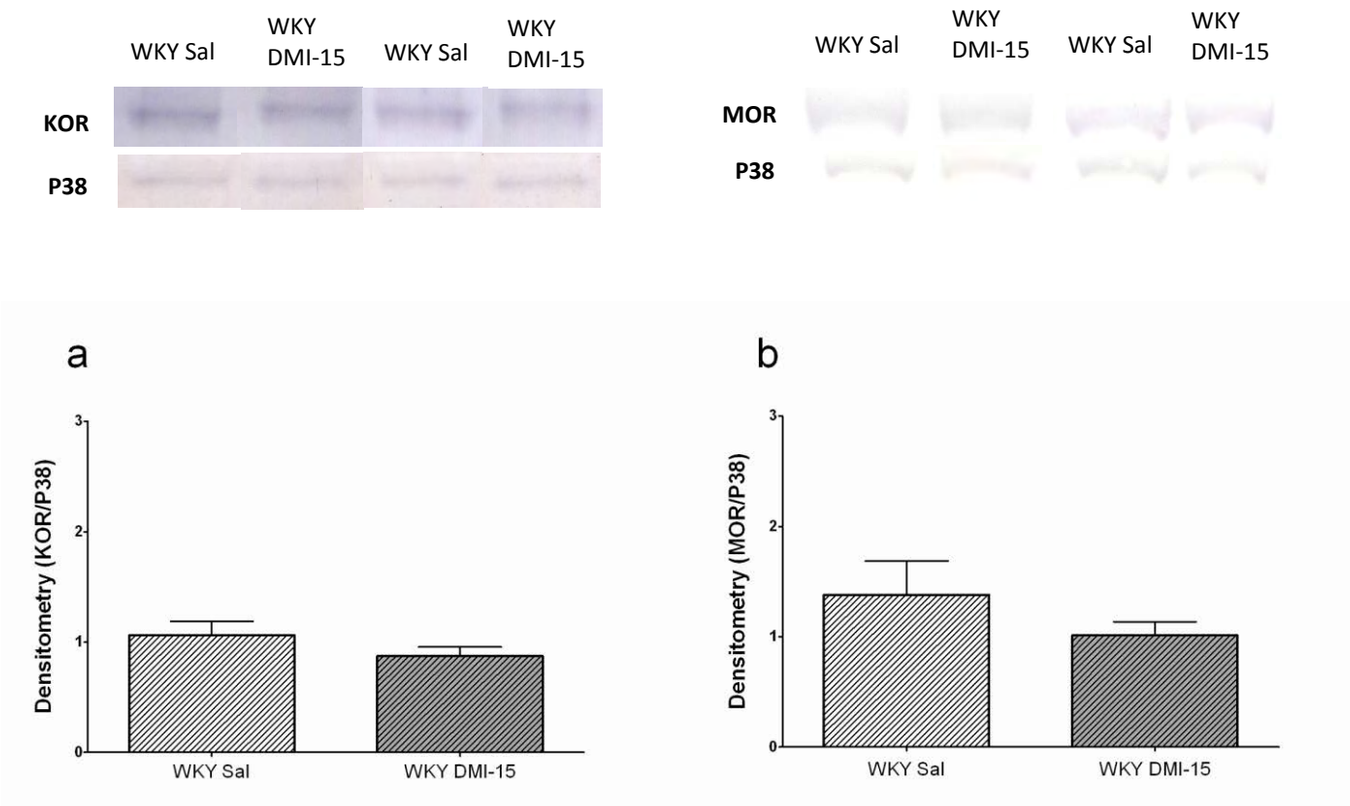


Figure 2.7: KOR and MOR density in the PFC of WKY/NCrl rats, determined by Western blot analysis. Western blots revealed no significant difference in KOR (a) and MOR (b) density between WKY Sal and WKY DMI-15 rat groups. Unpaired t-test (n = 5/group). Data presented as mean ± SEM.

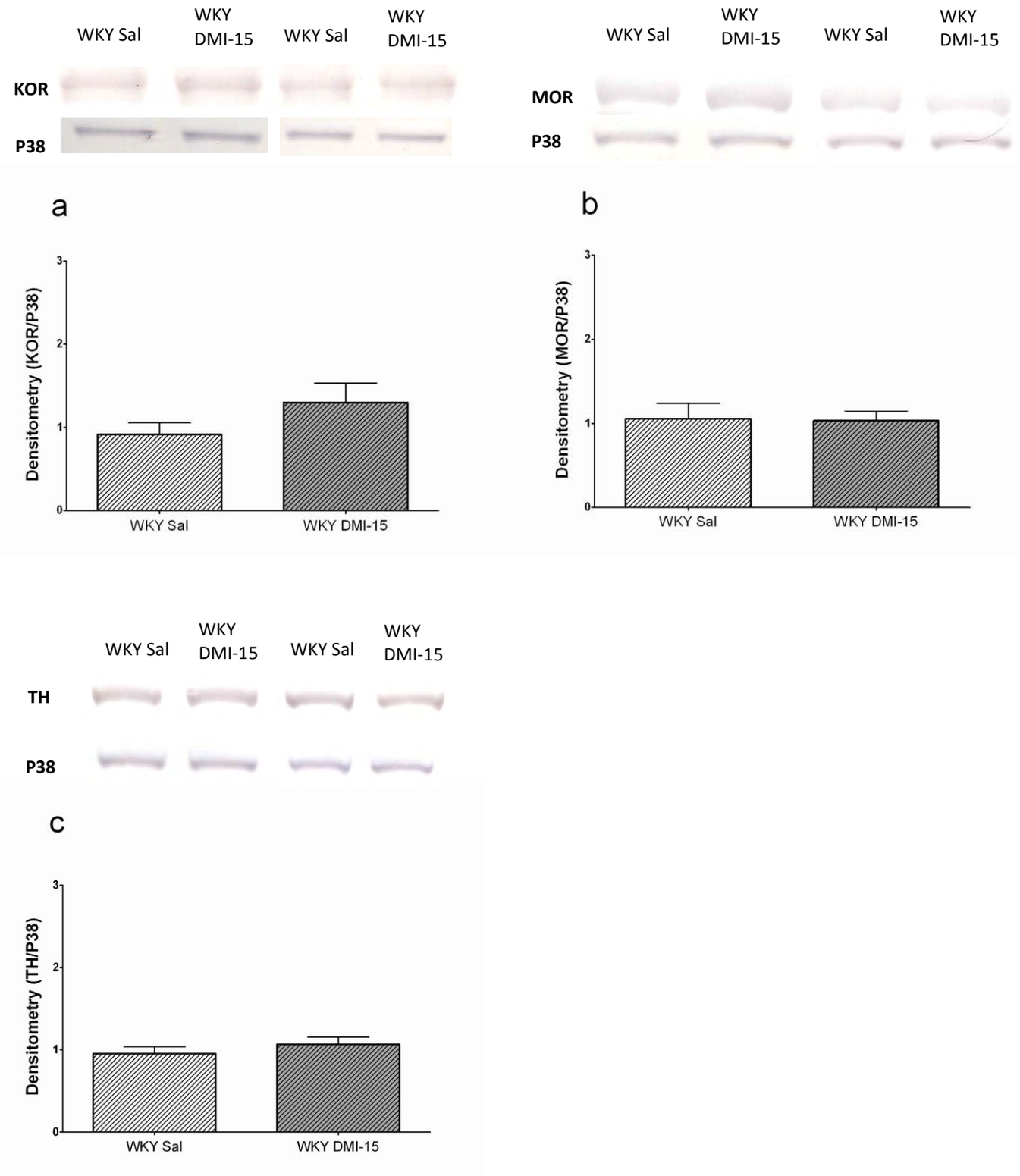


Figure 2.8: KOR, MOR and TH density in the NAcC of WKY/NCrl rats, determined by Western blot analysis. Western blots revealed no significant difference in KOR (a), MOR (b) and TH (c) density between WKY Sal and WKY DMI-15 rats. Unpaired t-test (n = 5/group). Data presented as mean ± SEM.

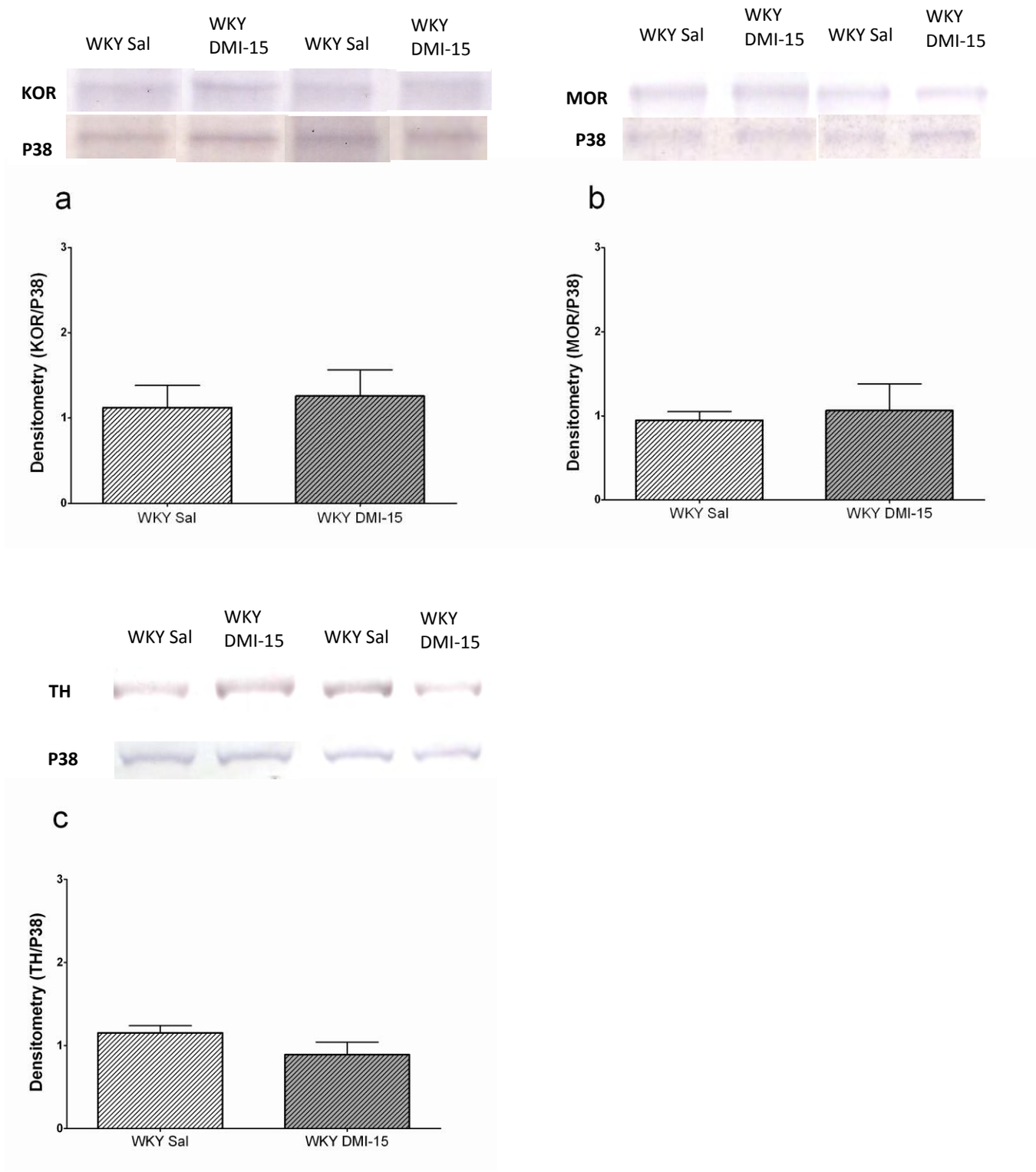


Figure 2.9: KOR, MOR and TH density in the NAcS of WKY/NCrl rats, determined by Western blot analysis. Western blots revealed no significant difference in KOR (a), MOR (b) and TH (c) density between WKY Sal and WKY DMI-15 rats. Unpaired t-test (n = 5/group). Data presented as mean ± SEM.

2.4 Discussion

This study is consistent with the use of WKY/NCrl as a model of depression: (1) WKY/NCrl rats were less active in the OFT and FST than WKY/NHsd and Wistar rats and (2) desipramine treatment decreased depression-like behaviour in the FST.

In experiment 1, the WKY rats displayed various behavioural characteristics generally found in depression namely a lack in general activity (psychomotor retardation) and spent less time in the inner zone (anxiety-like behaviour) of the OFT, a significant amount of time in the center zone of the EPM (indecisiveness) and a significant amount of time immobile in the FST ("despair") compared to the Wistar rats. Furthermore, the WKY/NCrl displayed less activity in the OFT and FST than the WKY/NHsd substrain. The current study is in agreement with various studies that have measured depression-like behaviour specifically in WKY from Charles River Laboratories (Lopez-Rubalcava and Lucki 2000; Pardon et al. 2002; Paré 2000; Tejani-Butt et al. 2003). Time spent in the center zone of the EPM is in frequently measured but provides an indication of indecisiveness (Nosek et al. 2008). The current study is in agreement with previous studies comparing the amount of time the WKY rats spent in the center zone relative to other rat strains (Nam et al. 2014; Nosek et al. 2008). Previous studies also found behavioural differences in activity in the OFT, FST and a visual discrimination task between substrains of WKY obtained from different suppliers (Browne et al. 2015; Paré and Kluczynski 1997; Sagvolden et al. 2008) which they attributed to differences in inbreeding programs and the fact that WKY were not fully inbred prior to distribution (Kurtz et al. 1989; Paré and Kluczynski 1997; Sagvolden et al. 2009). However, the study by Browne et al. (2015) showed that WKY rats from Taconic (WKY/NTac) displayed decreased immobility in the FST compared to WKY/NCrl and WKY/NHsd. In contrast to the present study, no significant difference was found in immobility between WKY/NCrl and WKY/NHsd (Browne et al. 2015). It is therefore important when reporting results and making comparisons between studies to indicate the source of WKY rats (Sagvolden et al. 2009).

In experiment 2, 8 mg/kg desipramine had no significant effect after 4 days, 9 days or 14 days of treatment in the first 5 min of a single 15-min FST session. Therefore, 8 mg/kg desipramine was not sufficient to cause an antidepressant effect or it is also possible that a pretest-swim is required for an antidepressant effect to be detected more easily (Borsini et al. 1989; Slattery and Cryan 2012). Previously either the inclusion of a pretest-swim (Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003) or a single swim session (Lahmame et al. 1997; Paré 1992) revealed antidepressant activity in WKY rats.

However, it was also found that a single 5-min FST in prepubertal rats treated chronically with 8 mg/kg desipramine had no antidepressant effects (Malkesman et al. 2009). This was investigated in experiment 3 which included a pretest-swim and a higher desipramine dosage of 15mg/kg.

In experiment 3, 15 mg/kg desipramine decreased immobility and increased active struggling behaviour of WKY/NCrl rats in the 5-min test session of the FST. Similar to experiment 2, desipramine treatment had no effect on the pretest-swim behavior of the rats. The results are therefore in agreement with previous studies in which desipramine similarly decreased immobility in WKY/NCrl rats but in some cases, struggling rather than swimming behaviour was increased (Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003). Similar to studies with SD rats (Detke and Lucki 1995; Lucki 1997), the noradrenaline uptake inhibitor also showed a selective increase in struggling behaviour in WKY/NCrl rats. The results therefore revealed the necessity of a pretest-swim to detect significant differences between treatment groups since neither 8 mg/kg or 15 mg/kg affected immobility behavior in a single swim session of the FST.

Western blot analysis revealed no significant difference in opioid receptor (KOR and MOR) and TH densities in the PFC, NAcC and NAcS between WKY rats treated with saline and WKY rats treated with desipramine. It is therefore possible that these neurotransmitter systems in the NAc and PFC brain areas are unrelated to the response to desipramine treatment (Berrocoso and Mico 2009; Plaznik et al. 1985; Scuvée-Moreau and Svensson 1982). Another reason for the non-significant effect of desipramine on KOR, MOR and TH, may originate from the methodology. The increased sensitivity of the DAB detection was suggested to enhance sensitivity by adding metal salts of cobalt and nickel to the reaction (Adams 1981). This colorimetric detection method have several advantages over other detection methods such as an easier methodology with less trial and error, more cost-effectiveness as well as a signal that does not fade over time. However, this method is limited to proteins of high abundance such as MOR, KOR and TH and therefore sensitivity is inferior to detection methods such as chemiluminescence when detecting low abundance proteins.

In conclusion, the WKY/NCrl were found to be a robust animal model of depression evidenced by high immobility levels in the FST compared to other strains as well as a treatment response to 15 mg/kg of antidepressant drug, desipramine.

Chapter 3

Behavioural changes during antidepressant treatment of the maternally separated Wistar-Kyoto rat model of depression

3.1 Introduction

Stress experienced during the early stages of life can impact negatively on the affective state of the individual in adulthood, especially individuals who are genetically predisposed in developing depression (Kim et al. 2007; Taylor et al. 2004). Rodent models of early life stress have been used in an effort to better understand the impact of early life adversity on behaviour and biochemistry of the brain. MS of pups from the dam for 3 h per day in the early stages of their development, when pups are completely dependent on their mother, is considered stressful and a well-established animal model of early developmental stress that results in depression-/anxiety-like behaviour later in life (Daniels et al. 2004; Dimatelis et al. 2012b; Kalinichev et al. 2002; Lee et al. 2007; Sánchez et al. 2001). However, the response to developmental stress is highly dependent on the genetic predisposition (Neumann et al. 2005) but the exact interaction remains unknown.

USVs in animals, as with human speech, provide information on social status (to direct specific behaviour) (Farrell and Alberts 2002; Takahashi et al. 1983) as well as emotional state (Knutson et al. 2002; Seyfarth and Cheney 2003). However, the emotional and communicational functions of USVs are not necessarilyseparate from each other (Brudzynski and Pniak 2002). Adolescent and adult rats are able to emit 50-kHz vocalizations in appetitive situations such as play, mating, anticipation of food reward and electrical brain stimulation (Burgdorf et al. 2000; Burgdorf et al. 2008; Knutson et al. 1998; Panksepp and Burgdorf 2000). The 50-kHz vocalizations have been further characterized into flat and FM calls. The flat 50-kHz calls are emitted during anticipation of social contact, as opposed to FM calls which are indicative of the emotional state (Brudzynski and Pniak 2002; Wohr et al. 2008).

The delayed onset of currently available antidepressant drugs suggests that their therapeutic effects are mediated by changes that occur beyond their immediate effects on the noradrenergic and serotonergic systems. Glycogen synthase kinase-3 (GSK3) plays a central role in many converging intracellular signalling pathways and is suggested to be involved in both neuroprotection and psychiatric disorders (Hunsberger et al. 2009; Rowe et al. 2007). The MAPK/ERK pathway is important for synaptic plasticity (Thomas and Huganir 2004) and has been shown to play a crucial role in depression and the action of antidepressants (Fumagalli et al. 2005; Musazzi et al. 2010; Qi et al. 2009). Furthermore, ERK can be considered an integrator of neuroprotective pathways (Almeida et al. 2005; Bravo et al. 2009; Colucci-D'Amato et al. 2003) and inhibition of GSK3 results in increased phosphorylation of ERK1/2 in cell lines (Wang et al. 2006). Opioid receptors are well recognized to play a role in depression. Specifically, opioid receptors play a role in depression-like behavior in adult rats as a results of early developmental stress (Dimatelis et al. 2012b). Recent evidence also reported the role of opioid receptors having a neuroprotective effect through GSK3-induced apoptosis (Olianas et al. 2011; Polakiewicz et al. 1998).

Despite the involvement of both genetic and environmental factors in depression, only a few studies determined depression-/anxiety-like behaviour and antidepressant response in genetic rat models of depression exposed to early developmental stress (El Khoury et al. 2006; Musazzi et al. 2010; Neumann et al. 2005; Petersén et al. 2008; Womersley et al. 2011). The aim of this study was to (1) use the WKY/NCrl and MS WKY/NCrl models of depression to measure changes in behaviour during antidepressant drug treatment in comparison with the Wistar that served as the control group and (2) to investigate molecular changes in dopamine and serotonin levels, opioid receptors and their signalling proteins (phospho (ser9)-GSK3β (p-GSK), phospho-ERK1/2 (p-ERK)) to provide further insight into the molecular pathways that are affected by antidepressant drug treatment and MS in the WKY model of depression..

It was hypothesized that MS WKY rats would display exaggerated depression-/anxiety-like behaviour and increased response to desipramine treatment compared to normally reared WKY rats. Wistar rats were used as a reference strain for behaviour. Furthermore, it was hypothesized that desipramine treatment would increase the number of high frequency USVs emitted by the rat remaining in the home cage after removal of the cage mate(s) (signalling communication and desire for social contact with the litter mates) and normalize serotonin and dopamine neurotransmitters, opioid receptors and their signalling proteins.

3.2 Materials and methods

3.2.1 Animals

This study was conducted in accordance with the guidelines of the South African National Standard: The care and use of animals for scientific purposes (1st edition, 2008) and approved by the University of Cape Town Faculty of Health Sciences Animal Ethics Committee (#010/036). A total number of 76 animals were obtained from the University of Cape Town Animal Unit. Housing conditions were similar as described in chapter 2. This study consisted of 3 rat groups: normally reared WKY/NCrl (WKY), maternally separated WKY/NCrl (MS WKY) and Wistar control rats. The rat groups were randomly divided into treatment groups: (a) normally reared WKY/NCrl treated with Saline (WKY Sal, n =15), (b) normally reared WKY/NCrl treated with desipramine (WKY DMI, n =5-6), (c) maternally separated WKY/NCrl treated with Saline (MS WKY Sal, n =17), (d) maternally separated WKY/NCrl treated with desipramine (MS WKY DMI, n = 9–10), (e) Wistar rats treated with Saline (Wistar Sal, n =15) and (f) Wistar rats treated with desipramine (Wistar DMI, n =13). A total of 9 rats were lost to the study before the endpoint was reached (dying/euthanased) and this led to transferring 6 rats from the desipramine group to the saline group before treatment started.

WKY/NCrl pups were separated from their dams for 3 h per day between 09h00 and 13h00 from P2 to P14. Some of the WKY/NCrl litters were normally reared and left undisturbed in their home cage. At P21, all pups were weaned and males were separated from the females. Wistar rats were obtained from the Animal Unit at a minimum of 1 week before the start of the experiment. For 4 days prior to baseline USV recording (P52 - P55), rats were transferred to the room in which the USVs were to be recorded, where they were handled briefly and then returned to the rat facility. One day after the last day of brief handling, baseline USVs were recorded for 4 days (P56 – P59) prior to treatment.

Desipramine treatment started at P60 for 15 days at a dose of 15 mg/kg during which time USV‟s were recorded daily in the dark phase (20h00–00h00) until the end of treatment (P74). Recording started immediately after the cage mate(s) had been removed from the home cage and continued for 6 min. The USVs of the cage mate(s) were recorded on the same evening with a minimum interval of 1 h between recordings of the cage mates. At P71 (17 h after desipramine/saline injection) each rat was allowed to swim for 15-min (pretest-swim). After 24 h, the rats were exposed to a 5-min test swim session (17 h after desipramine/saline injection). The EPM test was performed on rats at P74 (17 h after desipramine/saline injection) and immediately followed by the OFT. On P74, rats were

decapitated and brain areas rapidly dissected, snap frozen and stored in liquid nitrogen until later biochemical analysis. The rats with immobility data closest to the mean value for their group (12 WKY Sal, 6 WKY DMI, 13 MS WKY Sal and 9 MS WKY DMI) were selected for determination of serotonin and dopamine levels. Rats (9 WKY Sal, 6 WKY DMI, 9 MS WKY Sal and 8 MS WKY DMI) were similarly selected for western blot analysis.

3.2.2 Maternal Separation

Postnatal day 0 (P0) was designated as the date of birth. On P2, rats that were born in the Satellite Animal Facility were culled to 8 pups per litter to allow equal nourishment between litters. MS was carried out for 3 h per day between 09h00 and 13h00 from P2 to P14 (Daniels et al. 2004). Pups were transferred (together with some home cage bedding to avoid handling of the pups) to a clean cage and housed in a different room (31–33 °C, to prevent hypothermia) to prohibit communication between pups and dam by means of USVs. After 3 h of separation, pups were returned to the home cage with the dam. Care was taken to prevent handling of the pups. Non-maternally separated control rats were normally reared and left undisturbed during the MS procedure. Cages of MS and non-maternally separated rats were cleaned twice a week, half of the soiled bedding was replaced with fresh bedding to prevent disturbance of the pups. At P21, all pups were weaned; males were separated from females and housed 2–3 in a cage.

3.2.3 Behaviour

Behavioural tests were conducted on adult male rats in the same conditions as in chapter 2.

3.2.3.1 Elevated plus maze

The EPM were performed on rats at P74 (17 h after desipramine/saline injection) as described in chapter 2.

3.2.3.2 Open field test

The OFT was performed immediately after the EPM as described in chapter 2

3.2.3.3 Forced swim test

The FST was carried out as described in chapter 2 for experiment 1 and 3. At P71 (17 h after desipramine/saline injection) the rats were placed in individual transparent cylinders (40×19 cm) containing 30 cm of water (23–25 °C). Each rat was allowed to swim for 15 min (habituation). After 24 h, the rats were exposed to a 5-min test swim session (17 h

after desipramine/saline injection) during which FST behaviour was recorded for later analysis as described in chapter 2.

3.2.3.4 Ultrasonic vocalizations

The USV protocol was carried out according to Wohr et al. (2008) who showed that the rat remaining in the home cage after removal of its cage mates emits an increased number of 50-kHz USVs (Wohr et al. 2008). Rats were housed 2–3 in their home cage (36×20×18 cm). USVs were measured in the dark phase (20h00–00h00). For 4 days prior to baseline USV recording (P52 - P55), rats were transferred to the room in which the USVs were to be recorded, where they were handled briefly and then returned to the rat facility. This was necessary in order for the rats to get accustomed to the handling procedure as well as the room conditions. One day after the last day of brief handling, baseline USVs were recorded for 4 days (P56 – P59) prior to treatment. Desipramine treatment started at P60 during which USV"s were recorded daily until the end of treatment (P74). Rats were left for a minimum of 2 h to habituate to the room conditions before USV recording. No other rats were present in the USV room during a USV recording session. Recording started immediately after the cage mate(s) had been removed from the home cage and continued for 6 min. The USVs of the cage mate(s) were recorded on the same evening with a minimum interval of 1 h between recordings of the cage mates. No significant differences in baseline USVs were found between rats recorded first, second and third. Rat calls were recorded with a P48 Electret ultrasound microphone (Avisoft Bioacoustics) positioned 30 cm above the base of the cage. The microphone was sensitive to frequencies of 10–120 kHz and connected with the E-MU 0404 Audio/midi interface (E-MU Systems, USA) to a computer with a sampling rate of 192 kHz and 16 bit resolution. Acoustic data were displayed in real-time on Sea Pro Ultra version 2.0 software and recorded for later analysis. Analysis of data was conducted with a Fast Fourier Transform (2048 FFT, Hanning window, 1024 window size and a 75 % time window overlap). Spectrograms were produced at a frequency resolution of 187.5 Hz and a time resolution of 0.667 ms. Calls were manually counted as total number of calls, number of FM calls and number of flat calls between 40 kHz and 96 kHz. Calls that were counted were based on the selection criteria described by Wright et al. (2010). Calls had to meet certain spectrographic criteria: they had to occur within a range of 40 kHz to 96 kHz, with maximum interruption of 20 ms and a sound intensity and structure that were clearly distinct from background under optimal viewing settings. A call that was classified as flat had minimum change in frequency with a mean slope between −0.2 and 0.2 kHz/ms.

3.2.4 Drug Treatment

On P60, rats were either injected with 15 mg/kg desipramine hydrochloride (Sigma-Aldrich, St Louis, MO) or an equivalent volume of 0.9 % sterile saline between 13h00 and 14h00 for 15 days. Desipramine and saline were administered intraperitoneally in a volume equivalent to 2.5 ml/kg. Desipramine was freshly prepared each morning as described in chapter 2. On P74, rats were decapitated and brain areas rapidly dissected, snap frozen and stored in liquid nitrogen until later biochemical analysis.

3.2.5 Biochemistry

3.2.5.1 Dopamine and Serotonin determination by Enzyme-linked immunosorbent assay (ELISA)

The NAcS and PFC brain tissue samples of drug-treated WKY rat groups (WKY Sal, WKY DMI, MS WKY Sal and MS WKY DMI) were used to determine dopamine and serotonin levels respectively. Brain samples were weighed and sonicated for 10 s in 1ml RIPA buffer/50mg tissue (150 mM NaCl, 1% Triton X-100, 0.1% SDS, 20 mM Tris base (pH 7.5), 1% deoxycholate, 1 mM EGTA and 2 µl/1ml protease inhibitor cocktail). Following sonication, samples were mixed on a vortex and centrifuged at 17200 x g at 4 °C for 30 min. A volume of 20 µl of each sample was used for ELISA and the rest stored in liquid nitrogen for western blotting. The dopamine and serotonin concentration of the supernatant was determined according to the manufacturer‟s instructions (IBL international, Hamburg, Germany). Determinations were in duplicate. Results are expressed as ng/mg wet weight (ww).

3.2.5.2 Western blotting with chemiluminescence detection

The NAcC, NAcS and PFC brain tissue samples were prepared for western blotting as described above (section 3.2.5.1). Twenty micrograms of total protein was loaded onto 12% SDS-polyacrylamide gels and electrophoresed at 150 V for 2 h. Electrophoresed proteins were transferred onto nitrocellulose membranes (Amersham Hybond-ECL, GE Healthcare, USA) at 100 V for 1 h. The membranes were blocked for 1h with 5 % bovine serum albumin (BSA) in TBS-Tween for membranes with phospho- and total proteins, 5 % BSA in PBS-Tween for membranes with MOR proteins and 5 % milk in PBS-Tween for membranes with KOR proteins. Following blocking, membranes were immunolabeled with rabbit polyclonal primary antibody against MOR-1 (C-20, sc-7488-R, Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:12000), KOR-1 (H70, sc-9112; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:14000) and phospho-p44/42 MAPK (p-ERK,

Thr202/Tyr204, #9101; Cell Signaling Technology, Beverly, MA, USA) (1:7000) and with rabbit monoclonal primary antibody against p-GSK3β (Ser9)(#9323S; Cell Signaling Technology, Beverly, MA, USA) (1:2000). Membranes were incubated with their respective antibodies overnight at 4 °C. Following incubation, membranes were incubated with the appropriate secondary HRP-conjugated antibodies for 2 h. Blots were developed using Clarity Western ECL Substrate (Biorad laboratories Inc., USA). Blots with p-GSK and p-ERK were stripped and reprobed with rabbit monoclonal primary antibody against total GSK-3β (GSK, #9315; Cell Signaling Technology, Beverly, MA, USA) (1:8000) and with rabbit polyclonal primary antibody against total p44/42 MAPK (ERK, #9102L; Cell Signaling Technology, Beverly, MA, USA) (1:8000) respectively. After detection of total proteins with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, USA), all membranes were stripped and reprobed with anti-p38 MAP kinase antibody (P38, Sigma, Missouri, USA) (1:9000) to confirm equal loading of proteins. Densitometric analysis was performed using Un-Scan-It gel analysis software (Silk Scientific, Inc, USA). The density of each band was determined and expressed as a percentage of the mean density of all the bands of that specific protein.

3.2.6 Statistical Analysis

Behavioural and biochemical data were normally distributed (Shapiro-Wilk test) and were analyzed by means of analysis of variance (ANOVA), followed by Tukey‟s post-hoc test with correction for multiple comparisons. USV data were not normally distributed (Shapiro-Wilk test) and were analyzed with the Kruskal–Wallis ANOVA, followed by Dunn‟s post-hoc test with correction for multiple comparisons. The behavioural (EPM, OFT and FST) and biochemical data are presented as mean ± SEM and USV data are presented as median and interquartile range.

3.3 Results

3.3.1 Behaviour

3.3.1.1 Elevated plus maze

Time spent in open arms

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups (WKY, MS WKY and Wistar) ($F_{(2, 69)} = 6.51$, $p < 0.01$) in time spent in the open arms in the EPM. Post-hoc analysis indicated that the MS WKY DMI rats spent a greater amount of time in the open arms of the EPM than MS WKY Sal

rats ($p < 0.05$; Fig. 3.1a). The MS WKY DMI rats spent more time in the open arms than Wistar DMI rats ($p < 0.01$). The WKY Sal rats similarly spent more time in the open arms than the Wistar Sal rats ($p < 0.05$).

Time spent in closed arms

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F_{(2, 69)} = 19.24$, $p < 0.001$) in time spent in the closed arms of the EPM. Post-hoc analysis indicated that the MS WKY Sal rats spent more time in the closed arms than WKY Sal ($p < 0.05$; Fig. 3.1b). The Wistar Sal spent more time in the closed arms than the WKY Sal ($p < 0.001$) and MS WKY Sal rats ($p < 0.05$). The Wistar DMI rats spent more time in closed arms than WKY DMI ($p < 0.01$) and MS WKY DMI rats ($p < 0.05$).

Time spent in the center zone

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F_{(2, 69)} = 14.52$, $p < 0.01$) and a rat group × drug treatment interaction ($F_{(2, 69)} = 3.30$, $p < 0.05$) in time spent in the center zone of the EPM. Post-hoc analysis indicated that the MS WKY DMI rats spent less time in the center zone than WKY DMI rats ($p < 0.01$; Fig. 3.1c). The WKY DMI rats spent more time in the center zone than Wistar DMI ($p < 0.01$) and MS WKY DMI rats ($p < 0.01$). The Wistar Sal rats spent less time in the center zone than WKY Sal ($p < 0.001$) and MS WKY Sal rats ($p < 0.05$). The MS WKY DMI rats spent less time in the center zone than MS WKY Sal rats ($p < 0.01$).

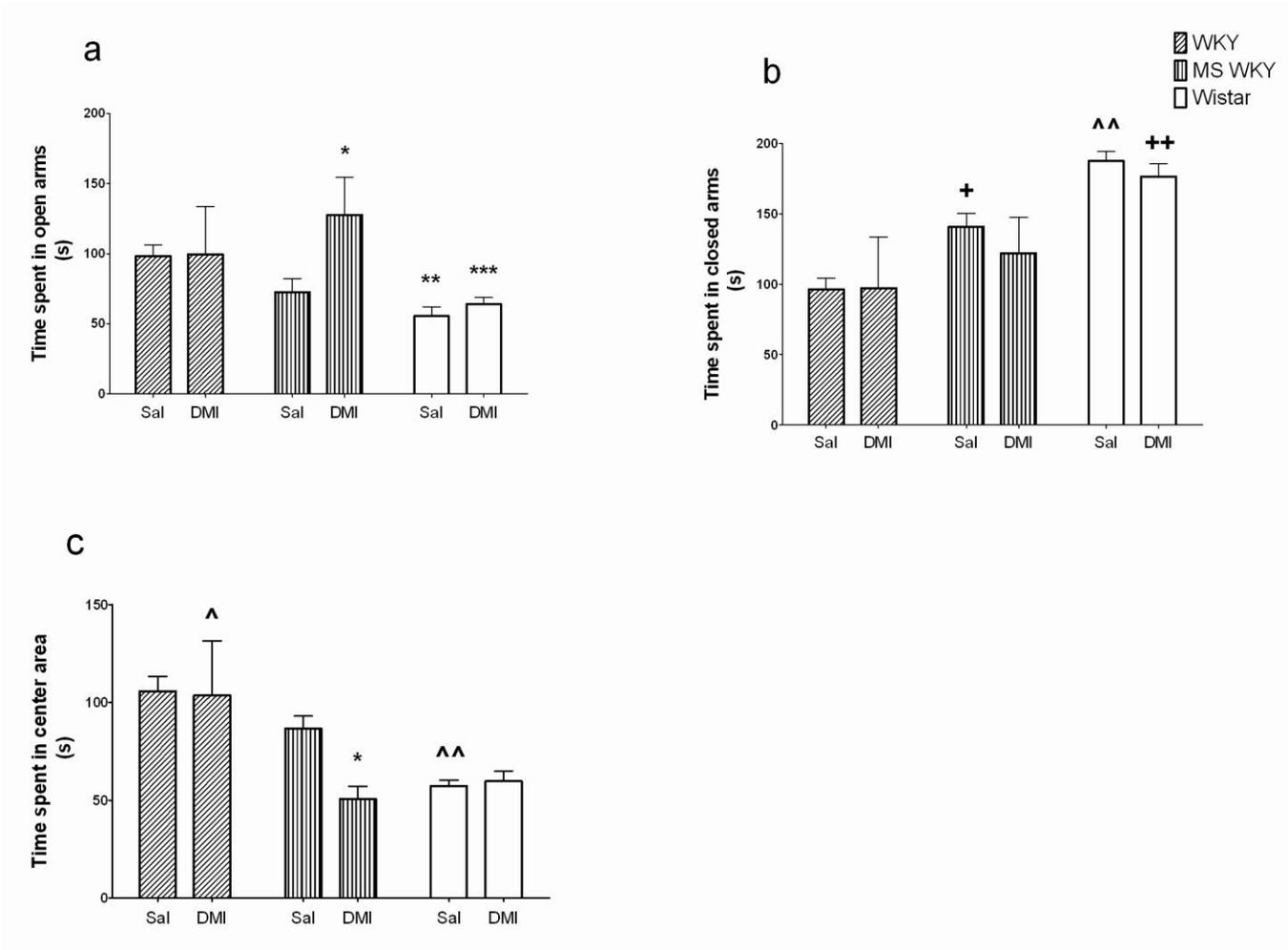


Figure 3.1: Time spent in the open arms, closed arms and center area by WKY, MS WKY and Wistar rats in the EPM following treatment with saline/desipramine. Desipramine treatment decreased anxiety (a) and indecisiveness (c) in MS WKY rats in the EPM. The MS WKY spent more time in the closed arms than the WKY (b). * MS WKY DMI significantly different from MS WKY Sal, $p < 0.05$; ** Wistar Sal significantly different from WKY Sal, $p < 0.05$; *** Wistar DMI significantly different from MS WKY DMI, $p < 0.01$; ^ WKY DMI significantly different from MS WKY DMI and Wistar DMI, $p < 0.05$; ^^ WKY Sal and MS WKY Sal significantly different from Wistar Sal, $p < 0.01$; + MS WKY Sal significantly different from WKY Sal, $p < 0.05$; ++ Wistar DMI significantly different from WKY DMI and MS WKY DMI; Tukey"s post-hoc test (WKY sal: n = 15; WKY DMI: n = 6; MS WKY sal: n = 17; MS WKY DMI: n = 9; Wistar Sal: n = 15; Wistar DMI: n = 13). Data presented as mean ± SEM.

3.3.1.2 Open field test

Distance travelled

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F_{(2, 69)} = 96.10$, $p < 0.001$), a drug treatment effect ($F_{(1, 69)} = 41.53$, $p < 0.001$) and a rat group × drug treatment interaction ($F_{(2, 69)} = 9.92$, $p < 0.001$) in total distance travelled in the open field. Post-hoc analysis revealed that the WKY DMI rats travelled a shorter distance than the WKY Sal rats and MS WKY DMI travelled a shorter distance than MS WKY Sal rats ($p < 0.001$; Fig. 3.2). The Wistar Sal rats travelled a greater distance than WKY Sal ($p < 0.001$) and MS WKY Sal rats ($p < 0.001$). The Wistar DMI rats travelled a greater distance than MS WKY DMI ($p < 0.001$) and WKY DMI rats ($p < 0.001$).

3.3.1.3 Forced swim test

Immobility

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F_{(2, 67)} = 34.86$, $p < 0.001$), a drug treatment effect ($F_{(1, 67)} = 30.86$, $p < 0.001$) and a rat group × drug treatment interaction ($F_{(2, 67)} = 9.88$, $p < 0.001$) for time spent immobile in the FST. Post-hoc analysis revealed that the WKY DMI rats spent less time immobile than WKY Sal rats ($p < 0.01$; Fig. 3.3a) and the MS WKY DMI rats spent less time immobile than MS WKY Sal rats ($p < 0.001$). Wistar Sal rats spent less time immobile than WKY Sal and MS WKY Sal rats ($p < 0.001$) and the Wistar DMI rats spent less time immobile than the WKY DMI rats ($p < 0.05$).

Swimming

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F_{(2, 67)} = 21.84$, $p < 0.001$), a drug treatment effect ($F_{(1, 67)} = 23.47$, $p < 0.001$) and a rat group × drug treatment interaction ($F_{(2, 67)} = 8.64$, $p < 0.001$) in time spent swimming in the FST. Post-hoc analysis revealed that WKY DMI rats spent more time swimming than WKY Sal rats ($p < 0.01$; Fig. 3.3b) and the MS WKY DMI rats spent more time swimming than MS WKY Sal rats ($p < 0.001$). Wistar Sal rats spent more time swimming than WKY Sal and MS WKY Sal rats ($p < 0.001$).

Struggling

Two-way ANOVA (rat group and drug treatment as factors) showed a significant difference between rat groups ($F(2, 67) = 35.36$; $p < 0.001$), a drug treatment effect ($F_{(1, 67)}$

= 23.09, $p < 0.001$) and a rat group × drug treatment interaction ($F_{(2, 67)} = 6.50$, $p < 0.01$) in time spent struggling in the FST. Post-hoc analysis revealed that MS WKY DMI rats spent more time struggling than MS WKY Sal rats ($p < 0.001$; Fig. 3.3c). Wistar Sal rats spent more time struggling than WKY Sal ($p < 0.001$) and MS WKY Sal rats ($p < 0.001$). The Wistar DMI rats spent more time struggling than WKY DMI rats ($p < 0.001$).

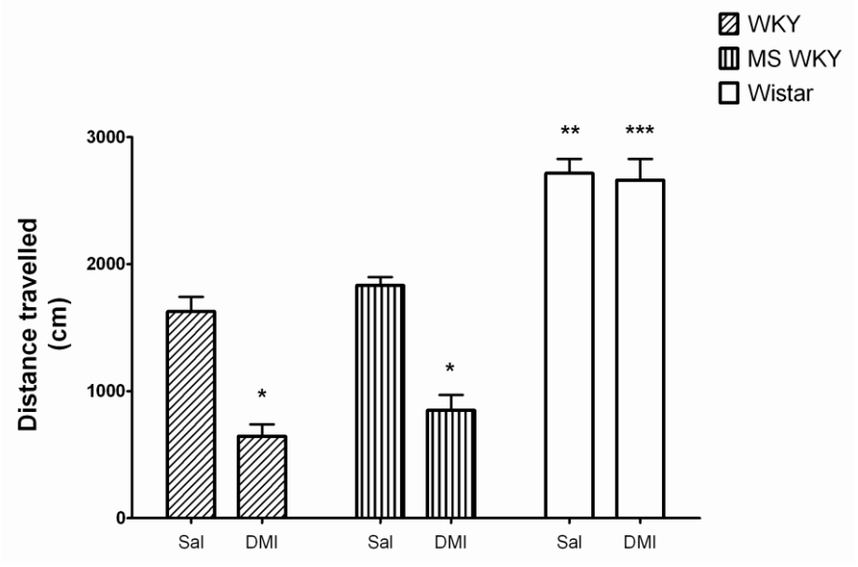


Figure 3.2: Distance travelled by WKY, MS WKY and Wistar rats in the OFT following treatment with saline/desipramine. Desipramine treatment decreased distance travelled by the WKY rats in the OFT without effect on the Wistar rats. * WKY DMI and MS WKY DMI significantly different from WKY Sal and MS WKY Sal rats, $p < 0.001$; ** Wistar Sal significantly different from WKY Sal and MS WKY Sal, $p < 0.001$; *** Wistar DMI rats significantly different from WKY DMI and MS WKY DMI rats, $p < 0.001$; Tukey's post-hoc test (WKY sal: n = 15; WKY DMI: n = 6; MS WKY sal: n = 17; MS WKY DMI: n = 9; Wistar Sal: n = 15; Wistar DMI: n = 13)). Data presented as mean ±SEM.

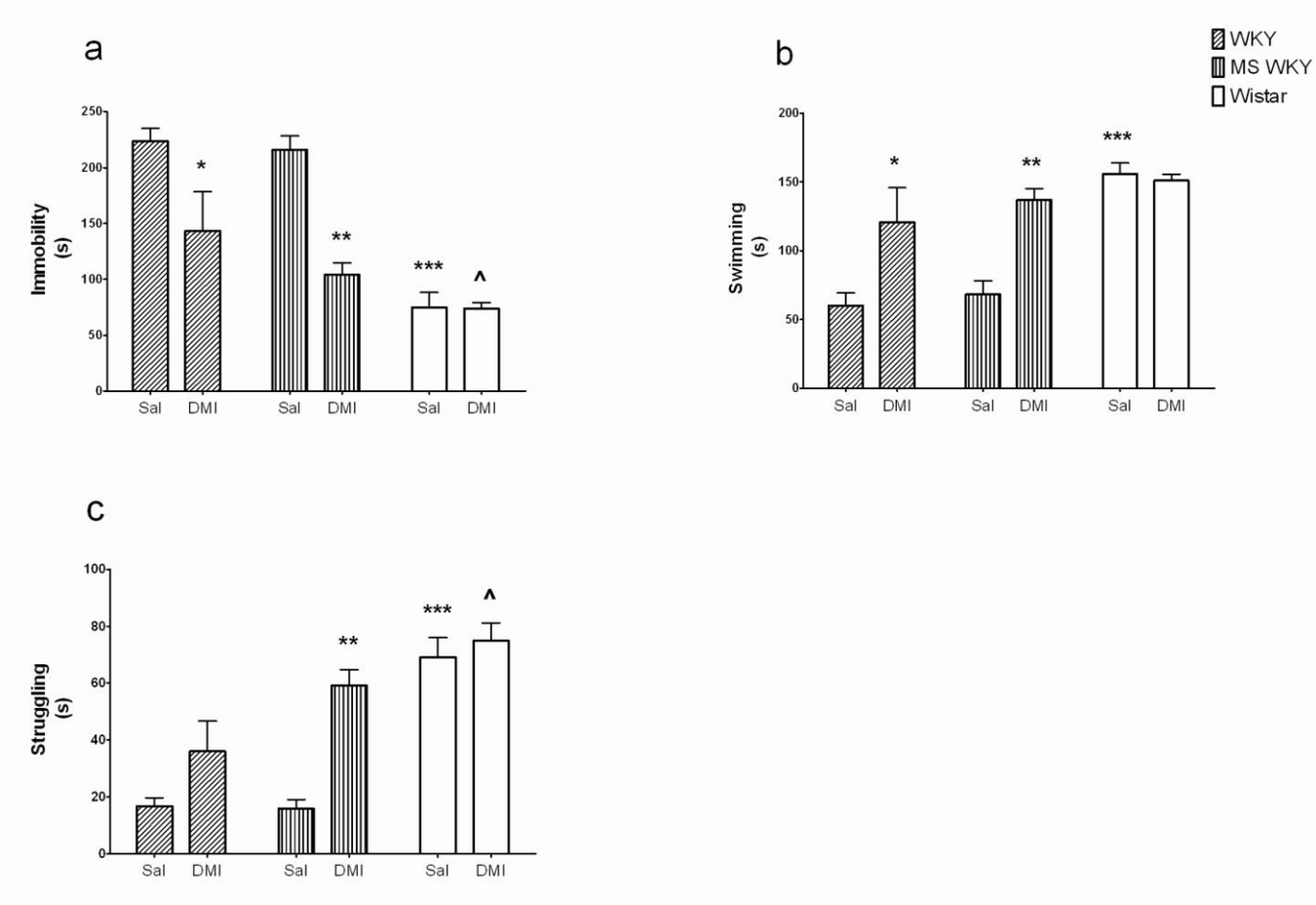


Figure 3.3: Immobility, swimming and struggling behaviours of WKY, MS WKY and Wistar rats in the FST following treatment with saline/desipramine. Desipramine treatment decreased immobility (a) and increased active swimming (b) and struggling (c) behaviours in the MS WKY and WKY rats without affecting the Wistar rats in the FST. * WKY DMI significantly different from WKY Sal, $p < 0.05$; ** MS WKY DMI significantly different from MS WKY Sal, $p < 0.001$; *** Wistar Sal significantly different from WKY Sal and MS WKY Sal, $p < 0.001$; ^ Wistar DMI significantly different from WKY DMI, $p < 0.05$; Tukey"s post-hoc test (WKY sal: n = 15; WKY DMI: n = 6; MS WKY sal: n = 17; MS WKY DMI: n = 10; Wistar Sal: n = 13; Wistar DMI: n = 13). Data presented as mean ± SEM.

3.3.1.4 Ultrasonic vocalizations

Frequency modulated calls

The Kruskal–Wallis ANOVA showed a significant difference between drug/saline-treated rat groups (WKY Sal, WKY DMI, MS WKY Sal, MS WKY DMI, Wistar Sal and Wistar DMI rats) in FM calls on day 11 ($H_{(5,N = 99)} = 37.42$, $p < 0.001$), day 13 ($H_{(5,N = 76)} = 27.12$, $p < 0.001$) and day 15 ($H_{(5,N = 75)} = 33.11$, $p < 0.001$) of drug treatment. Post-hoc analysis revealed that WKY DMI rats vocalized less than WKY Sal rats ($p < 0.05$; Fig. 3.4b) on day 11 of treatment. Wistar Sal rats vocalized less than MS WKY Sal ($p < 0.001$) and

WKY Sal rats ($p < 0.01$). At day 13 of treatment, Wistar Sal rats vocalized less than MS WKY Sal ($p < 0.01$) and WKY Sal rats ($p < 0.01$; Fig. 3.4b). At day 15 of treatment, MS WKY DMI vocalized less than MS WKY Sal rats ($p < 0.05$; Fig. 3.4b). Wistar Sal vocalized less than MS WKY Sal rats ($p < 0.05$).

Flat calls

The Kruskal–Wallis ANOVA revealed a significant difference between drug-treated rat groups in flat calls on day 11 ($H_{(5,N = 99)} = 20.89$, $p < 0.001$) and day 15 ($H_{(5,N = 75)} = 11.15$, $p < 0.05$). Post-hoc analysis showed that, Wistar Sal rats vocalized less than MS WKY Sal rats ($p < 0.01$; Fig. 3.5b) on day 11 of treatment.

Total number of calls

The Kruskal–Wallis ANOVA showed a significant difference between drug/saline-treated rat groups in the total number of calls on day 11 ($H_{(5,N = 99)} = 33.13$, $p < 0.001$), day 13 ($H_{(5,N = 76)} = 27.03$, $p < 0.001$) and day 15 ($H_{(5,N = 75)} = 25.03$, $p < 0.001$). Post-hoc analysis revealed that Wistar Sal rats vocalized less than WKY Sal ($p < 0.01$) and MS WKY Sal ($p < 0.001$; Fig. 3.6b) on day 11 of treatment. At day 13 of treatment, Wistar Sal rats vocalized less than WKY Sal ($p < 0.05$) and MS WKY Sal rats ($p < 0.01$; Fig. 3.6b).

3.3.2 Biochemistry

3.3.2.1 Serotonin and dopamine concentration in the prefrontal cortex and nucleus accumbens shell

Two-way ANOVA (rat group and drug treatment) showed no effect of rat group or drug treatment on serotonin (Fig. 3.7) and dopamine (Fig. 3.8) concentration in the PFC and NAcS respectively.

3.3.2.2 Density of opioid receptor proteins in the nucleus accumbens core and shell

Two-way ANOVA (rat group and drug treatment) revealed no effect of rat group or drug treatment on MOR and KOR in the NAcC (Fig. 3.9) and NAcS (Fig. 3.10)

3.3.2.3 Density of phospho-GSK3β and phospho-ERK1/2 in the prefrontal cortex

Two-way ANOVA (rat group and drug treatment) showed a drug treatment effect ($F_{(1.28)} = 4.74$; $p < 0.05$) on the density of p-GSK3β in the PFC and no effect on p-ERK1/2 (Fig.

3.11b) in the PFC. Post-hoc analysis showed higher phosphorylated GSK3β in WKY DMI than WKY Sal rats ($p < 0.05$; Fig. 3.11a).

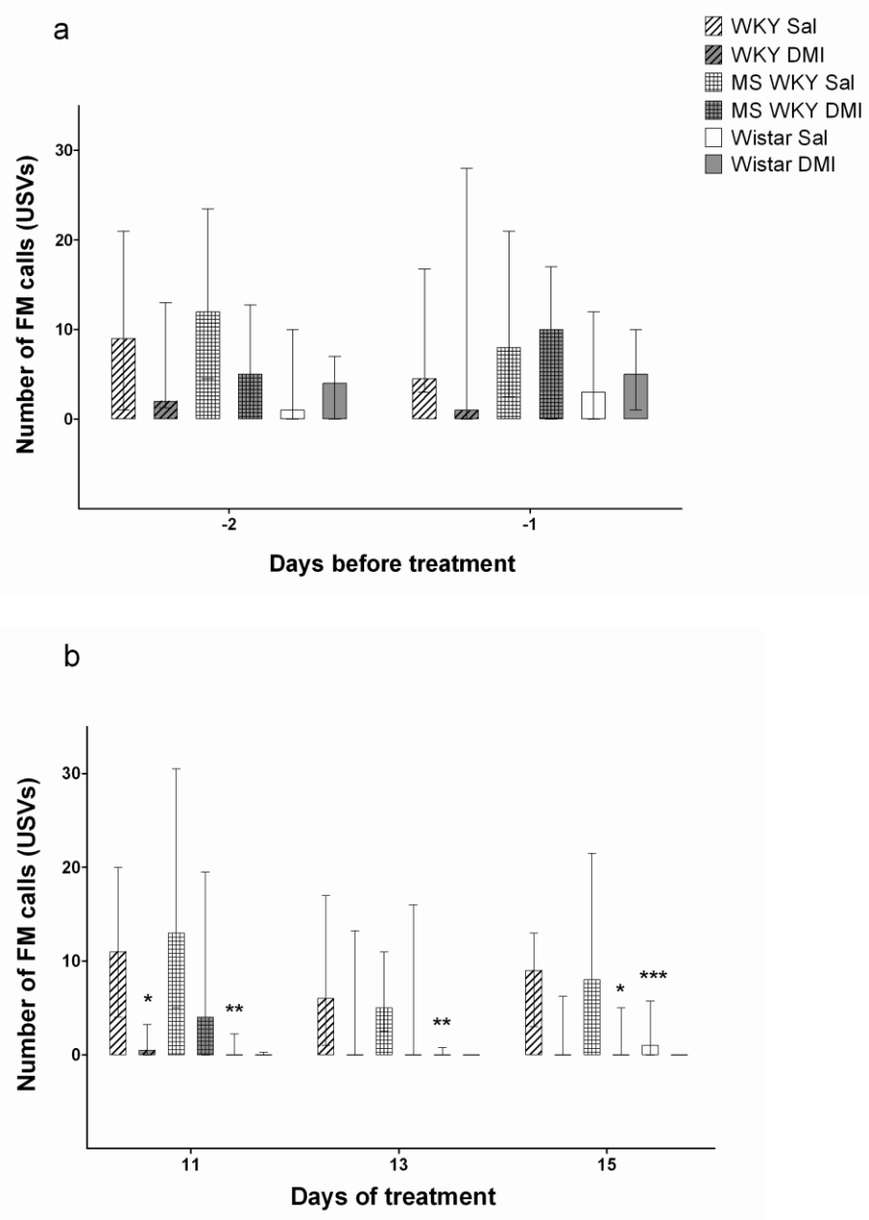


Figure 3.4: Number of FM USVs of WKY, MS WKY and Wistar rats treated with saline/desipramine. No significant difference found in the number of FM calls before treatment (a). Desipramine treatment (b) decreased FM calls in the WKY at day 11 of treatment and MS WKY rats at day 15 of treatment. The MS WKY Sal and WKY Sal rats vocalized more than Wistar Sal rats at day 11, 13 and 15 of treatment. * WKY DMI significantly different fromWKY Sal, $p < 0.05$; ** Wistar Sal significantly different from WKY Sal and MS WKY Sal, $p < 0.01$; *** Wistar Sal significantly different from MS WKY Sal, $p < 0.05$; Dunn‟s multiple comparisons post-hoc test (WKY sal: n = 12-18; WKY DMI: n = 8-10; MS WKY sal: n = 13-21; MS WKY DMI: n = 6-14; Wistar Sal: n = 12-19; Wistar DMI: n = 11-18). Data presented as median and interquartile range.

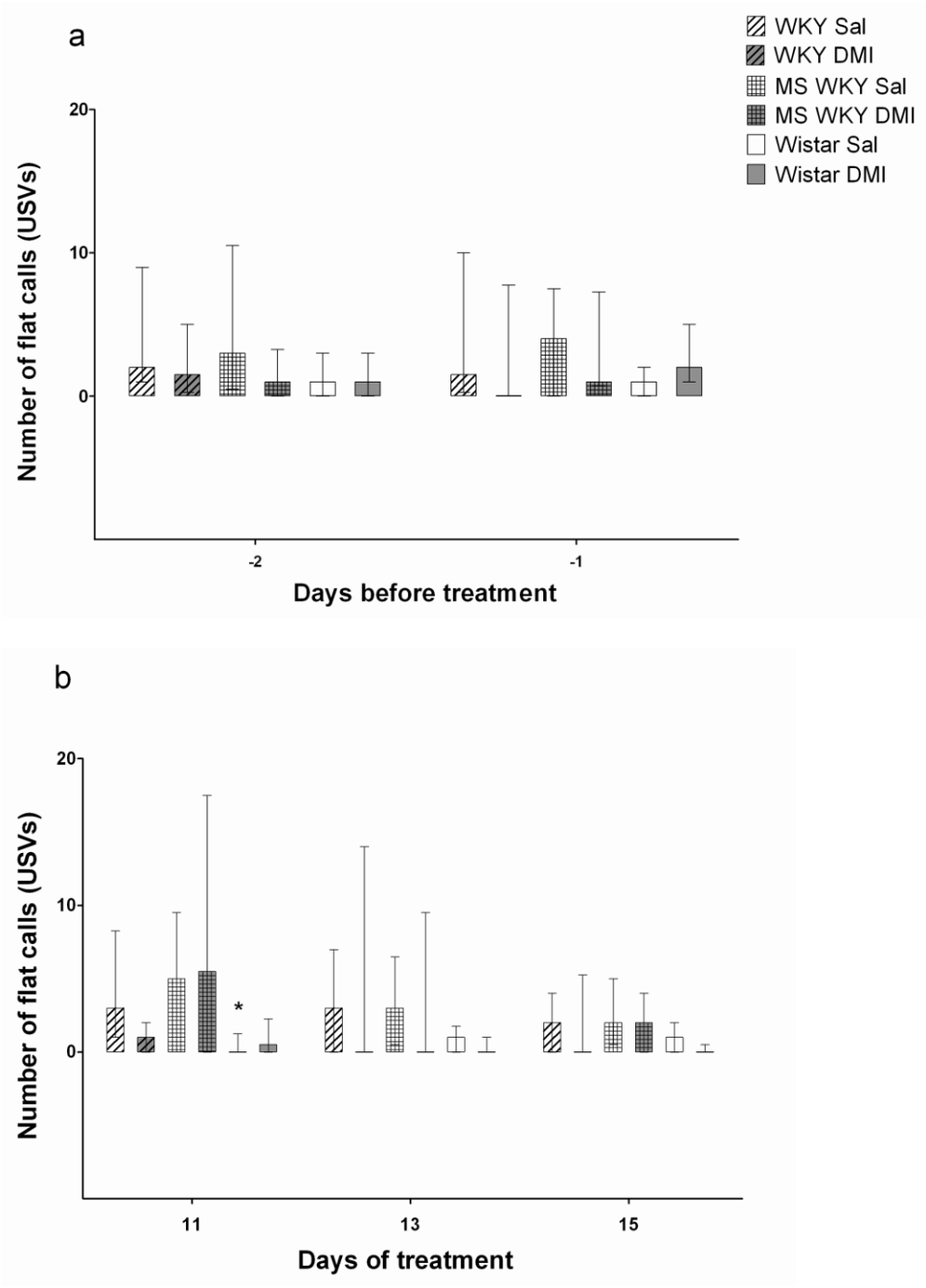
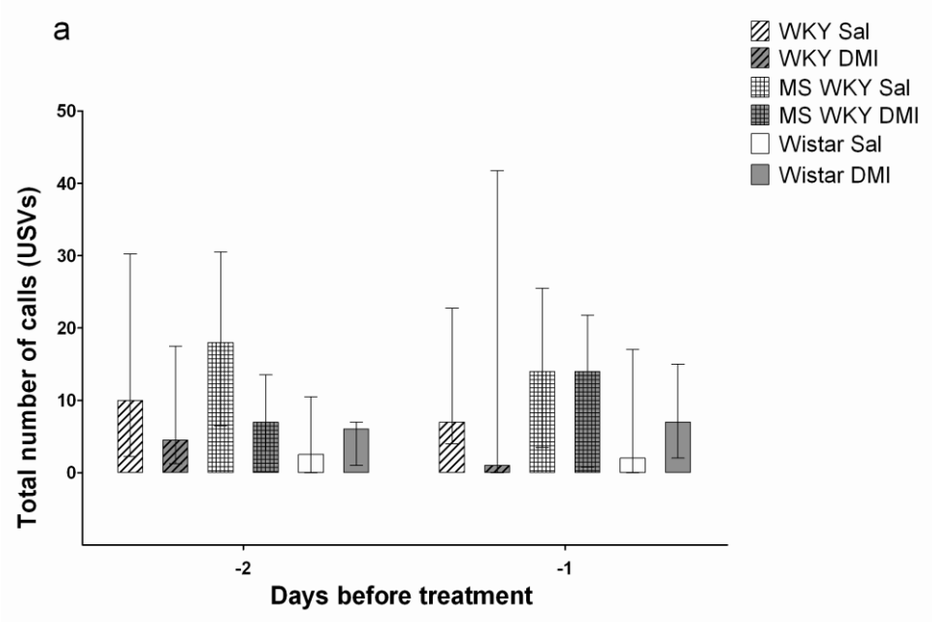


Figure 3.5: Number of flat USVs of WKY, MS WKY and Wistar rats treated with saline/desipramine. No significant difference found in the number of flat calls before treatment (a). Desipramine treatment (b) had no significant effect on the number of flat calls made by WKY, MS WKY and Wistar rats. * Wistar Sal significantly different from MS WKY Sal, $p < 0.01$; Dunn‟s multiple comparisons post-hoc test (WKY sal: n = 12-18; WKY DMI: n = 8-10; MS WKY sal: n = 13-21; MS WKY DMI: n = 6-14; Wistar Sal: n = 12-19; Wistar DMI: n = 11-18). Data presented as median and interquartile range.



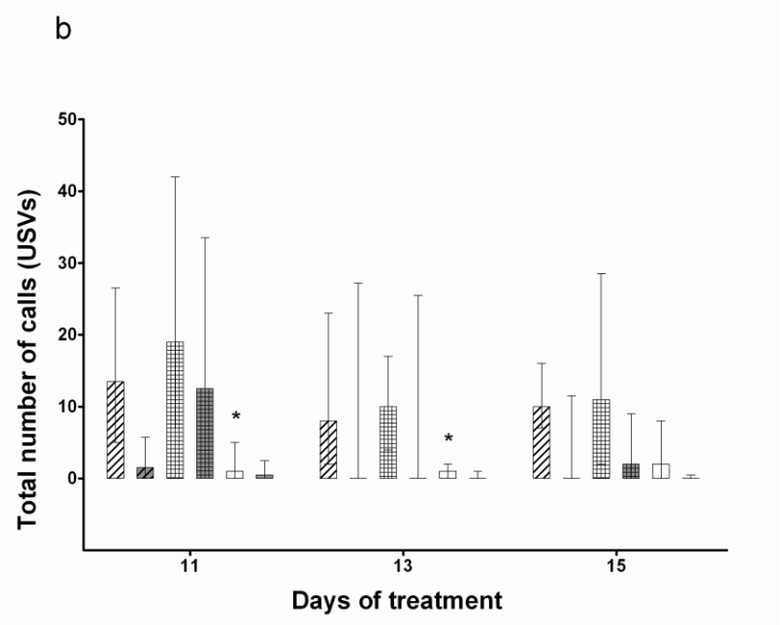


Figure 3.6: Total number of USVs of WKY, MS WKY and Wistar rats treated with saline/desipramine. No significant difference found in the total number of calls before treatment (a). Desipramine treatment (b) had no significant effect on the total number of calls made by WKY, MS WKY and Wistar rats. The MS WKY Sal and WKY Sal rats vocalized more than Wistar Sal rats at day 11and 13 of treatment. * Wistar Sal significantly different from WKY Sal and MS WKY Sal, $p < 0.05$; Dunn‟s multiple comparisons post-hoc test (WKY sal: n = 12-18; WKY DMI: n = 8-10; MS WKY sal: n = 13-21; MS WKY DMI: n = 6-14; Wistar Sal: n = 12-19; Wistar DMI: n = 11-18). Data presented as median and interquartile range.

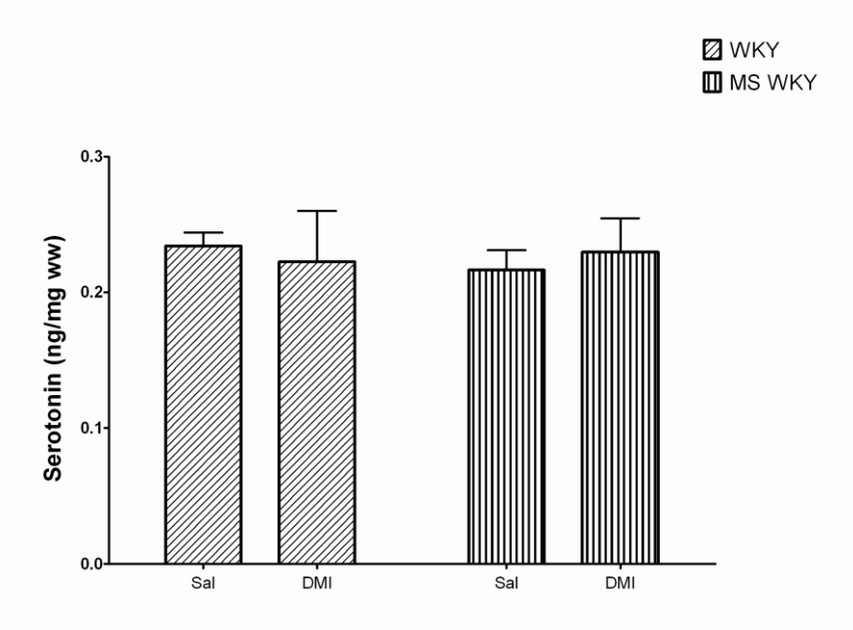


Figure 3.7: Serotonin levels in the PFC of WKY and MS WKY rats following treatment with saline/desipramine. No significant difference found in serotonin levels in PFC between WKY Sal, WKY DMI, MS WKY Sal and MS WKY DMI rats (WKY sal: n = 12; WKY DMI: n = 6; MS WKY sal: n = 12; MS WKY DMI: n = 9). Data presented as mean ± SEM.

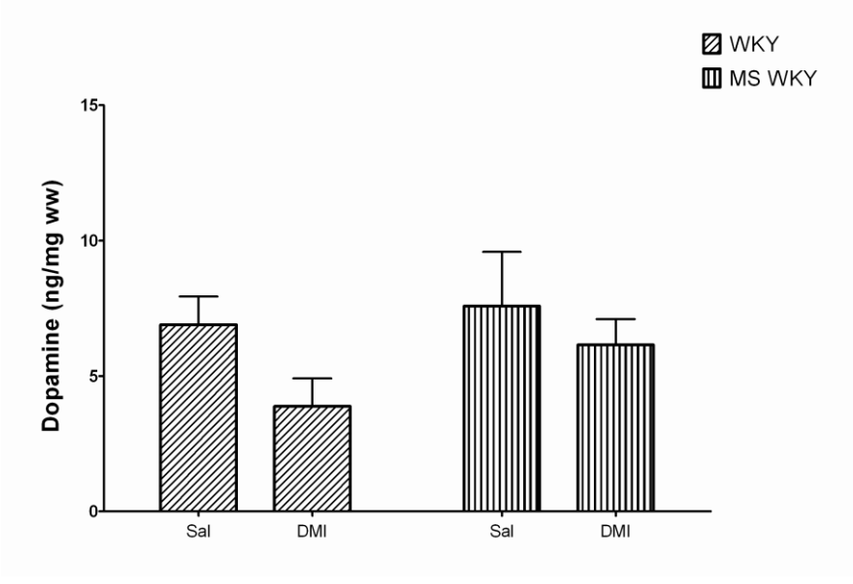


Figure 3.8: Dopamine levels in the NAcS of WKY and MS WKY rats following treatment with saline/desipramine. No significant difference found in dopamine levels in NAcS between WKY Sal, WKY DMI, MS WKY Sal and MS WKY DMI rats (WKY sal: n = 12; WKY DMI: n = 6; MS WKY sal: n = 13; MS WKY DMI: n = 9). Data presented as mean ± SEM.

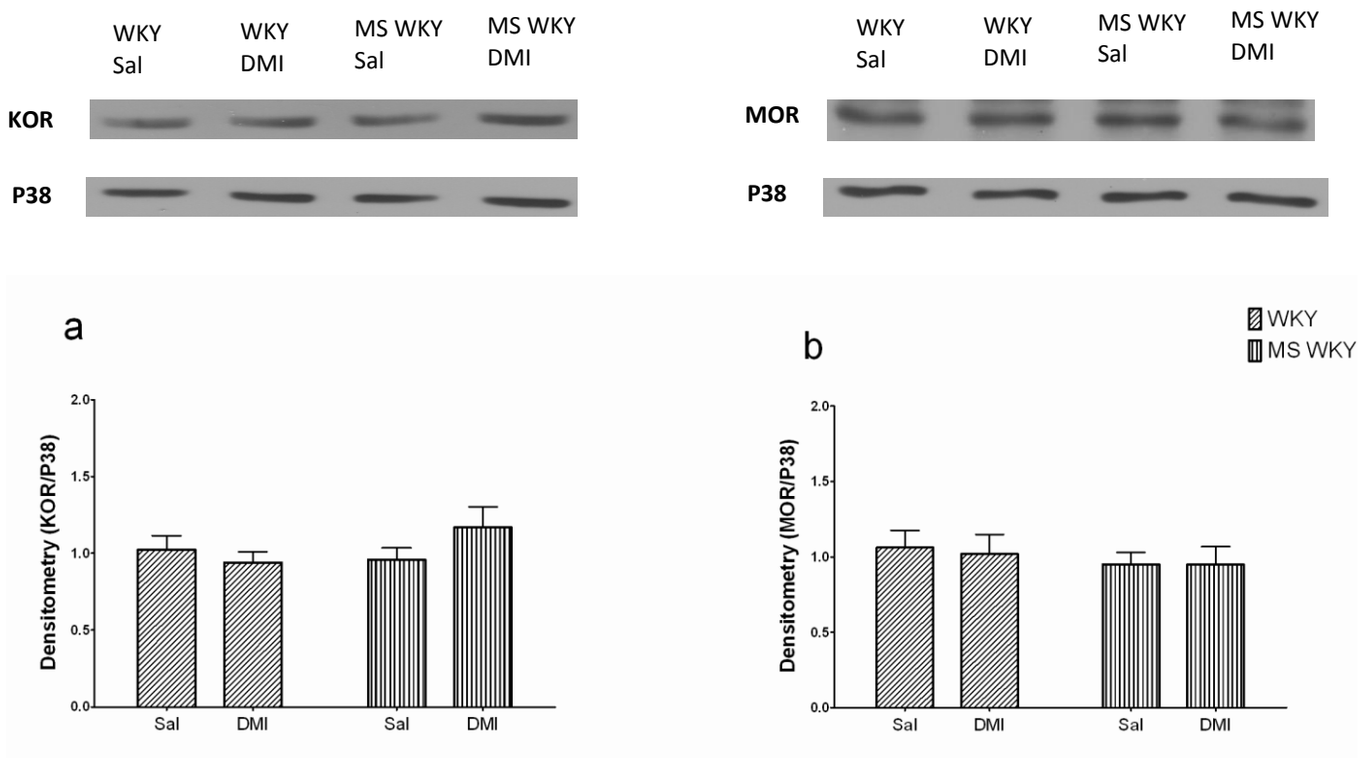


Figure 3.9: Density of KOR and MOR in the NAcC of WKY and MS WKY rats following treatment with saline/desipramine. No significant difference found in density of KOR (a) and MOR (b) in the NAcC between WKY Sal, WKY DMI, MS WKY Sal and MS WKY DMI rats. KOR and MOR normalized to P38 loading control (WKY sal: n = 9; WKY DMI: n = 6; MS WKY sal: n = 9; MS WKY DMI: n = 8). Data presented as mean ± SEM.

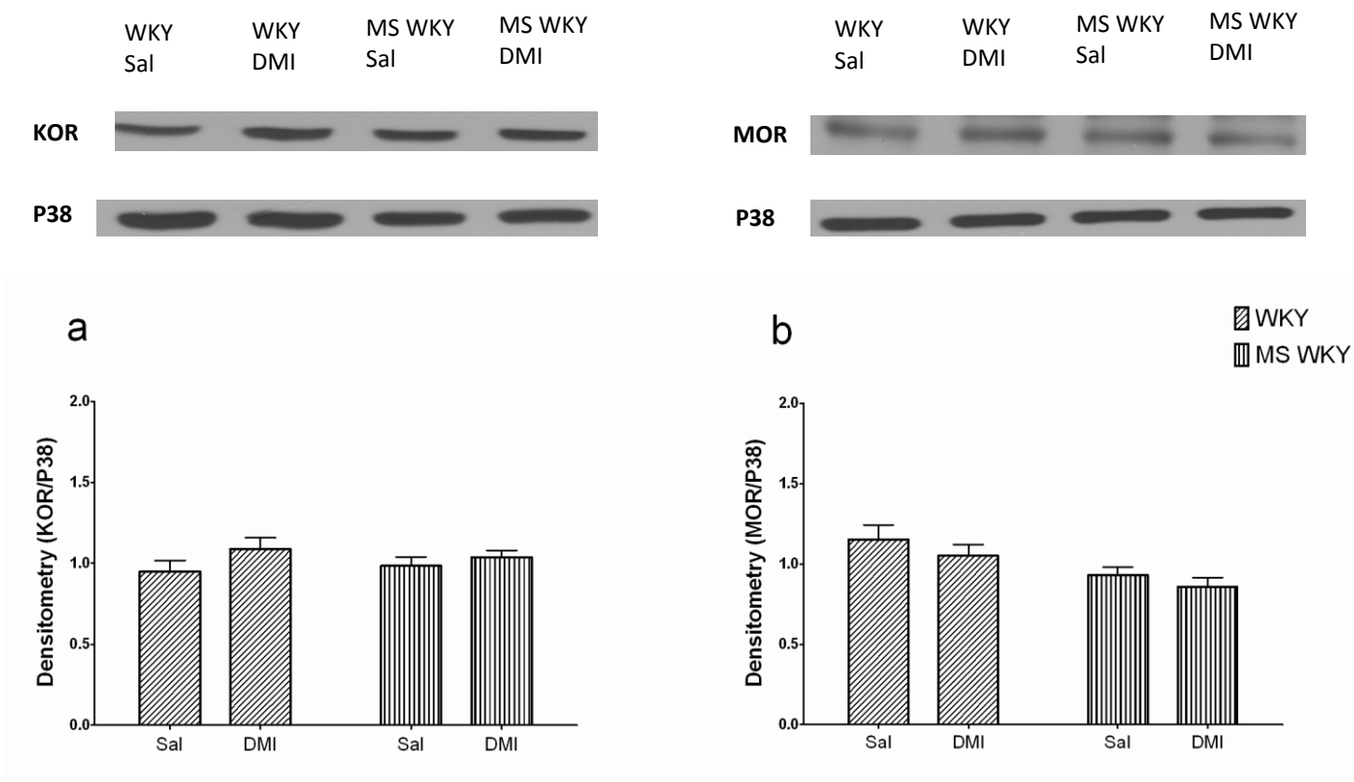


Figure 3.10: Density of KOR and MOR in the NAcS of WKY and MS WKY rats following treatment with saline/desipramine. No significant difference found in density of KOR (a) and MOR (b) in the NAcS between WKY Sal, WKY DMI, MS WKY Sal and MS WKY DMI rats. KOR and MOR normalized to P38 loading control (WKY sal: n = 9; WKY DMI: n = 6; MS WKY sal: n = 9; MS WKY DMI: n = 8). Data presented as mean ± SEM.

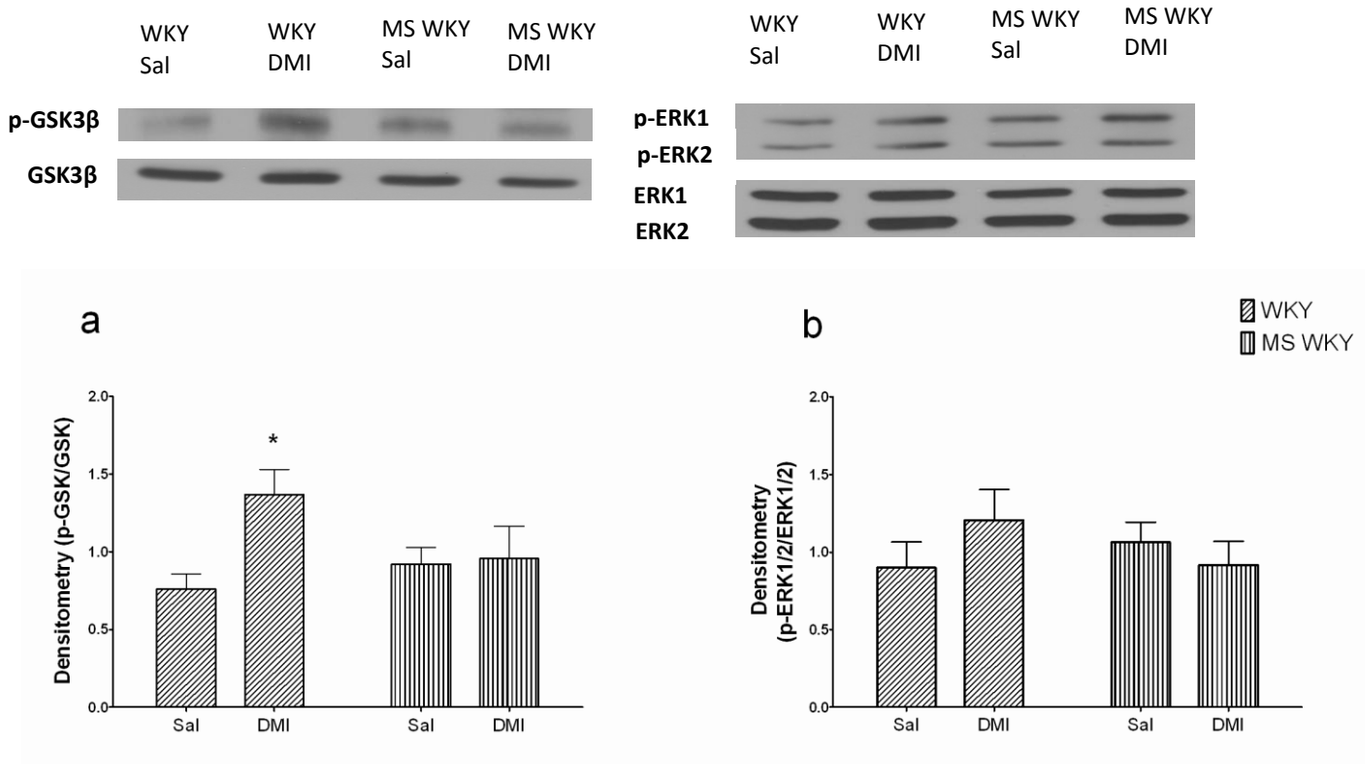


Figure 3.11: Density of p-GSK3β and p-ERK in the PFC of WKY and MS WKY rats following treatment with saline/desipramine. Desipramine treatment increased density of p-GSK3β in the PFC of WKY rats (a) and without effect on p-ERK/1/2 in the PFC (b) * WKY DMI significantly different from WKY Sal rats, $p < 0.05$; Tukey post-hoc test. Phospho-GSK3β and p-ERK1/2 normalized to total GSK and total ERK1/2 respectively (WKY sal: n = 9; WKY DMI: n = 6; MS WKY sal: n = 9; MS WKY DMI: n = 8). Data presented as mean ± SEM.

3.4 Discussion

The main findings of this study are consistent with the use of MS WKY/NCrl as a robust model of depression-/anxiety-like behaviour, (1) MS, a model of depression in itself, increased anxiety-like behaviour of WKY/NCrl, enhancing the potential of WKY/NCrl to serve as a model of anxiety (2) consistent with a model of depression, desipramine treatment decreased depression-like behaviour in MS WKY/NCrl and normally reared WKY/NCrl rats, (3) together with the decreased depression-like behaviour, desipramine treatment decreased the need of WKY/NCrl rats to reconnect with the cage mate after social isolation, evidenced by the decreased number of FM USVs in response to removal of the cage mate(s), and (4) desipramine increased p-GSK3β in the WKY/NCrl, an effect that was blocked by MS of WKY/NCrl rats.

Desipramine decreased immobility and increased active swimming and struggling behaviours of WKY/NCrl rats in the FST without affecting the Wistar rats. The results of the current study are therefore in agreement with previous studies (Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003). These previous studies also found that desipramine increased swimming behaviour in the WKY/NCrl. This is contrary to findings with other rat strains (Detke and Lucki 1995; Lucki 1997). Noradrenaline uptake inhibitors were shown to selectively increase climbing behaviour and serotonin uptake inhibitors selectively increased swimming behaviour in SD rats (Detke and Lucki 1995; Lucki 1997). Indeed, it was suggested that desipramine treatment rats may involve changes in the serotonergic system (Racagni and Brunello 1984) therefore also affecting swimming behaviour (Lopez-Rubalcava and Lucki 2000). In agreement with previous studies, desipramine did not affect the performance of the control Wistar rats in the FST (Jeannotte et al. 2009; Tejani-Butt et al. 2003). In the current study, both the normally reared WKY/NCrl and MS WKY/NCrl rats responded to the antidepressant effect of desipramine. MS did not enhance the depression-like behaviour in the WKY/NCrl rats.

MS WKY/NCrl displayed more anxiety-like behaviour than normally reared WKY/NCrl. They spent more time in the closed arms of the EPM than normally reared WKY/NCrl and also responded to desipramine treatment evidenced by the increased amount of time they spent in the open arms and decreased amount of time spent in the center zone of the EPM. Similarly, prepubertal MS WKY rats spent more time in the closed arms of the EPM during the first minute of testing compared to non-separated WKY (Womersley et al. 2011). However, the WKY/NCrl showed less anxiety in the open arms and closed arms of the EPM compared to the Wistar rats. WKY/NCrl rats spent more time in the open arms of the EPM and less time in the closed arms than the Wistar reference strain. It appeared that the WKY/NCrl rats were less anxious, possibly as a result of additional handling during the daily injections and USV recordings. Previous studies found that repeated handling can reduce anxiety-like behaviour in the EPM (Costa et al. 2012; Schmitt and Hiemke 1998). The center zone of the EPM provides a decision point at which the rat chooses to enter either the open arms or closed arms of the EPM through risk assessment (Nosek et al. 2008). Rats that are indecisive/ambivalent will spend more time in the center zone (Nosek et al. 2008; Rodgers and Johnson 1995). Desipramine decreased the amount of time the MS WKY spent in the center zone of the EPM, apparently reducing indecisiveness which is one of the core symptoms of depression (American Psychiatric Association 2013). The present results therefore demonstrated the heightened anxiety of the MS WKY/NCrl compared to the normally reared WKY/NCrl, evidenced by the increased amount of time spent in the closed arms of the EPM. They

also responded to the anxiolytic effects of desipramine in terms of increased time spent in the open arms and by spending less time in the center zone of the EPM.

In the OFT, desipramine treatment reduced the locomotor activity of the WKY/NCrl without affecting Wistar rats. This is in agreement with some studies (Kulkarni and Dandiya 1973; Mitchell et al. 2006; Tucker and File 1986) and in contrast to others (Lahmame and Armario 1996; Tejani-Butt et al. 2003). The reasons for discrepancies between studies in locomotor activity following antidepressant treatment could be attributed to differences in experimental design, duration of treatment, time after last drug administration, as well as the rat strain being tested. Several studies measured the acute effects of desipramine whereas desipramine administered chronically, as in the present study, involved a complex sequence of changes in affinity, function and number of α1-,α2-, β1- and β2-adrenoceptors both pre- and postsynaptically (Deupree et al. 2007; Paetsch and Greenshaw 1993; Racagni and Brunello 1984; Subhash et al. 2003; Zhang et al. 2009). Reduced noradrenergic firing rate, TH activity and cortical levels of normetanephrine all indicate reduced noradrenergic activity following chronic antidepressant treatment (Grant and Weiss 2001; Nestler et al. 1990; Racagni et al. 1983). In addition, WKY rats have a higher density of noradrenaline transporter binding sites than control rats that only decrease in the cortex, hippocampus, amygdala, hypothalamus and locus coeruleus after repeated stressors (Tejani-Butt et al. 1994; Zafar et al. 1997). Therefore, reduced noradrenaline transporter function resulting from exposure of WKY rats to repeated stressors (FST and OFT) may have further enhanced extracellular levels of noradrenaline and ultimately reduced locomotor activity.

The USVs is indicative of communication of the animal‟s emotional state (Mällo et al. 2007). In the current study, it was found that the WKY/NCrl emitted more calls (predominantly FM calls) than the Wistar control rats and desipramine treatment decreased FM calls in the WKY/NCrl. The decreased number of USVs after antidepressant treatment is therefore not in line with the hypothesis that high frequency USVs are indicative of a positive emotional state. However, some experimental situations that are not seen as rewarding are also known to induce high frequency vocalizations (Miczek et al. 1995; Tornatzky and Miczek 1995; Vivian and Miczek 1991; Wohr et al. 2008). For example, it was previously found that an intruder rat emitted high frequency (31–70 kHz) and low frequency calls when introduced to an environment of a resident rat or environment where it had been previously defeated by a resident rat (Miczek et al. 1995; Takahashi et al. 1983; Tornatzky and Miczek 1995). Anxiolytic drugs were effective in reducing the high frequency calls in response to anticipation of a threat.

Similarly, high frequency USVs were recorded during morphine withdrawal (Vivian and Miczek 1991). Apart from USVs having an emotional function (Burgdorf and Panksepp 2006), it also provides a mechanism of communication of specific information between rats regarding their well-being as well as directing specific behaviours (Burman et al. 2007; Portfors 2007). Therefore, USVs in aversive situations could serve a social function and in the current study may play a role in trying to re-establish social contact with the cage mate (Brudzynski and Pniak 2002; Wohr et al. 2008). In this study, FM calls were more affected than the flat calls which is in line with previous findings that FM calls play a role in emotion (Burgdorf et al. 2011). The social isolation resulting from removal of the cage mate may therefore have induced social signalling to the cage mate containing emotional information regarding the need to re-connect. The social signalling to re-establish contact with the cage-mate was found to be more evident in the WKY/NCrl compared to the Wistar rats. The WKY/NCrl therefore appeared to be more affected by separation from the cage mate, hence the need for more social signalling to be reunited with the cage mate. Treatment with desipramine reduced the number of FM USVs in the WKY/NCrl rat model of depression without affecting the control Wistar rats. However, the number of USVs emitted by the Wistar rats was already low and could possibly not be further reduced by desipramine treatment. The increased USVs in WKY/NCrl compared to Wistar rats and decreased FM USVs after desipramine treatment, suggest that USVs in this study occurred in response to an aversive situation that induced social signalling in an attempt to re-establish social contact with the cage mate(s).

Serotonin and dopamine levels in the PFC and NAcS respectively and opioid receptor (MOR and KOR) densities in the NAcC and NAcS were unaffected by desipramine treatment and found to be similar in the different rat groups (WKY, MS WKY and Wistar). It is therefore possible that these neurotransmitter systems in the NAc and PFC brain areas are unrelated to the response to desipramine treatment as well as the depression-/anxiety-like behaviour of normally reared WKY/NCrl and MS WKY/NCrl rat models (Berrocoso and Mico 2009; Page et al. 1999; Plaznik et al. 1985).

Desipramine treatment increased p-GSK3β in the PFC of normally reared WKY/NCrl rats but had no effect on p-GSK3β in MS WKY/NCrl rats. Furthermore, p-ERK was unaffected by desipramine treatment in WKY/NCrl rats. Desipramine had no effect on these proteins in Wistar rats. This is the first study to show an increase in p-GSK3β after chronic desipramine treatment. Previous studies showed that antidepressant drugs that are more selective in blocking the reuptake of serotonin, increased p-GSK3β (Li et al. 2004; Liu et al. 2012; Okamoto et al. 2010; Sutton and Rushlow 2011). Furthermore,

phosphorylation of GSK3β was found to be regulated by serotonin $5HT_{1A}$ and $5HT_2$ receptors (Li et al. 2004). It is unlikely that increased p-GSK3β by desipramine was modulated by serotonin signalling mechanisms in the current study, since desipramine had no effect on serotonin levels in the PFC. However, the exact mechanism by which antidepressants regulate phosphorylation of GSK3β remains unknown although it has been shown that the serotonin reuptake inhibitor, citalopram increased p-GSK3β through the Wnt signalling pathway (Liu et al. 2012) whereas other antidepressants were suggested to regulate GSK3β through phosphorylated protein kinase B (Akt) (Sutton and Rushlow 2011). Previous studies have shown that antidepressant drugs also regulated signalling proteins in the MAPK/ERK pathway in the hippocampus and frontal cortex (Balu et al. 2008; Bravo et al. 2009; Fumagalli et al. 2005; Nibuya et al. 1995) while other studies failed to show this (Budziszewska et al. 2010; Dias et al. 2003). In the current study, phosphorylated and total ERK1/2 were unaffected by desipramine treatment in WKY/NCrl rats. These discrepancies in the results could be in fact ascribed to the different rat strains, experimental procedures, differential effects of antidepressant drugs, and treatment regimens. Indeed, it has been shown that different classes of antidepressant drugs regulated ERK1/2 differently and which is specific to the brain region being measured (Fumagalli et al. 2005). For example, chronic desipramine treatment decreased p-ERK in the hippocampus in a restraint stress rat model of depression (Bravo et al. 2009). Furthermore, fluoxetine treatment decreased p-ERK in the PFC but not in the striatum whereas imipramine did not reduce p-ERK in the hippocampus but increased p-ERK1 in the PFC (Fumagalli et al. 2005). On the other hand, acute desipramine treatment had no effect on p-ERK in the PFC, hippocampus and striatum of mice (Di Benedetto et al. 2013). However, further studies are necessary to determine the chronic effect of desipramine on p-ERK in a wider variety of limbic brain areas in the WKY rats.

MS had no effect on p-GSK and p-ERK in in the PFC of WKY/NCrl rats. Stress is known to activate the MAPK/ERK signalling pathway and thereby increasing p-ERK protein levels (Bhat et al. 1998; Meller et al. 2003; Musazzi et al. 2010). However, the rat strain may be the determining factor in the signalling pathways being affected. A previous study, using the MS FSL genetic model of depression, also found no effect of MS on p-ERK in the FSL rats but a higher levels of p-ERK compared to the control, FRL rat (Musazzi et al. 2010). The fact that MS had no effect on p-ERK in FSL rats was suggested to be the result of the already innate sustained increase in p-ERK. It is therefore possible that p-ERK levels were already elevated in the normally reared WKY rats in this study and could therefore not be further elevated by MS.

In conclusion, the MS WKY/NCrl rats were found to be an animal model of depression-/anxiety-like behaviour. In addition to the depression-like behaviour and response to antidepressant treatment, MS WKY/NCrl rats displayed more anxiety-like behaviour than normally reared WKY/NCrl rats and responded to the anxiolytic effects of desipramine treatment. Together with diminished depression-/anxiety-like behaviour, diminished USVs could signal reduced attempts to maintain social contact in response to isolation stress in the WKY/NCrl following chronic desipramine treatment. Since GSK3β has been involved in neurogenesis (Lange et al. 2011) and changes in neurogenesis have been linked to depression (Banasr et al. 2011), it may be suggested that as least some of the antidepressant behavioural effects may be linked to enhanced neurogenesis in the PFC in WKY/NCrl rats; an effect that is blocked by MS. Therefore, in addition to its depression-like behaviour, MS enhanced the validity of the WKY/NCrl as a model to study anxiety-like behaviour.

Chapter 4

Behavioural and biochemical changes in maternally separated Sprague-Dawley rats exposed to restraint stress

4.1 Introduction

Chronic exposure to stressful life events is considered a major risk factor in developing various psychological conditions, including major depression (Kendler et al. 1998; Kendler et al. 1999). Moreover, individuals with a history of childhood trauma are more vulnerable in developing depression compared to individuals with no childhood trauma (Harkness et al. 2006). In addition, the type of childhood trauma or recent stressful event has differential effects on the symptomatology of depression (van Veen et al. 2013).

Various animal models, including chronic restraint stress, have been developed to study the effects of chronic stress on the neurobiology of depression. Restraint stress has proved to be a valid stress-inducing animal model of depression and anxiety (Bombi et al. 2013; Chiba et al. 2012; Naert et al. 2011; Ulloa et al. 2010). Furthermore, it was evident from previous studies that restraint stress induced depression-like behaviour in rats that were subjected to MS in early life (Marais et al. 2008; Uchida et al. 2010).

It has been reported that chronic stress that results in depression-like behaviour in rodents, decreased BDNF levels in the hippocampus and PFC (Ray et al. 2011; Xu et al. 2004;Xu et al. 2006) although several inconsistencies have been reported with some studies showing an increase (Adlard et al. 2004; Larsen et al. 2010; Naert et al. 2011) or no effect on BDNF (Kuroda and McEwen 1998; Rosenbrock et al. 2005).

However, depression is a multifaceted disorder with various causative factors present during childhood and adulthood involving multiple signalling pathways that regulate the release of many neurotransmitters and neurotrophins (Covington et al. 2010; Stepanichev et al. 2014). It is therefore necessary to further investigate differently regulated proteins to provide further insight into the stress-induced pathology of depression-like behaviour in rats. Proteomic analysis with iTRAQ labeling allows separation and

identification of many proteins and is proving to be an effective method to identify the molecular changes associated with depression.

The aim of the current study was therefore to measure BDNF concentration in the ventral hippocampus with ELISA and further to follow with proteomic analysis of the PFC in rats exposed to early-life MS and restraint stress in adulthood. This was achieved in order to identify differential protein expression, involved in the development of depression-like behaviour. A mild stressor such as restraint stress was selected to minimize the stressful experience of the animal as much as possible. This was the first proteomic study to assess protein regulation in the PFC of the early life MS model of depression that was also subjected to stress in adulthood.

4.2 Materials and Methods

4.2.1 Animals

A total of 50 SD rats that were bred in the Satellite Animal Facility at the University of Cape Town, were used for the study. Rats were kept under the same conditions as described in chapter 2. The study was conducted in accordance with the guidelines of the South African National Standard: The care and use of animals for scientific purposes (2008) and approved by the University of Cape Town Faculty of Health Sciences Animal Ethics Committee (#010/036). This study consisted of 4 groups: control SD (Ctr, n =12), maternally separated SD (MS, n =12), restraint stressed SD (RS, n =13) and maternally separated SD rats that had been restraint stressed in adulthood (MS+RS, n = 13).

SD pups were separated from their dams for 3 h per day between 09h00 and 13h00 from P2 to P14. Some of the SD litters were normally reared and left undisturbed in their home cages. At P21, all pups were weaned and males separated from the females. Some of the MS rats were chronically restrained for 4 h on 5 consecutive days between 09h00 – 13h00, starting at P61-P67. Following 5 days of restraint, rats were left undisturbed for 7 days before each rat was allowed to swim for 15 min (pretest-swim; P72-P78). After 24 h, the rats were exposed to a 5-min test swim session. Four days after the FST, rats were tested in the EPM and the OFT. On P79-P85, rats were killed and brain areas (ventral hippocampus and PFC) collected and stored in liquid nitrogen until analysis. A total of 10 rats/group were selected for determination of BDNF levels and 6 rats/group selected for proteomic analysis, according to their immobility scores in the FST. The rats with scores closest to the mean value of their respective group (Ctr, MS, RS and MS+RS) were selected.

4.2.2 Maternal separation

MS was performed according to the procedure and under similar conditions as described in chapter 3. Pups were weaned at P21; males were separated from females and housed 2–4 in a cage.

4.2.3 Restraint Stress

According to the procedure by Marais et al. (2008), some rats were chronically restrained in Plexiglas tubes for 4 h on 5 consecutive days between 09h00 – 13h00, starting at P61-P67. Following 5 days of restraint, rats were left undisturbed for 7 days to overcome the short-term effects of stress before depression-like behaviour was measured in the FST.

4.2.4 Behaviour

Behavioural tests were conducted on adult male rats under the same conditions as decribed in chapter 2.

4.2.4.1 Forced swim test

Seven days after the last restraint stress (P72-P78), the FST was carried out as described in chapter 2 for experiment 1 and 3. The FST behaviours were measured manually since external interferences during the process of placing rats in the cylinder affected automatic ethovision detection. Ethovision identifies the subject as the pixel difference between the reference image and the image of interest. However, any change in the image of interest due to any interference (e.g. light reflection), results in ethovision tracking the artifact as well as the rat. The total time spent immobile, swimming and struggling was measured by an experienced observer blind to the experimental conditions.

4.2.4.2 Elevated plus maze

Four days after the FST, rats were tested in the EPM as described in chapter 2.

4.2.4.3 Open field test

The OFT was performed immediately after the EPM as described in chapter 2.

4.2.5 Biochemistry

4.2.5.1 BDNF determination with ELISA

The ventral hippocampus brain tissue samples of the SD behavioural rat groups (Ctr (n = 10), MS (n = 10), RS (n = 10) and MS+RS (n = 10)) were used to determine BDNF concentration. Brain samples were weighed and sonicated for 15 s in 300 µl lysis buffer

(137 mM NaCl, 20 mM Tris-HCl (pH = 8), 1 % NP40, 10 % glycerol, 1 mM PMSF, 10 µg/ml aprotinin, 1 µg/ml leupeptin and 0.5 mM sodium vanadate). Following sonication, samples were mixed on a vortex mixer and centrifuged at 12 000 x g at 4 °C for 30 min. A volume of 40 µl of each sample was used for ELISA. The BDNF concentration of the supernatant was determined according to the manufacturer"s instructions (Promega Corporation, Madison, WI, USA). Determinations were in duplicate. Results are expressed as pg/mg wet weight (ww).

4.2.5.2 Proteomics

Samples of the PFC obtained from 3 SD rats were pooled (3 SD rats/digest), sonicated in 1 M triethylammonium bicarbonate (TEAB) buffer and centrifuged at 17200 x g for 30 min at 4°C. Supernatants were collected and protein concentration, iTRAQ labeling and liquid chromatography mass spectrometry (LC-MS) were carried out by the Centre for Proteomic and Genomic Research (CPGR) at the University of Cape Town.

Protein concentration of the supernatant was determined with a nanodrop at 280 nm. The samples were adjusted to 10 µg/µl with 50 mM TEAB and incubated for 1 h at 60 °C with 100 mM Tris (2-carboxyethyl) phosphine (TCEP) reducing agent. Methyl methanethiosulfonate (MMTS) was added to the samples to block reduced cysteines followed by incubation for 15 min at room temperature. Following incubation, samples were adjusted to 45 µl with 50 mM TEAB and 1 µg/µl trypsin added. The reaction was allowed to proceed at 37 °C for 18 h whereafter the samples were suspended in 0.1 % trifluoroacetic acid (TFA). The digested samples were reduced to a volume of 10 µl and made up to a final volume of 10 µl with 1 M TEAB to adjust the pH (> 7.5). The iTRAQ labeling was carried out using an 8-plex labeling kit for Ctr, MS and RS rat groups (2 pooled PFC digests for each group) and a 4-plex labeling kit for the Ctr and MS+RS rat groups (2 pooled PFC digests for each group). Labeling was performed at room temperature for 2 h according to the manufacturer"s instructions (Applied Biosystems).

Label confirmation was performed by applying the sample mixture to a C-18 column conditioned with 20 µl of 100 % acetonitrile (ACN) and equilibrated with 20 µl of 0.1% TFA. A volume of 1 µl of each sample were pooled and desalted using a C_{18} ZipTip, washed with 20 µl of 0.1 % TFA and eluted with 10 µl of 50 % ACN and 0.1% TFA. The desalted sample was mixed with α-cyano-4-hydroxycinnamic acid (CHCA, Sigma Aldrich) solution and spotted on a MALDI target plate. Labeling was confirmed using tandem mass spectroscopy (MS/MS). After label confirmation, 100 µl of Milli Q water were added to each sample and incubated at room temperature for 30 min. The samples

were pooled and the volume reduced to 100 µl. The pooled sample was stored for further LC-MS analysis.

The LC was performed using a Dionex Ultimate 3000 nano-HPLC system. The HPLC fractionated peptides were dissolved in sample loading buffer (95% water, 5% Acetonitrile, 0.05% TFA) and loaded on a C18 trap column (100 µm×20 mm×5 µm). Chromatographic separation was performed with an Acclaim Pepmap (Thermo Fisher Scientific, USA) C18 column (75 µm×250 mm×3 µm). The mobile phases consisted of solvent A (0.1% formic acid in water) and solvent B (80% ACN, 10% water, and 0.1% formic acid). The gradient was delivered at 250 nl/min and consisted of a linear gradient of mobile phase B initiating with solvent B: 6–30% over 148 min.

Mass spectroscopy was performed using an electron spray ion source coupled to a Q-Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, USA). The MS was operated in positive electron spray ionized mode with a capillary temperature of 250 °C. The applied electrospray voltage was set at 1.95 kV. Data were collected in the full scan mode and data-dependent MS/MS modes with a maximum ion injection time of 50 ms. In full scan mode, ions were collected in the mass range of 350-2000 m/z and acquired at a resolution of 70,000 at m/z 200.

Data analysis was performed using Scaffold software (Proteome software Inc., Portland, OR) using the Rattus sequence database (Source: UniprotKB-www.uniprot.org , filtered by "Organism – „Rattus"", dated 22/03/2014) with the MASCOT (Matrix Science, London, U.K.; version 2.1) search algorithm. All identified peptides had an ion score above the Mascot peptide identity threshold (a high confidence score of 99% and a low false discovery rate (FDR) of 1%), and a target protein was considered identified if at least two such unique peptide matches were apparent for the protein. Acquired intensities in the experiment were globally normalized across all acquisition runs. Individual quantitative samples were normalized within each acquisition run. Intensities for each peptide were normalized within the assigned protein. The reference channels were normalized to produce a 1:1 fold change. All normalization calculations were performed using medians to multiplicatively normalize data. Values for duplicate rat groups were averaged and MS, RS and MS+RS rat groups expressed as fold difference relative to the Ctr group. Rat groups were considered to be increased or decreased if they were ± 0.2 fold different from Ctr rats.

4.2.6 Statistical Analysis

Behavioural and BDNF data were normally distributed (Shapiro-Wilk test). The data were analyzed by means of analysis of variance (ANOVA), followed by Tukey's post-hoc test with correction for multiple comparisons. Behavioural and BDNF data are presented as mean ± SEM.

4.3 Results

4.3.1 Behaviour

4.3.1.1 Forced swim test

One-way ANOVA revealed a significant effect of stress on immobility ($F_{(3, 46)} = 19.56$, $p < 0.001$) and swimming ($F_{(3, 46)} = 32.03$, $p < 0.001$) in the FST. Post-hoc comparisons revealed that both MS ($p < 0.01$) and MS + RS rats ($p < 0.05$) spent significantly more time immobile than Ctr rats and the RS rats spent significantly less time immobile than Ctr ($p < 0.05$), MS ($p < 0.001$) and MS + RS rats ($p < 0.001$; Fig. 4.1a). Both MS ($p < 0.001$) and MS + RS rats ($p < 0.05$) spent significantly less time swimming than Ctr rats and RS rats spent significantly more time swimming than Ctr, MS and MS + RS rats ($p < 0.001$; Fig. 4.1b).

4.3.1.2 Elevated plus maze

One-way ANOVA showed no effect of stress on the amount of time spent in the open arms (Fig. 4.2a), closed arms (Fig. 4.2b) or center area (Fig. 4.2c) of the EPM.

4.3.1.3 Open field test

One-way ANOVA revealed a significant effect of stress on distance travelled ($F_{(3, 47)} = 8.03$, $p < 0.001$) in the OFT. Post-hoc comparisons revealed that both MS ($p < 0.001$) and MS + RS rats ($p < 0.05$) travelled a significantly longer distance than Ctr rats (Fig. 4.3).

4.3.2 Biochemistry

4.3.2.1 BDNF concentration in the ventral hippocampus

One-way ANOVA showed no effect of stress on BDNF concentration (Fig. 4.4) in the ventral hippocampus.

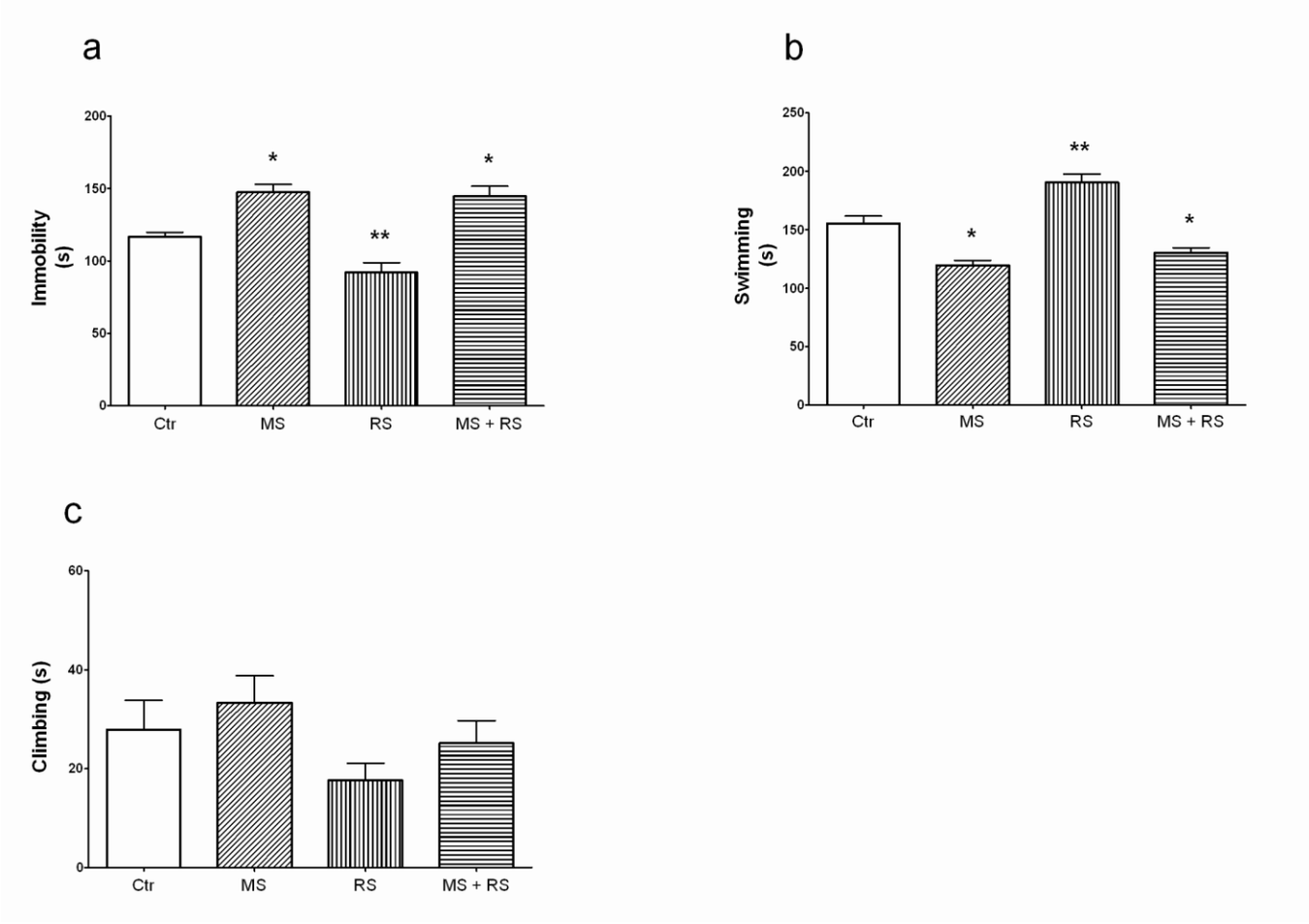


Figure 4.1: Immobility, swimming and climbing behaviours of control SD, MS SD, restraint stressed SD and MS SD rats subjected to restraint stress in the FST. MS and MS+RS increased immobility (a) and decreased swimming (b) behaviours. Restraint stress decreased immobility (a) and increased swimming (b) behaviours in the FST. Restraint stress had no additional effect on MS. * MS and MS+RS rats significantly different from Ctr rats, $p < 0.05$; ** RS rats significantly different from Ctr, MS and MS+RS rats, $p < 0.001$; Tukey‟s post-hoc test (n = 12-13/group). Data presented as mean ± SEM.

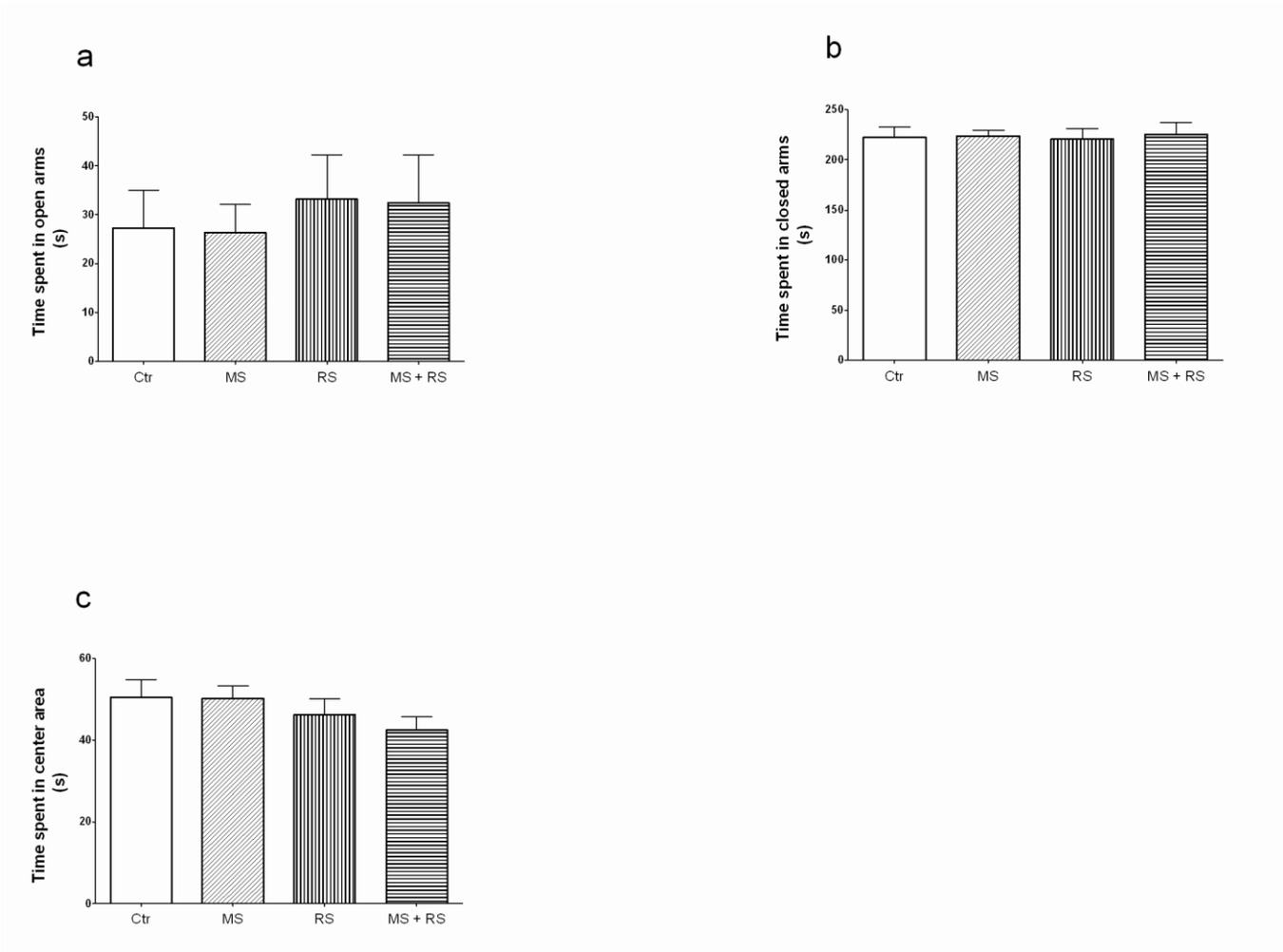


Figure 4.2: Time spent in the open arms, closed arms and center area by control SD, MS SD, restraint stressed SD and MS SD rats subjected to restraint stress in the EPM. The EPM revealed no difference between Ctr, MS, RS, and MS+RS rats in time spent in the open arms (a) and closed arms (b) and center area (c) (n = 12-13/group). Data presented as mean ± SEM.

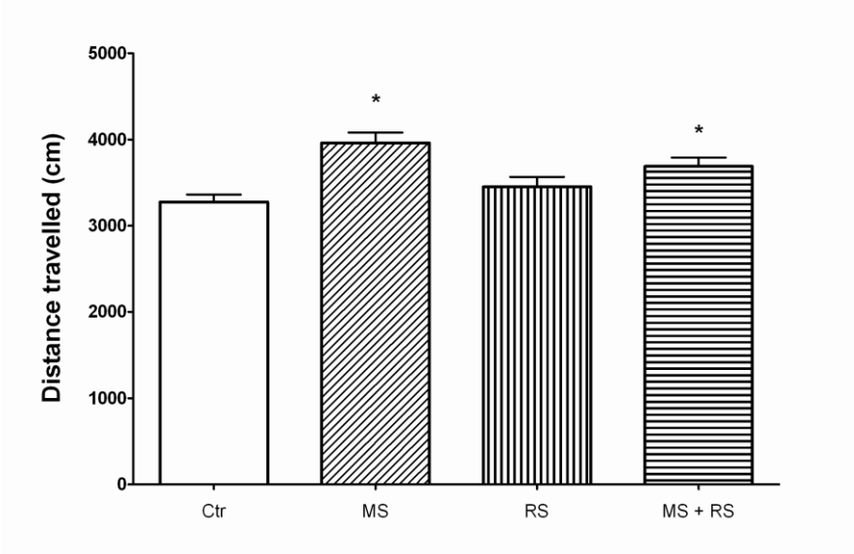


Figure 4.3: Distance travelled by control SD, MS SD, restraint stressed SD and MS SD rats subjected to restraint stress in the OFT. MS and MS+RS increased activity in the OFT and restraint stress had no effect on MS. *MS and MS + RS rats significantly different from Ctr rats, $p < 0.05$; Tukey's post-hoc test (n = 12-13/group). Data presented as mean ± SEM.

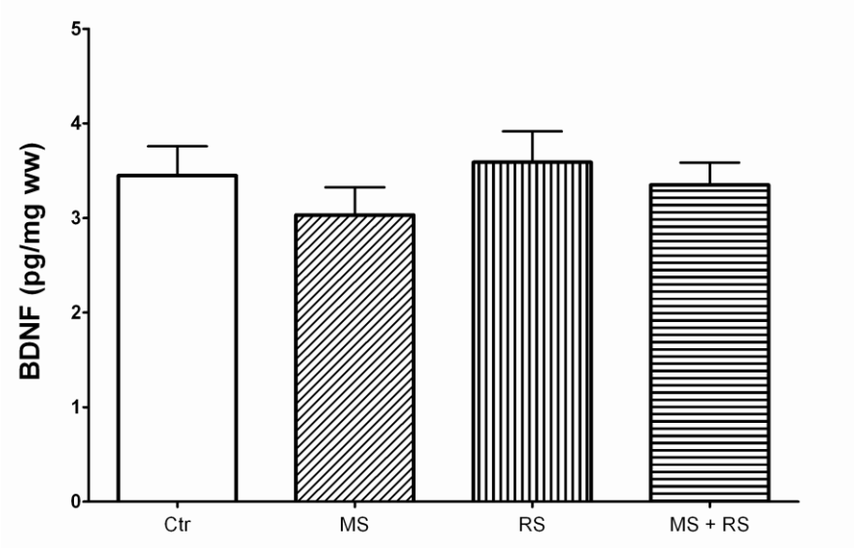


Figure 4.4: BDNF levels in the ventral hippocampus of control SD, MS SD, restraint stressed SD and MS SD rats subjected to restraint stress. No significant difference found in BDNF levels in the ventral hippocampus between Ctr, MS, RS and MS + RS rats (n = 10/group). Data presented as mean ± SEM.

4.3.2.2 Proteomics

Using the Rattus sequence database, 814 proteins were identified for the 8-plex (appendix A; table A1) and 869 proteins identified for the 4-plex (appendix A; table A2) with > 95 % confidence. In the 8-plex, 32 proteins were identified to be increased or decreased in the PFC of MS and RS relative to the Ctr rats (Table 4.1). In the 4-plex, 29 proteins were identified to be increased or decreased in the PFC of MS+RS relative to the Ctr rats (Table 4.2).

The 8-plex (table 4.1) quantification revealed that MS and RS rats had decreased actin-associated structural proteins (Actin related protein 2/3 complex, subunit 3, Alpha II spectrin or ARP10 actin-related protein 10 homolog) compared to Ctr rats. The 8-plex and 4-plex (table 4.1 and 4.2) quantification revealed that MS, RS and MS+RS rats had decreased mitochondrial energy-related proteins (Trifunctional enzyme subunit alpha, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, ATP synthase subunit O, Cytochrome b-c1 complex subunit 1, ATP synthase gamma chain, Mitochondrial 2-oxoglutarate/malate carrier protein) compared to Ctr rats. Neurotransmission/signalling proteins in MS, RS and MS+RS rats were increased or decreased compared to Ctr rats. However, proteins involved in glutamate and opioid signalling (Opioid-binding protein/cell adhesion molecule, Glutamate receptor 2) were decreased in the MS+RS compared to Ctr rats. Furthermore, the 4-plex revealed a decrease in proteins involved in protein synthesis (Protein Tardbp, 40S ribosomal protein S12, RCG45615, isoform CRA_a and Tyrosine--tRNA ligase) and increase in proteins involved in protein degradation (F-box only protein 2 and COP9 (Constitutive photomorphogenic) homolog, subunit 7a) in MS+RS compared to Ctr rats. In further support of the disruption in protein synthesis/degradation in MS+RS rats, carboxypeptidase E was found to be decreased.

Table 4.1: Proteins that differed by more than 20% (1.2-fold change) from non-maternally separated, non-stressed rats (Ctr) in the proteomic profile of the PFC of MS and restraint stressed (RS) rats (in red). Data presented as average fold difference relative to Ctr (in blue).

Protein / function	Accession no	Ctr 1	Ctr 2	Avg	Ctr/ Ctr	MS 1	MS 2	Avg	MS/ Ctr	RS 1	RS 2	Avg	RS/ Ctr
Cytoskeletal/Structural													
Actin related protein 2/3 complex, subunit 3	tr | B2GV73 |	1	1.20	1.10	1.00	0.80	0.80	0.80	**0.73**	1.20	1.20	1.20	1.09
Alpha II spectrin	sp | P16086 |	1	1.10	1.05	1.00	0.80	0.70	0.75	**0.71**	0.90	1.30	1.10	1.05
ARP10 actin-related protein 10 homolog (S. cerevisiae)	tr | Q5M9F7 |	1	1.00	1.00	1.00	1.10	0.70	0.90	0.90	0.80	0.80	0.80	**0.80**
Myosin regulatory light chain	tr | Q63781 |	1	1.00	1.00	1.00	1.20	1.20	1.20	**1.20**	1.00	1.10	1.05	1.05
Myelin basic protein transcript variant 1	tr | I7FKL4 |	1	0.50	0.75	1.00	0.90	0.80	0.85	1.13	1.20	1.00	1.10	**1.47**
Energy metabolism													
Trifunctional enzyme subunit alpha, mitochondrial	sp | Q64428 |	1	1.10	1.05	1.00	0.40	1.00	0.70	**0.67**	0.90	0.70	0.80	**0.76**
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	sp | Q561S0 |	1	0.70	0.85	1.00	0.50	0.70	0.60	**0.71**	0.70	0.70	0.70	0.82
ATP synthase subunit O, mitochondrial	sp | Q06647 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84	0.80	0.70	0.75	**0.79**
ADP/ATP translocase 1	sp | Q05962 |	1	0.70	0.85	1.00	0.60	0.70	0.65	**0.76**	0.80	0.70	0.75	0.88
Neurotransmission/Signalling													
Adaptor protein complex AP-1, sigma 1 (Predicted), isoform CRA_b	tr | B5DFI3 |	1	1.00	1.00	1.00	1.10	1.30	1.20	**1.20**	1.10	1.00	1.05	1.05
SH3-containing GRB2-like protein 3-interacting protein 1	sp | P0DJJ3 |	1	1.00	1.00	1.00	1.20	1.30	1.25	**1.25**	1.20	1.10	1.15	1.15
Guanine nucleotide-binding protein G(k) subunit alpha	sp | P08753 |	1	0.70	0.85	1.00	1.10	1.10	1.10	**1.29**	0.80	0.90	0.85	1.00
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	sp | P36876 |	1	1.10	1.05	1.00	1.80	1.00	1.40	**1.33**	1.00	1.10	1.05	1.00
Diphosphoinositol polyphosphate	sp | Q566C7 |	1	1.00	1.00	1.00	1.10	1.40	1.25	**1.25**	0.90	0.90	0.90	0.90

phosphohydrolase 1													
MAP/microtubule affinity-regulating kinase 3	sp | Q8VHF0 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**	1.00	1.00	1.00	1.00
Visinin-like protein 1	sp | P62762 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05	1.20	1.20	1.20	**1.20**
Annexin A3	sp | P14669 |	1	1.30	1.15	1.00	0.70	0.90	0.80	**0.70**	0.90	1.20	1.05	0.91
Neuronal pentraxin receptor (Fragment)	sp | O35764 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84	0.70	0.80	0.75	**0.79**
Protein synthesis/neurotrophic													
RNA binding motif protein, X-linked-like-1	sp | D4AE41 |	1	0.80	0.90	1.00	0.80	0.70	0.75	0.83	0.70	0.70	0.70	**0.78**
Scaffold attachment factor B1	sp | O88453 |	1	1.00	1.00	1.00	1.10	0.90	1.00	1.00	0.80	0.70	0.75	**0.75**
Polyadenylate-binding protein 1	sp | Q9EPH8 |	1	0.90	0.95	1.00	1.10	1.00	1.05	1.11	0.80	0.70	0.75	**0.79**
60S ribosomal protein L19	sp | P84100 |	1	1.00	1.00	1.00	1.20	1.20	1.20	**1.20**	0.90	0.90	0.90	0.90
Peptidyl-prolyl cis-trans isomerase FKBP1A	sp | Q62658 |	1	1.00	1.00	1.00	1.20	1.20	1.20	**1.20**	1.00	1.00	1.00	1.00
Other													
Ferritin	tr | F1M5T1 |	1	0.90	0.95	1.00	1.20	1.10	1.15	**1.21**	1.10	0.80	0.95	1.00
Nitrilase 1, isoform CRA_a	tr | F7ESM5 |	1	0.90	0.95	1.00	1.10	1.20	1.15	**1.21**	1.10	1.00	1.05	1.11
Adenine phosphoribosyltransferase	sp | P36972 |	1	1.10	1.05	1.00	1.40	1.20	1.30	**1.24**	1.20	1.20	1.20	1.14
Cysteine and glycine-rich protein 1	sp | P47875 |	1	1.00	1.00	1.00	1.30	1.10	1.20	**1.20**	1.00	1.20	1.10	1.10
Neuromodulin	sp | P07936 |	1	0.90	0.95	1.00	1.10	0.90	1.00	1.05	1.20	1.20	1.20	**1.26**
Programmed cell death 6-interacting protein	sp | Q9QZA2 |	1	0.90	0.95	1.00	1.10	1.20	1.15	**1.21**	0.90	1.00	0.95	1.00
Protein RGD1304884	tr | D4A3C2 |	1	1.00	1.00	1.00	1.30	1.10	1.20	**1.20**	1.10	1.10	1.10	1.10
Protein RGD1309586	tr | D3ZN21 |	1	1.50	1.25	1.00	1.40	1.70	1.55	**1.24**	1.90	1.70	1.80	1.44
6-phosphogluconolactonase	sp | P85971 |	1	0.90	0.95	1.00	1.00	1.10	1.05	1.11	1.10	1.20	1.15	**1.21**

Table 4.2: Proteins that differed by more than 20% (1.2-fold change) from non-maternally separated, non-stressed rats (Ctr) in the proteomic profile of the PFC of MS and restraint stressed (MS+RS) rats (in red). Data presented as average fold difference relative to Ctr (in blue).

Protein / function	Accession No	Ctr 1	Ctr 2	Avg	Ctr/Ctr	MS+RS 1	MS+RS 2	Avg	MS+RS/ Ctr
Cytoskeletal/Structural									
Tropomyosin alpha isoform	tr | Q91XN7 |	1	1.00	1.00	1.00	1.20	1.30	1.25	**1.25**
Protein Twf2	tr | B0BMY7 |	1	1.00	1.00	1.00	1.20	1.20	1.20	**1.20**
Neuronal migration protein doublecortin	sp | Q9ESI7 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Paralemmin-1	sp | Q920Q0 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Energy metabolism									
Cytochrome b-c1 complex subunit 1, mitochondrial	sp | Q68FY0 |	1	0.80	0.90	1.00	0.80	0.60	0.70	**0.78**
Cytochrome c oxidase subunit 6B1	tr | D3ZD09 |	1	0.70	0.85	1.00	0.70	0.60	0.65	**0.76**
ATP synthase F(0) complex subunit B1, mitochondrial	sp | P19511 |	1	0.70	0.85	1.00	0.80	0.50	0.65	**0.76**
ATP synthase gamma chain	tr | Q6QI09 |	1	0.80	0.90	1.00	0.80	0.60	0.70	**0.78**
Mitochondrial 2-oxoglutarate/malate carrier protein	tr | G3V6H5 |	1	0.90	0.95	1.00	0.80	0.70	0.75	**0.79**
Neurotransmission/signalling									
Opioid-binding protein/cell adhesion molecule	sp | P32736 |	1	1.00	1.00	1.00	0.80	0.70	0.75	**0.75**
Glutamate receptor 2	tr | F1LNE4 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Voltage-dependent anion-selective channel protein 1	sp | Q9Z2L0 |	1	0.70	0.85	1.00	0.80	0.50	0.65	**0.76**
Dynein light chain 2, cytoplasmic	sp | Q78P75 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**

Protein Ranbp1	tr | D4A2G9 |	1	1.00	1.00	1.00	1.30	1.20	1.25	**1.25**
Mitochondrial 2-oxoglutarate/malate carrier protein	tr | G3V6H5 |	1	0.90	0.95	1.00	0.80	0.70	0.75	**0.79**
Rho guanine nucleotide exchange factor 2	sp | Q5FVC2 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Regulator of G-protein-signaling 6	tr | F1LS67 |	1	0.90	0.95	1.00	1.20	1.20	1.20	**1.26**
Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B	sp | Q01066 |	1	0.90	0.95	1.00	0.70	0.80	0.75	**0.79**
Protein synthesis/neurotrophic									
Protein Tardbp	tr | I6L9G6 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
40S ribosomal protein S12	tr | M0R9I8 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**
RCG45615, isoform CRA_a	tr | B2RYU2 |	1	0.90	0.95	1.00	0.70	0.80	0.75	**0.79**
Tyrosine--tRNA ligase, cytoplasmic	sp | Q4KM49 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Protein degradation									
F-box only protein 2	tr | G3V774 |	1	0.90	0.95	1.00	1.40	1.10	1.25	**1.32**
COP9 (Constitutive photomorphogenic) homolog, subunit 7a (Arabidopsis thaliana) (Predicted)	tr | G3V8Z9 |	1	0.90	0.95	1.00	1.10	1.20	1.15	**1.21**
Carboxypeptidase E	sp | P15087 |	1	1.10	1.05	1.00	0.80	0.80	0.80	**0.76**
Other									
Uncharacterized protein	tr | M0R9D5 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**
Protein LOC679748	tr | D3ZE63 |	1	1.10	1.05	1.00	0.80	0.70	0.75	**0.71**
Thiosulfate sulfurtransferase	sp | P24329 |	1	1.00	1.00	1.00	1.10	1.60	1.35	**1.35**
C-terminal-binding protein 1	sp | Q9Z2F5 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Ig kappa chain C region, A allele	sp | P01836 |	1	1.00	1.00	1.00	1.10	1.30	1.20	**1.20**

4.4 Discussion

The main findings of the current study showed that MS SD rats or MS SD rats subjected to restraint stress displayed depression-like behaviour without anxiety-like behaviour and that MS was not affected by chronic restraint stress in adulthood. Biochemical studies of the ventral hippocampus revealed no effect of MS and restraint stress on BDNF levels. Proteomic analysis of the PFC revealed a decrease in actin-associated proteins in MS SD rats (Actin related protein 2/3 complex, subunit 3, Alpha II spectrin) and non-separated SD rats subjected to restraint stress (ARP10 actin-related protein 10 homolog) as well as a decrease in mitochondrial energy-related proteins in MS SD rats (Trifunctional enzyme, NADH dehydrogenase, ADP/ATP translocase 1), non-separated SD rats subjected to restraint stress (Trifunctional enzyme and ATP synthase) and MS SD rats subjected to restraint stress (cytochrome subunits, ATP synthase and malate carrier protein). Furthermore, a decrease in proteins involved in protein synthesis (Protein Tardbp, RCG, 40s ribosomal protein and tyrosine-tRNA ligase) and an increase in proteins involved in protein degradation (F-box protein and COP9) were found in MS SD rats subjected to restraint stress.

Rats subjected to MS or MS and restraint stress displayed depression-like behaviour as evidenced by increased immobility and decreased swimming behaviours in the FST. Restraint stress decreased immobility and increased swimming behaviours in the FST in SD rats. Restraint stress had no effect on MS rats. The depression-like behaviour of MS SD rats in the FST is in agreement with previous studies using the same MS protocol (Desbonnet et al. 2010; Dimatelis et al. 2012b; Lambás-Señas et al. 2009; Yoo et al. 2013). However, the current study showed that restraint stress decreased immobility in the FST. This is in contrast to studies that showed a stress-induced increase in immobility (Bombi et al. 2013; Chiba et al. 2012; Ulloa et al. 2010). This paradoxical effect of restraint stress on immobility in the FST has been reported previously (Platt and Stone 1982; Swiergiel et al. 2007), an effect further supported by data demonstrating that chronically administered corticosterone decreased immobility in the FST (Brotto et al. 2001). There is no understandable explanation for the decreased immobility following restraint stress but has been previously suggested to be the result of adaptation to chronic stress and changes in the β-adrenergic receptor (Platt and Stone 1982; Swiergiel et al. 2007). The inconsistent effect of restraint stress on immobility could also probably be ascribed to the different restraint stress protocols since different durations and intensity of restraint stress have different effects on immobility in the FST (Cancela et al. 1991; Suvrathan et al. 2010).

To exclude the possibility that the depression-like behaviour observed in the MS rats may be due to a change in motor activity, the distance travelled by the rat was determined in the OFT. Rats subjected to MS or MS and restraint stress displayed increased locomotor activity in the OFT compared to control rats. This is in line with results obtained by Lambás-Señas et al. (2009) who showed that MS SD rats displayed increased motor activity in a novel environment, using a similar MS protocol. Therefore, the results in the FST could not be related to a decrease in motor activity per se.

Furthermore, MS and restraint stress did not induce anxiety-like behaviour as measured by time spent in the closed arms of the EPM. This is consistent with various studies that found no anxiety-like behaviour in adult rats subjected to MS in early life (Dimatelis et al. 2012b; Rüedi-Bettschen et al. 2005; van Heerden et al. 2010; Yoo et al. 2013). Differences in both MS and restraint stress procedures between studies may explain differences in reported results such as anxiety-like behaviour. This is not only true for the duration of restraint stress or MS, but also for the method of restraint stress (restraint in tubes or with wire mesh as well as by taping the limbs to restrict movement) or MS (removing the pups or removing the dam from the home cage during MS as well as separation with a whole litter or separation in isolation) (Glavin et al. 1994; Vetulani 2013).

BDNF is highly expressed in the hippocampus (Yan et al. 1997) and the results of its action include neuronal sprouting, synaptic reorganization and neurogenesis (Lindvall et al. 1994; McAllister et al. 1999). In the current study, MS and restraint stress had no effect on BDNF concentration in the ventral hippocampus. The results are therefore in contrast with various studies that showed decreased BDNF expression in the hippocampus of MS or restraint stress rats (Roceri et al. 2002; Xu et al. 2006). However, the neuronal mechanisms involved seem complex, since other studies found increased (Greisen et al. 2005) or even no effect of MS or restraint stress on BDNF expression in the hippocampus (Kuroda and McEwen 1998; Reagan et al. 2007; Roceri et al. 2004; Rosenbrock et al. 2005). However, it is not surprising that BDNF results were inconsistant since the methodology such as time of stressor for both MS and restraint stress are quite variable between these studies. For example, it has previously been shown that variations in the duration of restraint stress have different effects on BDNF. This was evidenced by reduced BDNF concentration after 21 days compared to 7 days of restraint stress in mice (Capoccia et al. 2013). It is also likely that the neurobiology of the depression-like behaviour in MS rats or MS rats subjected to restraint stress is related to diverse and regionally specific neurotrophin function (Berton et al. 2006). This could be due to the

fact that BDNF is highly complex (eight exons, multiple splice variants and alternate polyadenylation sites), providing the potential for multiple BDNF transcripts that are differently regulated by stress and therefore resulting in contrasting functional effects (Groves 2007; Liu et al. 2006).

To further evaluate the effect of restraint stress and MS on rats, a proteomic analysis of the PFC was performed. Proteins that were differently regulated in the PFC of rats subjected to MS, restraint stress or MS and restraint stress, fall into five broadly defined functional categories: (a) cytoskeletal, (b) energy metabolism, (c) neurotransmission, (d) protein synthesis and (e) protein degradation. These functional categories will be further discussed according to current understanding of the neurobiology of animal models of stress-induced depression-like behaviour.

Cytoskeletal proteins such as microfilaments, microtubules and intermediate filaments play a central role in neuronal function and the creation and maintenance of cell shape (Pigino et al. 2006). In the current study, actin microfilament-associated proteins such as actin related protein 2/3, alpha II spectrin and actin-related protein 10 were reduced in rats subjected to MS or restraint stress. On the other hand, actin binding proteins, such as the myosin regulatory light chain and tropomyosin alpha isoform were increased in rats subjected to MS or MS and restraint stress. It has previously been shown that MS reduced actin microfilament-associated proteins in the NAc and ventral hippocampus suggesting altered neurotransmission and synaptic function (Daniels et al. 2012; Dimatelis et al. 2012a). Furthermore, it has also been shown that a combination of various stressors (restraint stress, forced swim stress and ether vapor) decreased structural proteins in the ventral hippocampus (Uys et al. 2008). In line with the current study, Daniels et al. (2012) found that MS increased the expression of tropomyosin-5 in the ventral hippocampus. Tropomyosin and myosin regulatory light chain not only bind to actin, but also increase axoplasmic transport, synaptic rearrangement, vesicle transport or neurotransmitter release (Lees-Miller et al. 1990; Mochida 1995). It therefore appeared that the stress of MS increased the expression of myosin regulatory light chain and tropomyosin alpha isoform in the rats subjected to MS or MS and restraint stress, to compensate for the loss of structural proteins. Therefore, the stress of MS and restraint stress mostly decreased actin-associated proteins, which function to maintain the cytoskeletal network and therefore may have altered neuronal function in the PFC.

The current study demonstrated a decrease in proteins involved in mitochondrial energy metabolism in the PFC of rats subjected to MS, restraint stress or MS and restraint stress. These proteins included trifunctional enzyme subunit alpha, ATP synthase subunit O,

ATP synthase gamma chain, cytochrome b-c1 complex subunit 1 and mitochondrial 2-oxoglutarate/malate carrier protein. Most of these proteins are involved in oxidative phosphorylation of ADP to produce ATP (ATP synthase subunit O, ATP synthase gamma chain, cytochrome b-c1 complex subunit 1 and mitochondrial 2-oxoglutarate/malate carrier protein). Dysfunction of the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of neuropsychiatric disorders such as depression (Tobe 2013) as well as being associated with cellular degeneration (Calabrese et al. 2001). The findings of the current study are in line with previous studies that showed decreased energy-related proteins in the hippocampus in response to chronic unpredictable stress (21 days) and MS stress (Dimatelis et al. 2012a; Mu et al. 2007). Furthermore, chronic mild stress (40 days) or restraint stress (7 - 21 days) in Wistar rats has been previously shown to decrease energy-related proteins in the PFC associated with oxidative phosphorylation (Madrigal et al. 2001; Réus et al. 2014). Therefore, dysfunction of mitochondrial energy metabolism may compromise neuronal function as a possible consequence of mitochondrial damage and degeneration in rats subjected to MS, restraint stress or MS and restraint stress.

Proteins in the 8-plex and 4-plex, especially the energy-related proteins, were found to be variable in most of the control duplicates. Both biological and technical variability have been previously reported (Gan et al. 2007) and may have contributed to the variation of duplicates in the current study. The technical variability is often introduced in the multiple steps of sample preparation, efficiency of enzymatic digestion, efficiency of chemical labeling, performance of instrumentation and overlapping of mass of reporter ions which have an impact on yield of fragmentation (Pottiez et al. 2012). Nonetheless, iTRAQ proteomic quantification is widely used in all fields of research and proved to be highly sensitive (Wu et al. 2006). Biological variability is introduced as variability that exists from animal-to-animal within experimental groups. Outbred rat strains are genetically undefined and show greater inherent biological variation than inbred strains (Zakharkin et al. 2005) which may be the cause of biological variability in the current study, since two samples of pooled tissue from 3 rats per group were analyzed separately. In the current study, most of the proteins in the 8-plex and 4-plex control duplicates that varied more than 20 %, showed similar fold differences in both analysis. The differences between control duplicate analyses may therefore be attributed to the biological variability. However, since some of the proteins in the control duplicates were also variable in the 4-plex and 8-plex, technical variability may also be a factor.

A number of proteins involved in neurotransmission/signalling were differentially expressed in the MS rats and non-maternally separated rats and MS rats subjected to restraint stress. The AMPA glutamate receptor 2 (GluR2) was decreased in the rats subjected to MS and restraint stress. The involvement of AMPA receptors in animal models of depression and mechanism of antidepressant action have been well documented (Chourbaji et al. 2008; Li et al. 2001; Martinez-Turrillas et al. 2002; Martínez-Turrillas et al. 2007). AMPA receptor knockout mice displayed increased learned helplessness, decreased serotonin and noradrenaline levels and increased NMDA receptor expression in the hippocampus (Chourbaji et al. 2008). Furthermore, the social defeat model of depression in mice showed decreased GluR2 in the NAc as measured by immunohistochemistry (Vialou et al. 2010). This was further supported by evidence showing decreased GluR2 mRNA expression in the hippocampus of Wistar rats previously exposed to MS (3 h daily for 21 days) (Pickering et al. 2006). Furthermore, AMPA receptor channel impermeability to calcium is dependent on the GluR2 subunit and cells that contain AMPA receptors lacking the GluR2 subunit show high calcium permeability and therefore vulnerability to excitotoxicity (Liu and Zukin 2007). AMPA receptors are also known to play an important role in neurotrophic/neuroprotective effects (Zarate, Jr. and Manji 2008). For example, in vivo and in vitro studies showed that treatment with kainic acid or ampakines (AMPA receptor modulator) increased BDNF and nerve growth factor mRNA and protein levels in the cerebral cortex and hippocampus of rats (Lauterborn et al. 2000; Lauterborn et al. 2003; Zafra et al. 1990). It is therefore evident that modifications of AMPA receptors alter synaptic function through various signalling proteins that underlie the neuropathology of psychiatric disorders such as depression.

The neuroprotective effects of GluR2 were further supported by evidence indicating an interaction between AMPA and opioid receptors (Fundytus 2001). The acute and chronic function of opioid receptors is regulated by the opioid-binding protein/cell adhesion molecule (OBCAM) (Loh and Smith 1996) and was found to be decreased in the present study. OBCAM is a glycosylphosphatidylinositol (GPI)-anchored protein with selectivity for the MOR ligands (Brown et al. 2012) and has been shown to regulate DOR function (Lane et al. 1992). Furthermore, OBCAM has been shown to be associated with individuals with depression in a genome-wide linkage analysis in a family-based study (Schol-Gelok et al. 2010). The DOR and MOR are also involved in the neurotrophic/neuroprotective effects of opioids as evidenced by increased BDNF mRNA expression in the frontal cortex, hippocampus and amygdala following administration of an opioid agonist or endogenous ligand (Torregrossa et al. 2004; Zhang et al. 2006).

Proteins involved in protein synthesis were decreased (Protein Tardbp, 40S ribosomal protein S12, RCG45615/ 60S ribosomal protein L12 and Tyrosine tRNA ligase) and proteins involved in protein degradation (F-box only protein 2 and COP9 homolog, subunit 7a) were increased in the PFC of rats subjected to both MS and restraint stress. Inhibition of protein synthesis is a common response of cells to severe forms of stress such as thermal, physical and metabolic stress and viral infection (Clemens et al. 2000; Clemens et al. 1998; Gale, Jr. et al. 2000; Sheikh and Fornace 1999). For instance, animal models of transient cerebral ischemia have shown reduced protein synthesis (Hu and Wieloch 1993; Mengesdorf et al. 2002). The involvement of protein synthesis in depression was evidenced by the antidepressant drug, ketamine that triggered protein synthesis by dephosphorylating and activating eukaryotic elongation factor 2 in the hippocampus of mice (Autry et al. 2011; Flight 2011). On the other hand, restraint stress for 1 h daily for 4 weeks showed increased protein degradation in the brain of SD rats as measured by the H_2O_2 degradation assay (Hong et al. 2014). The decreased protein synthesis and increased protein degradation in the current study may possibly result in atrophy and reduced function of PFC neurons in rats subjected to MS and restraint stress. In further support of disruptions in protein synthesis and degradation, carboxypeptidase E was found to be increased in the PFC of rats subjected to MS and RS. Carboxypeptidase E is the enzyme responsible for cleaving the C-terminally extended residues of peptide intermediates to produce bioactive peptides (Cawley et al. 2012). Additionally, this protein is also involved in depressive-like behaviour since a previous study in mice with a mutation in carboxypeptidase E that renders the protein inactive and unstable, displayed increased immobility in the FST and tail suspension test (Rodriguiz et al. 2013).

In conclusion, rats exposed to early life MS without or with additional restraint stress, displayed depression-like behaviour in the FST. Furthermore, reduced mitochondrial energy-related, structural actin-associated proteins as well as disruption in proteins associated with protein synthesis and degradation, may be the result of the stress of MS and restraint stress on the PFC.

Chapter 5

Effect of ketamine in Wistar-Kyoto and Sprague-Dawley rat models of depression

5.1 Introduction

The NMDA receptor antagonist, ketamine at subanaesthetic doses, has recently been used to treat depression. On the other hand, a subanaesthetic dose of ketamine has also been shown to produce symptoms of schizophrenia in healthy individuals and to exacerbate symptoms in patients with schizophrenia due to its psychotomimetic effects (Lahti et al. 1995; Lahti et al. 2001; Malhotra et al. 1997). Unfortunately, the adverse psychotomimetic effects and abuse potential of ketamine have limited its use as an antidepressant.

In rodents, antagonists of the NMDA receptor such as phencyclidine have been used in naïve rodents to produce a model of schizophrenia to gain insight into the mechanisms underlying some of the positive (hyperactivity, heightened stimulant sensitivity), negative (reduced social behaviour), affective (depression-like behaviour in the FST) and cognitive symptoms (impairment of executive function and reduced memory in object recognition tasks) and disruption of sensory motor gating (latent inhibition and prepulse inhibition) characteristic of schizophrenia (Egerton et al. 2005; Grayson et al. 2007; Kalinichev et al. 2008; Martinez et al. 1999; Noda et al. 1995; Sams-Dodd 1995). Similarly, ketamine, an NMDA receptor antagonist, has also recently been used to study schizophrenia-like behaviour in rodents (Becker et al. 2003; Becker and Grecksch 2004; Chatterjee et al. 2011; Chindo et al. 2012; Hou et al. 2013; Imre et al. 2006). At a dose of 30 – 50 mg/kg, ketamine decreased social behaviour in rats and increased immobility in the FST (Becker et al. 2003; Becker and Grecksch 2004; Chindo et al. 2012). However, acute and chronic treatment with ketamine produced differential effects. For example, acute treatment of mice with various doses (12 - 100 mg/kg) of ketamine induced hyperactivity in the OFT, impaired memory in the passive avoidance task and disrupted prepulse inhibition (Chatterjee et al. 2011; Hou et al. 2013; Imre et al. 2006) whereas chronic treatment of rodents with ketamine at a dose of 30 – 100 mg/kg for 5 days or longer increased immobility in the FST (Chatterjee et al. 2011; Chindo et al. 2012; Hou et al. 2013). In further support of the ketamine model of schizophrenia, behaviours such as reduced social

behaviour, increased activity in the OFT, reduced learning and memory in the passive avoidance task and increased immobility in the FST were reversed by antipsychotic drugs (risperidone and clozapine) (Becker and Grecksch 2004; Chatterjee et al. 2011; Chindo et al. 2012).

At lower doses, in various rodent models of depression, ketamine showed rapid antidepressant-like effects in the FST, learned helplessness paradigm and tail suspension test (Garcia et al. 2008; Koike et al. 2011; Li et al. 2010; Maeng et al. 2008; Tizabi et al. 2012). Ketamine treatment of rats at a dose of 10 mg/kg and 15 mg/kg has been shown to reduce the duration of immobility in the FST induced by the 15-min pretest-swim, at 60 min after treatment (Garcia et al. 2008). Furthermore, ketamine treatment at a dose of 10 mg/kg has been shown to reduce the number of escape failures in the learned helplessness paradigm, increased sucrose consumption and reduced latency to feed (measure of anxiety) in rats that had been exposed to chronic unpredictable stressors (Koike et al. 2011; Li et al. 2011). However, ketamine, at higher doses (80 mg/kg or 160 mg/kg), had no sustained antidepressant effect in the FST in rats at 24 h or 1 week after acute treatment (Li et al. 2010; Popik et al. 2008). Furthermore, repeated treatment (3 days, 7 days or 3 injections at 24, 5 and 1 h before test swim) of ketamine at a dose of 25 mg/kg had no immediate effect on immobility in the FST at 1 h after treatment in rats (Fraga et al. 2013; Gigliucci et al. 2013). Therefore, treatment of rodent models of depression with a lower dose of ketamine was required to produce rapid antidepressant effects.

Similarly, it has recently been shown that acute ketamine treatment of female WKY rats at low doses (2.5 mg/kg and 5 mg/kg) reversed their depressive-like behaviour as evidenced by reduced duration of immobility in the FST more than 20 min after treatment (Tizabi et al. 2012). Furthermore, reduced immobility induced by ketamine treatment at a dose of 5 mg/kg was still evident 1 week after treatment (Tizabi et al. 2012). The exact mechanism of ketamine‟s rapid and sustained antidepressant effect is still unknown. However, a direct role for the AMPA and NMDA receptor in the antidepressant effects of ketamine has been suggested (Koike et al. 2011; Maeng et al. 2008; Tizabi et al. 2012). Previous studies showed that ketamine treatment increased the AMPA/NMDA ratio in WKY rats and the antidepressant effect was enhanced by an AMPA receptor agonist (Akinfiresoye and Tizabi 2013; Tizabi et al. 2012) or blocked by an antagonist (Koike et al. 2011; Maeng et al. 2008). Furthermore, WKY rats have a unique neurochemical profile. Previous studies showed that the NMDA receptor density was reduced in several brain areas of WKY rats. NMDA receptor density was reduced in the cerebral cortex, striatum, NAc, hippocampus and substantia nigra of WKY compared to Wistar rats (Lei and Tejani-

Butt 2010; Lei et al. 2009). In addition, noradrenaline and dopamine uptake blockers such as desipramine and nomifensine have been shown to reduce depressive-like behaviour in the FST in WKY rats while selective serotonin uptake blockers (paroxetine and fluoxetine) were without effect (Lopez-Rubalcava and Lucki 2000; Paré 1992; Tejani-Butt et al. 2003). It was therefore suggested that the WKY may be a suitable model to study depression with resistance to specific antidepressant drugs (Lahmame et al. 1997; Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003) similar to patients who show a differential response to antidepressant drugs (Labermaier et al. 2013).

The aim of the current study was therefore to measure the antidepressant-like effects of ketamine in the WKY rat model of depression at low subanaesthetic doses (5-15 mg/kg) previously shown to induce antidepressant-like effects in animal models of depression (Garcia et al. 2008; Tizabi et al. 2012). The FST was used to measure antidepressant-like effects at different times (2 h, 48 h and 72 h) after a single injection of ketamine in order to determine both the rapid and sustained effects of ketamine. Since high frequency USVs have previously been shown to be associated with appetitive behaviour and communication of emotional information (Knutson et al. 2002), the effect of ketamine on isolation-induced social signalling was measured in WKY rats. MS of SD rats has been shown to produce a model of depression that is sensitive to a variety of antidepressant drugs (El Khoury et al. 2006; Huang and Lin 2010). Consequently, the effect of ketamine on the MS SD model of depression was also investigated.

5.2 Materials and Methods

5.2.1 Animals

A total of 75 male WKY/NCrl, 51 male Wistar and 123 male SD rats were used for experiment 1, 2 and 3. Wistar rats were used as the control for the WKY rats. All animals were obtained from the University of Cape Town Animal Unit except for SD rats that were bred in the Satellite Animal Facility in the Anatomy Building of the University of Cape Town for experiment 3. Rats were kept under the same conditions as described in section 2.2.1. The study was conducted in accordance with the guidelines of the South African National Standard: The care and use of animals for scientific purposes (2008) and approved by the University of Cape Town Faculty of Health Sciences Animal Ethics Committee (#012/045 for WKY and Wistar rats and #012/046 for SD rats).

Experiments 1-3 consisted of the following rat groups: WKY/NCrl (WKY) and Wistar rats for experiment 1, WKY/NCrl (WKY) rats for experiment 2 and non-maternally

separated SD (nMS SD) and maternally separated SD rats (MS SD) for experiment 3. The groups were randomly divided into drug treatment groups and further randomly divided into behavioural groups for experiment 1 and 3 according to treatment at different times before OFT and FST behavioural analysis (see behavioural group A and B in figure 5.1).

Experiment 1 consisted of 8 treatment groups: (a) WKY/NCrl tested 46 h and 72 h (Group A, n = 9) and 22 h and 48 h (Group B, n = 8) after intraperitoneal injection of saline (WKY Sal), (b) WKY/NCrl tested 46 h and 72 h (Group A, n = 5) and 22 h and 48 h (Group B, n = 5) after injection of 5 mg/kg ketamine (WKY Ket-5), (c) WKY/NCrl tested 46 h and 72 h (Group A, n = 6) and 22 h and 48 h (Group B, n = 5) after injection of 10 mg/kg ketamine (WKY Ket-10), (d) WKY/NCrl tested 46 h and 72 h (Group A, n = 5) and 22 h and 48 h (Group B, n = 5) after injection of 15 mg/kg ketamine (WKY Ket-15), (e) Wistar tested 46 h and 72 h (Group A, n = 9) and 22 h and 48 h (Group B, n = 8) after injection of saline (Wistar Sal), (f) Wistar tested 46 h and 72 h (Group A, n = 7) and 22 h and 48 h (Group B, n = 5) after injection of 5 mg/kg ketamine (Wistar Ket-5), (g) Wistar tested 46 h and 72 h (Group A, n = 6) and 22 h and 48 h (Group B, n = 6) after injection of 10 mg/kg ketamine (Wistar Ket-10) and (h) Wistar tested 46 h and 72 h (Group A, n = 5) and 22 h and 48 h (Group B, n = 5) after injection of 15 mg/kg ketamine (Wistar Ket-15).

Experiment 2 consisted of 2 treatment groups: WKY/NCrl treated with saline (WKY Sal, n = 13) and WKY/NCrl treated with 10 mg/kg ketamine (WKY Ket-10, n = 11).

Experiment 3 consisted of 4 treatment groups: (a) non-maternally separated SD (nMS SD) tested 46 h and 72 h (Group A, n = 15) and 22 h and 48 h (Group B, n = 17) after injection of saline (nMS SD Sal), (b) non-maternally separated SD (nMS SD) tested 46 h and 72 h (Group A, n = 16) and 22 h and 48 h (Group B, n = 16) after injection of 15 mg/kg ketamine (nMS SD Ket-15), (c) maternally separated SD (MS SD) tested 46 h and 72 h (Group A, n = 16) and 22 h and 48 h (Group B, n = 15) after injection of saline (MS SD Sal) and (d) maternally separated SD (MS SD) tested 46 h and 72 h (Group A, n = 15) and 22 h and 48 h (Group B, n = 15) after injection of 15 mg/kg ketamine (MS SD Ket-15).

In experiment 1 (WKY/NCrl and Wistar rats) and experiment 3 (MS SD and non-maternally SD rats), for 2 days prior to baseline USV recording (P60 – P61), rats were transferred to the room in which the USVs were to be recorded, where they were handled briefly and then returned to the rat facility. One day after the last day of brief handling, baseline USVs were recorded for 8 days (P62 – P69) prior to treatment. The USVs of the cage mate (Group B; figure 5.1) were recorded the next evening so that USVs were

recorded every alternate day for each rat. Each rat received a single ketamine injection at P70 - P71 and USV"s were recorded after 5 h and 29 h. At P72 (22 h and 46 h after ketamine/saline injection), the OFT was carried out and 2 h thereafter each rat was allowed to swim for 15 min in the FST (pretest-swim). After 24 h, the rats were exposed to a second 5-min test swim session (48 h and 72 h after ketamine/saline injection).

In experiment 2 (WKY/NCrl rats), each rat (P60) was allowed to swim for 15 min (pretest-swim) and 2 h before the test swim session, injected with 10 mg/kg ketamine/saline. At 24 h after the pretest-swim, the rats were exposed to a second 5-min swim session (2 h after ketamine/saline injection).

5.2.2 Drug treatment

At P70 (between 13h00-14h00), rats in experiment 1 were either injected with a single subanesthetic dose of ketamine hydrochloride (Anaket ®, Bayer Healthcare, Isando, RSA) (5, 10 or 15 mg/kg) or an equivalent volume of 0.9 % sterile saline (on day 1 and day 2 in figure 5.1). Ketamine and saline were administered intraperitoneally in a volume equivalent to 1 ml/kg as a single dose. Acute administration of 2.5 - 15 mg/kg ketamine have been shown previously to induce antidepressant-like effects in the FST (Garcia et al. 2008; Tizabi et al. 2012). It was therefore decided to use doses 5 mg/kg, 10 mg/kg or 15 mg/kg in the current study. Rats in experiment 2 and 3 were either injected with a single subanesthetic dose of 10 mg/kg or 15 mg/kg of ketamine, respectively, or an equivalent volume of sterile saline.

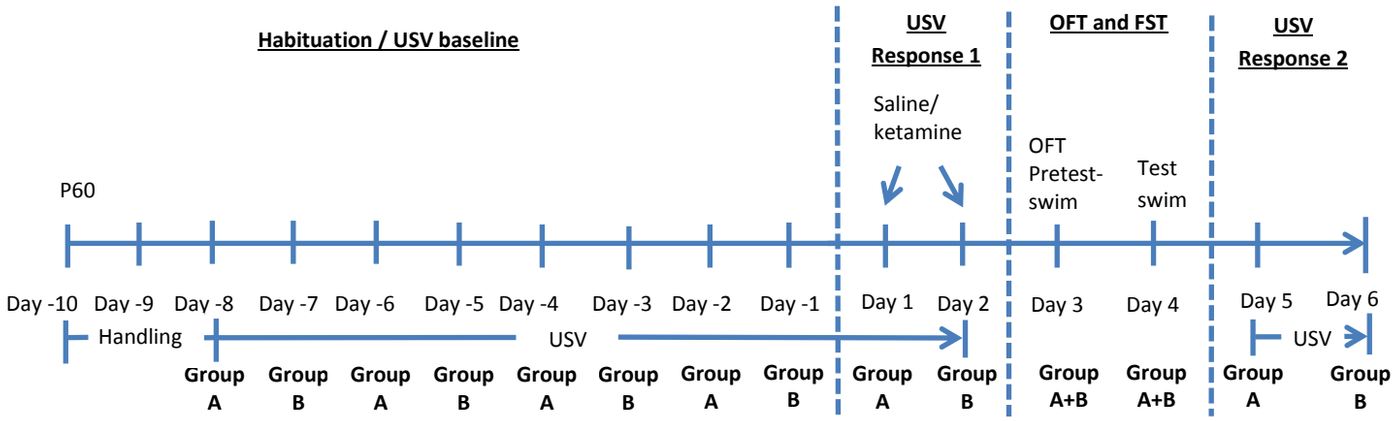
5.2.3 Maternal separation

MS was performed on SD rats according to the procedure and under similar conditions as described in chapter 3. Pups were weaned at P21; males were separated from females and housed 2–4 in a cage.

5.2.4 Behaviour

USVs were measured in the rats" dark phase (18h00-23h00) and OFT and FST tests were conducted in the light phase (06h00-13h00). Adult male rats were transferred to a room adjacent to the behavioural room on the morning of behavioural testing and allowed to habituate for 1 h before behavioural testing.

a



b

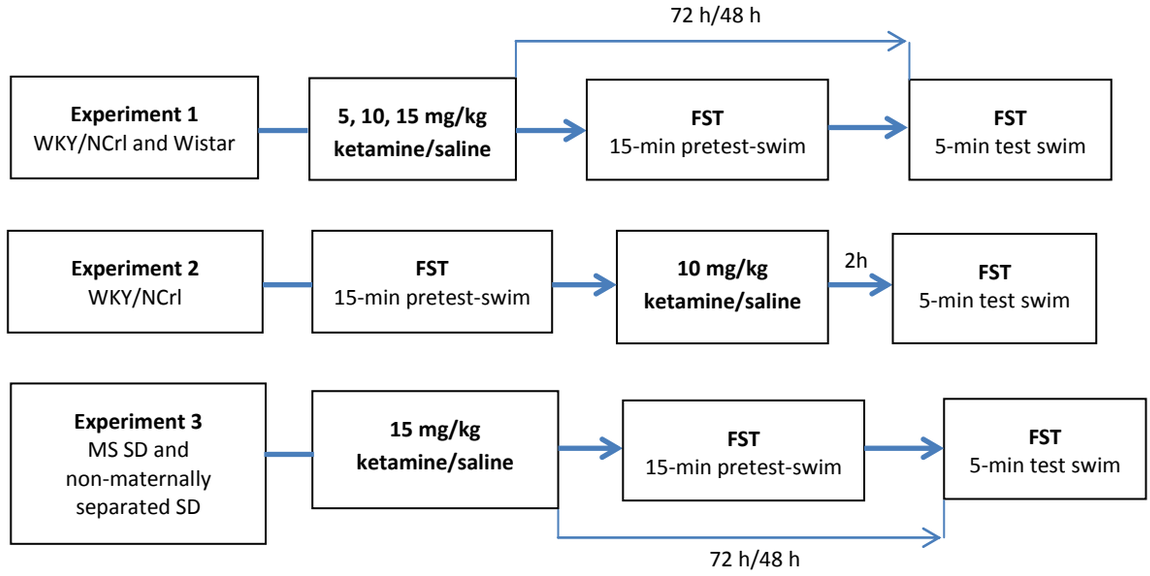


Figure 5.1: An illustration of the experimental protocol (a) (Experiment 1 and 3): Rats in behavioural groups A and B were tested on alternate days for USV, injected on different days (day 1 and day 2) and tested in the OFT and FST on the same day. The OFT and FST were therefore carried out at different time points after ketamine injection for group A (46 h for OFT and 72 h for test swim) and group B (22 h for OFT and 48 h for test swim). **(b)** (Experiment 1-3): Rats in experiment 1 (WKY/NCrl and Wistar) and 3 (MS SD and non-maternally separated SD) were treated with 5, 10 or 15 mg/kg ketamine/saline at 72 h/48 h before the 5-min test swim. WKY/NCrl rats in experiment 2 were treated with 10 mg/kg ketamine/saline 2 h before the 5-min test swim.

5.2.4.1 Open field test (experiments 1 and 3)

At P72 (22 h and 46 h after ketamine/saline injection), the OFT was carried out as described in section 2.2.2.2

5.2.4.2 Forced swim test (experiments 1-3)

At P72, 2 h after the OFT, the rats were placed in individual transparent cylinders (40×19 cm) containing 30 cm of water (23–25 °C). Each rat was allowed to swim for 15 min (habituation). After 24 h, the rats were exposed to a second 5-min swim session (48 h and 72 h after ketamine/saline injection for experiments 1 and 3 and 2 h after ketamine/saline injection for experiment 2) during which FST behaviour was recorded for later analysis as described in chapter 2. The total time spent immobile, swimming and struggling was tracked by Ethovision (automated scoring) and also measured manually for experiment 3. This was necessary to confirm that the FST results that were tracked by Ethovision were not influenced by the method of analysis (automated and manual scoring) since immobility for SD rats were previously manually scored (section 4.2.4.1).

5.2.4.3 Ultrasonic vocalizations (experiments 1 and 3)

Rats were housed 2–3 in their home cage (36×20×18 cm) in which USV‟s were recorded. For 2 days prior to baseline USV recording (P60 – P61), rats were transferred to the room in which the USVs were to be recorded, where they were handled briefly and then returned to the rat facility in order to habituate rats to the testing environment. One day after the last day of brief handling, baseline USVs were recorded for 8 days (P62 – P69) prior to treatment. Each rat received a single ketamine injection at P70 - P71 and USV‟s were recorded after 5 h and 29 h. Rats were left for a minimum of 1 h to habituate to the room conditions before USV recording. No other rats were present in the USV room during a USV recording session. Recording started immediately after the cage mate(s) had been removed from the home cage and continued for 6 min. The USVs of the cage mate (Group B; figure 5.1) were recorded the next evening so that USVs were recorded every alternate day for each rat. In cages with 3 rats, only 2 rats were used for USV recordings. The same recording equipment and analyses were used as described in section 3.2.3.4.

5.2.5 Statistical Analysis

The OFT and FST data were normally distributed (Shapiro-Wilk test) and were analyzed by means of analysis of variance (ANOVA), followed by Tukey‟s post-hoc test with

correction for multiple comparisons. A t-test was used to compare the behaviour of two groups of rats (group A and Group B) studied. USV data were not normally distributed and were analyzed with the Kruskal–Wallis ANOVA, followed by Dunn‟s post-hoc test with correction for multiple comparisons. The Friedman test for repeated measures was used to test for differences between USVs at different time points before and after treatment.. The OFT and FST data are presented as mean ± SEM and USV data are presented as median and interquartile range.

5.3 Results

5.3.1 Experiment 1: Sustained effects of acute ketamine on Wistar-Kyoto and Wistar rats

5.3.1.1 Open field test

Distance travelled

Two-way ANOVA (rat strain and drug as factors) showed a significant rat strain effect on total distance travelled in the open field at 22 h ($F_{(1, 39)} = 84.12$, $p < 0.001$) and 46 h ($F_{(1, 47)} = 40.64$, $p < 0.001$) after the ketamine/saline injection and no significant drug effect at 22 h and 46 h after ketamine/saline injection. Post-hoc analysis revealed that the WKY Sal rats travelled a shorter distance than Wistar Sal rats at 22 h (Fig. 5.2a) and 46 h (Fig. 5.2b) after the saline injection ($p < 0.001$).

Since there was no difference between treated rat groups at 22 h and 46 h, the distance travelled data for the two ketamine/saline-treated rat groups studied at 22 h (group B) and 46 h (group A) after ketamine/saline injection were combined (22 h + 46 h). Furthermore, these were different groups of rats at 22 h and 46 h and a t-test showed no significant difference in distance travelled by the two ketamine/saline-treated rat groups at these time points. Two-way ANOVA revealed a significant rat strain effect ($F_{(1, 94)} = 113.8$, $p < 0.001$) and no significant drug effect on distance travelled. Post-hoc analysis showed that the WKY Sal rats travelled a shorter distance than Wistar Sal rats (Fig. 5.2c, $p < 0.001$).

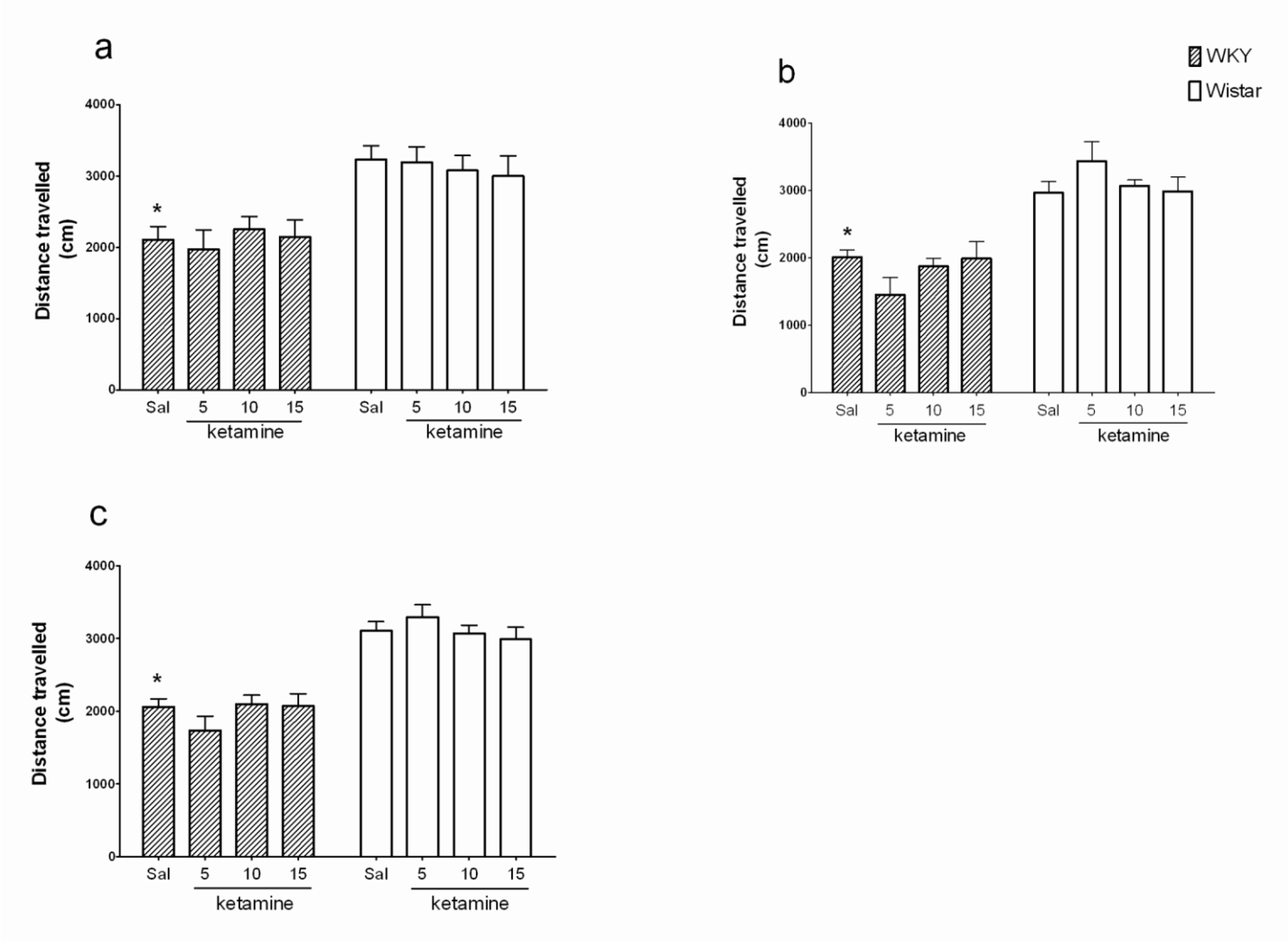


Figure 5.2: Distance travelled by WKY and Wistar rats in the OFT following treatment with saline/ketamine. Treatment with 5, 10 and 15 mg/kg ketamine had no effect on distance travelled by WKY and Wistar rats in the OFT at (a) 22 h (n = 5 – 9/group), (b) 46 h (n = 5 – 8/group) and (c) 22 h + 46 h (n = 10 – 17/group) after saline/ketamine injection. * WKY Sal significantly different from Wistar Sal, $p < 0.001$; Tukey's post-hoc test. Data presented as mean ± SEM.

5.3.1.2 Forced swim test

Immobility

Two-way ANOVA (rat strain and drug as factors) showed a significant rat strain effect on immobility in the FST at 48 h ($F_{(1,47)} = 239.7$, $p < 0.001$) and 72 h ($F_{(1,39)} = 122.60$, $p < 0.001$) after the ketamine/saline injection and no significant drug effect at 48 h and 72 h after the ketamine/saline injection. Post-hoc analysis showed that the WKY Sal rats spent

more time immobile than Wistar Sal rats at 48 h (Fig. 5.3a) and 72 h (Fig. 5.3b) after the saline injection ($p < 0.001$). WKY Ket-10 rats tended to spend more time immobile than WKY Sal rats at 48 h after the ketamine/saline injection ($p = 0.05$, Fig. 5.3a)

Since there was no difference between treated rat groups at 48 h and 72 h, the immobility data for ketamine/saline-treated rat groups at 48 h and 72 h after ketamine/saline injection were combined (48 h + 72 h). Furthermore, these were different groups of rats at 48 h (group B) and 72 h (groups A) and a t-test showed no significant difference in immobility by the two ketamine/saline-treated rat groups measured at these time points. Two-way ANOVA showed a significant rat strain effect ($F_{(1,94)} = 352.10$, $p < 0.001$) and drug effect ($F_{(3,94)} = 2.80$, $p < 0.05$) on immobility in the FST. Post-hoc analysis revealed that the WKY Sal rats spent more time immobile than Wistar Sal rats (Fig. 5.3c, $p < 0.001$).

One-way ANOVA of the combined 48 h and 72 h (Group A + Group B) data revealed a significant drug effect ($F_{(3,47)} = 3.28$, $p < 0.05$) on immobility in the FST. Post-hoc analysis revealed that the WKY Sal rats spent less time immobile than WKY Ket-10 rats ($p < 0.05$, Fig 5.3c).

Swimming

Two-way ANOVA (rat strain and drug as factors) revealed a significant rat strain effect on swimming behavior in the FST at 48 h ($F_{(1,47)} = 155.00$, $p < 0.001$) and 72 h ($F_{(1,39)} = 128.80$, $p < 0.001$) after the ketamine/saline injection, a rat strain × drug interaction ($F_{(3,47)} = 2.83$, $p < 0.05$) at 48 h after the ketamine/saline injection and no significant drug treatment effect at 48 h and 72 h after the ketamine/saline injection. Post-hoc analysis showed that the WKY Sal rats spent less time swimming than Wistar Sal rats at 48 h (Fig. 5.4a) and 72 h (Fig. 5.4b) after the saline injection ($p < 0.001$).

One-way ANOVA revealed a significant drug treatment effect ($F_{(3,24)} = 4.16$, $p < 0.05$) on swimming in the FST at 48 h after the ketamine/saline injection. Post-hoc analysis showed that the WKY Ket-10 rats spent less time swimming than WKY Sal rats at 48 h after the ketamine/saline injection ($p < 0.05$, Fig 5.4c).

Since there was no difference between treated rat groups at 48 h and 72 h, the swimming for ketamine/saline-treated rat groups at 48 h and 72 h after ketamine/saline injection were combined (48 h + 72 h). Furthermore, these were different groups of rats (Group A and Group B) and a t-test showed no significant difference in swimming behaviour at these two time points. Two-way ANOVA showed a significant rat strain effect ($F_{(1,94)} = 290.80$,

p < 0.001) and no significant drug effect. Post-hoc analysis revealed that the WKY Sal rats spent more time immobile than Wistar Sal rats (Fig. 5.4c, p < 0.001).

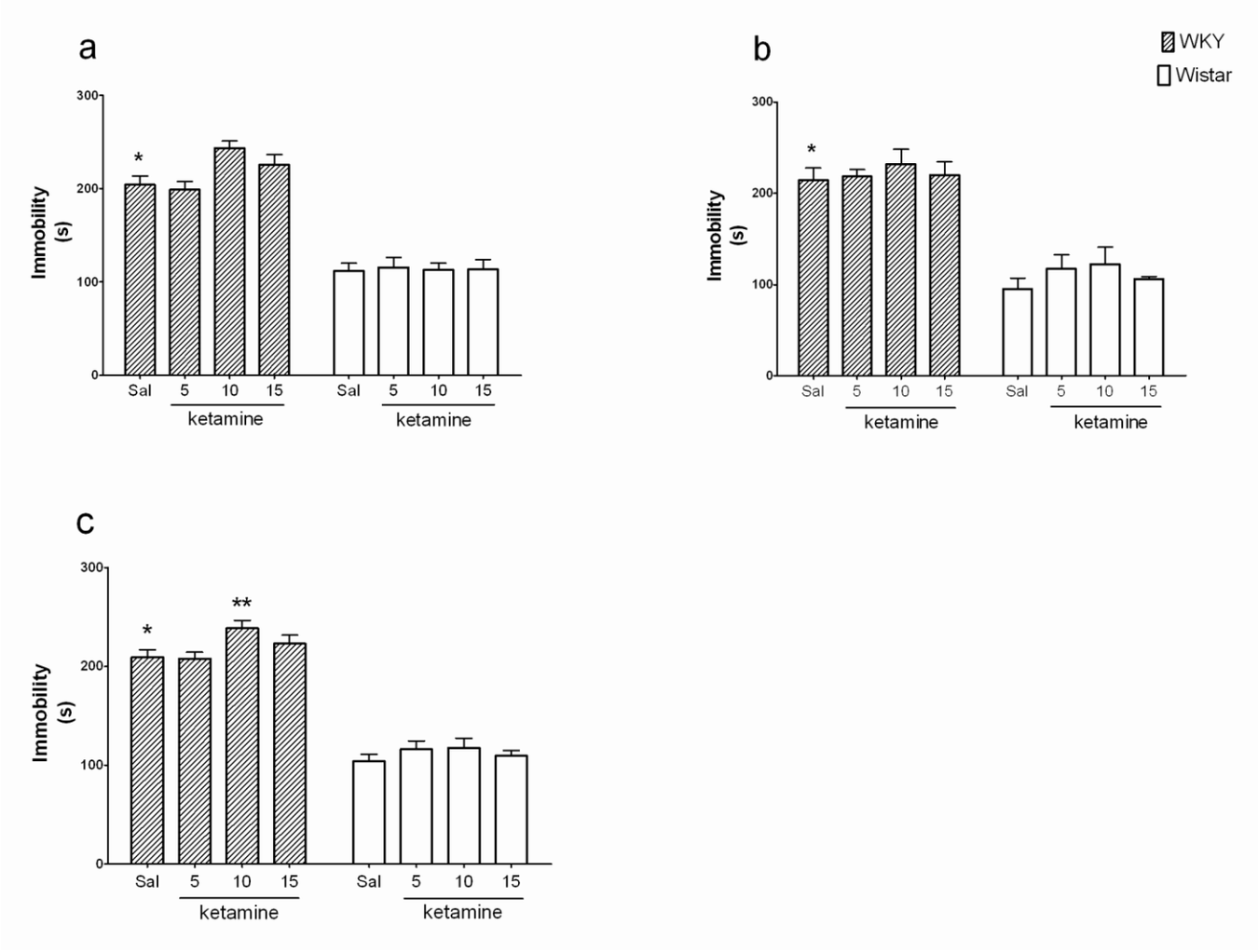


Figure 5.3: Immobility of WKY and Wistar rats in the FST following treatment with saline/ketamine. Treatment with 10 mg/kg ketamine increased immobility of the combined WKY groups at (c) 48 h + 72 h (n = 10 – 17/group) in WKY rats with no effect on Wistar rats. Treatment with 5, 10 and 15 mg/kg ketamine had no effect on immobility of WKY and Wistar rats in the FST at (a) 48 h (n = 5 – 9/group) and (b) 72 h (n = 5 – 8/group) after saline/ketamine injection. * WKY Sal significantly different from Wistar Sal, p < 0.001; ** WKY Sal significantly different from WKY Ket-10, p < 0.05; Tukey"s post-hoc test. Data presented as mean ± SEM.

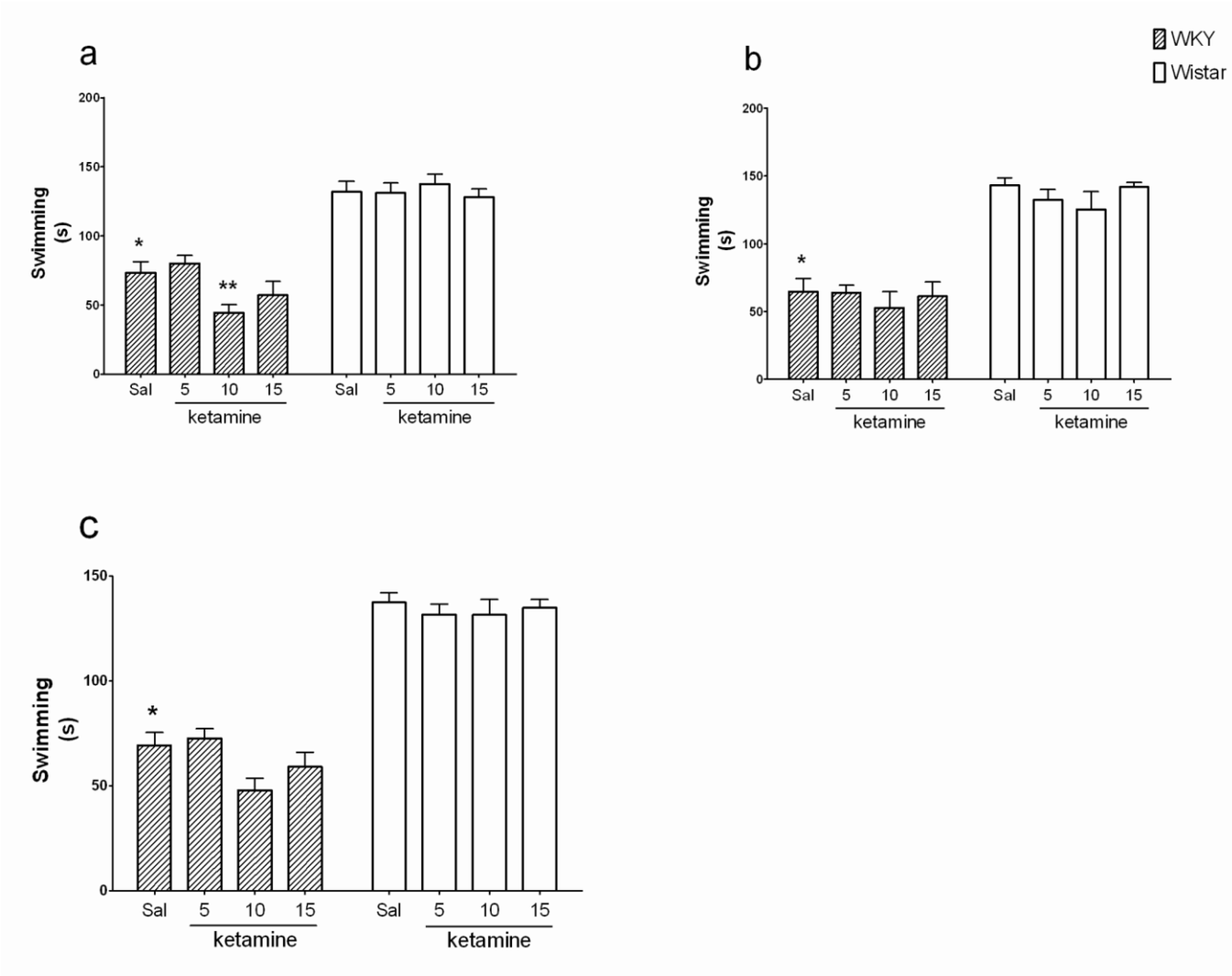


Figure 5.4: Swimming behaviour of WKY and Wistar rats in the FST following treatment with saline/ketamine. Treatment with 10 mg/kg ketamine decreased swimming after (a) 48 h (n = 5 – 9/group) in WKY rats without any drug effect in Wistar rats. Treatment with 5, 10 and 15 mg/kg ketamine had no effect on swimming after (b) 72h (n = 5 – 8/group) and (c) at the combined time points (48 h + 72 h) (n = 10 – 17/group) in WKY and Wistar rats. * WKY Sal significantly different from Wistar Sal, $p < 0.001$; ** WKY Sal significantly different from WKY Ket-10, $p < 0.05$; Tukey‟s post-hoc test. Data presented as mean ± SEM.

Struggling

Two-way ANOVA (rat strain and drug as factors) revealed a significant rat strain effect on struggling behaviour in the FST at 48 h ($F_{(1,47)} = 204.30$, $p < 0.001$) and 72 h ($F_{(1,39)} = 61.53$, $p < 0.001$) after the ketamine/saline injection and no significant drug treatment effect at 48 h and 72 h after the ketamine/saline injection. Post-hoc analysis showed that the WKY Sal rats spent less time struggling than Wistar Sal rats at 48 h (Fig. 5.5a) and 72 h (Fig. 5.5b) after the saline injection ($p < 0.001$).

Since there was no difference between treated rat groups at 48 h and 72 h, the struggling for ketamine/saline-treated rat groups at 48 h and 72 h after ketamine/saline injection were combined (48 h + 72 h). Furthermore, these were different groups of rats (Group A and Group B) and a t-test showed no significant difference in struggling behaviour of the two groups of rats at these two time points. Two-way ANOVA showed a significant rat strain effect ($F_{(1,94)} = 290.80$, $p < 0.001$) and no significant drug effect. Post-hoc analysis revealed that the WKY Sal rats spent less time struggling than Wistar Sal rats (Fig. 5.5c, $p < 0.001$).

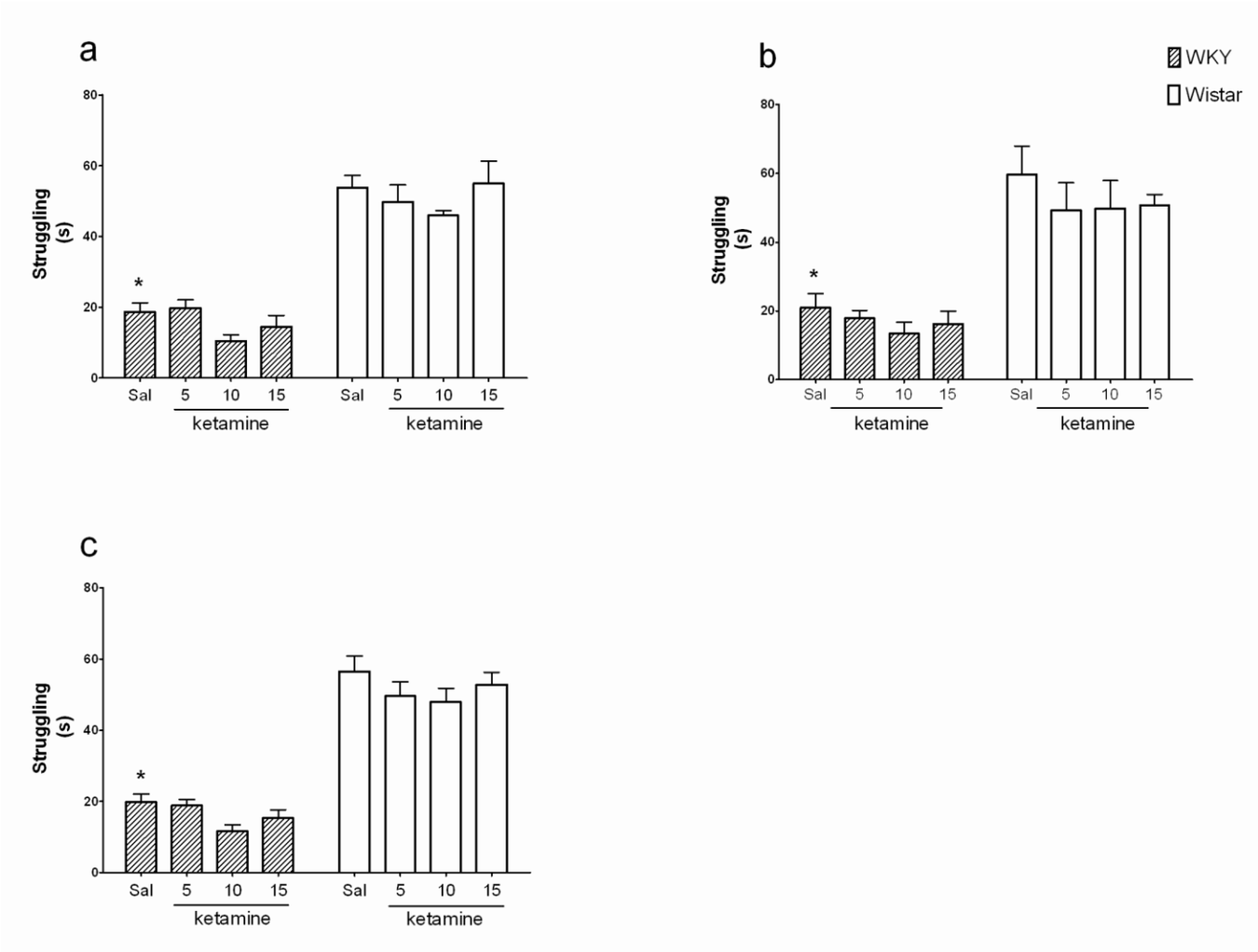


Figure 5.5: Struggling behaviour of WKY and Wistar rats in the FST following treatment with saline/ketamine. Treatment with 5, 10 and 15 mg/kg ketamine had no effect on struggling behaviour at (a) 48 h (n = 5 – 9/group), (b) 72 h (n = 5 – 8/group) and (c) at the combined time points (48 h + 72 h) (n = 10 – 17/group) in WKY and Wistar rats. * WKY Sal significantly different from Wistar Sal, $p < 0.001$; Tukey's post-hoc test. Data presented as mean ± SEM.

5.3.1.3 Ultrasonic vocalizations

Frequency modulated calls

The Kruskal-Wallis ANOVA revealed a significant difference between rat groups in FM calls on day -4 ($H_{(7,N = 94)} = 23.11$, $p < 0.01$) before the ketamine/saline injection. Post-hoc analysis showed that WKY Sal rats vocalized less than Wistar Sal rats on day -4 before treatment ($p < 0.05$, Fig. 5.6a).

Furthermore, the Friedman test for repeated measures revealed a significant difference in FM calls at different time points before and after treatment ($Q = 17.86$, $p < 0.01$). Post-hoc analysis showed that WKY rats on day -4 and day -3 before treatment vocalized less than at 29 h after ketamine/saline treatment ($p < 0.01$ and $p < 0.05$ respectively, Fig. 5.6).

Flat calls

The Kruskal-Wallis ANOVA showed a significant difference between rat groups in flat calls on day -4 ($H_{(7,N = 94)} = 22.43$, $p < 0.01$) before ketamine/saline injection. Post-hoc analysis showed that WKY Sal rats vocalized less than Wistar Sal rats ($p < 0.05$, Fig. 5.7a).

The Friedman test for repeated measures revealed a significant difference in flat calls at different time points and after treatment ($Q = 17.71$, $p < 0.01$). Post-hoc analysis showed that WKY rats on day -4 and day -3 before treatment vocalized less than at 29 h after ketamine/saline treatment ($p < 0.01$ and $p < 0.05$ respectively, Fig. 5.7).

Total number of calls

The Kruskal-Wallis ANOVA revealed a significant difference between rat groups in total number of calls (FM + flat calls) on day -4 ($H_{(7,N = 94)} = 24.03$, $p < 0.01$) before ketamine/saline injection. Post-hoc analysis showed that WKY Sal rats vocalized less than Wistar Sal rats ($p < 0.05$, Fig. 5.8a).

The Friedman test for repeated measures revealed a significant difference in total number of calls at different time points before and after treatment ($Q = 18.14$, $p < 0.01$). Post-hoc analysis revealed that WKY rats on day -4 and day -3 before treatment vocalized less than at 29 h after ketamine/saline treatment ($p < 0.01$ and $p < 0.05$ respectively, Fig. 5.8).

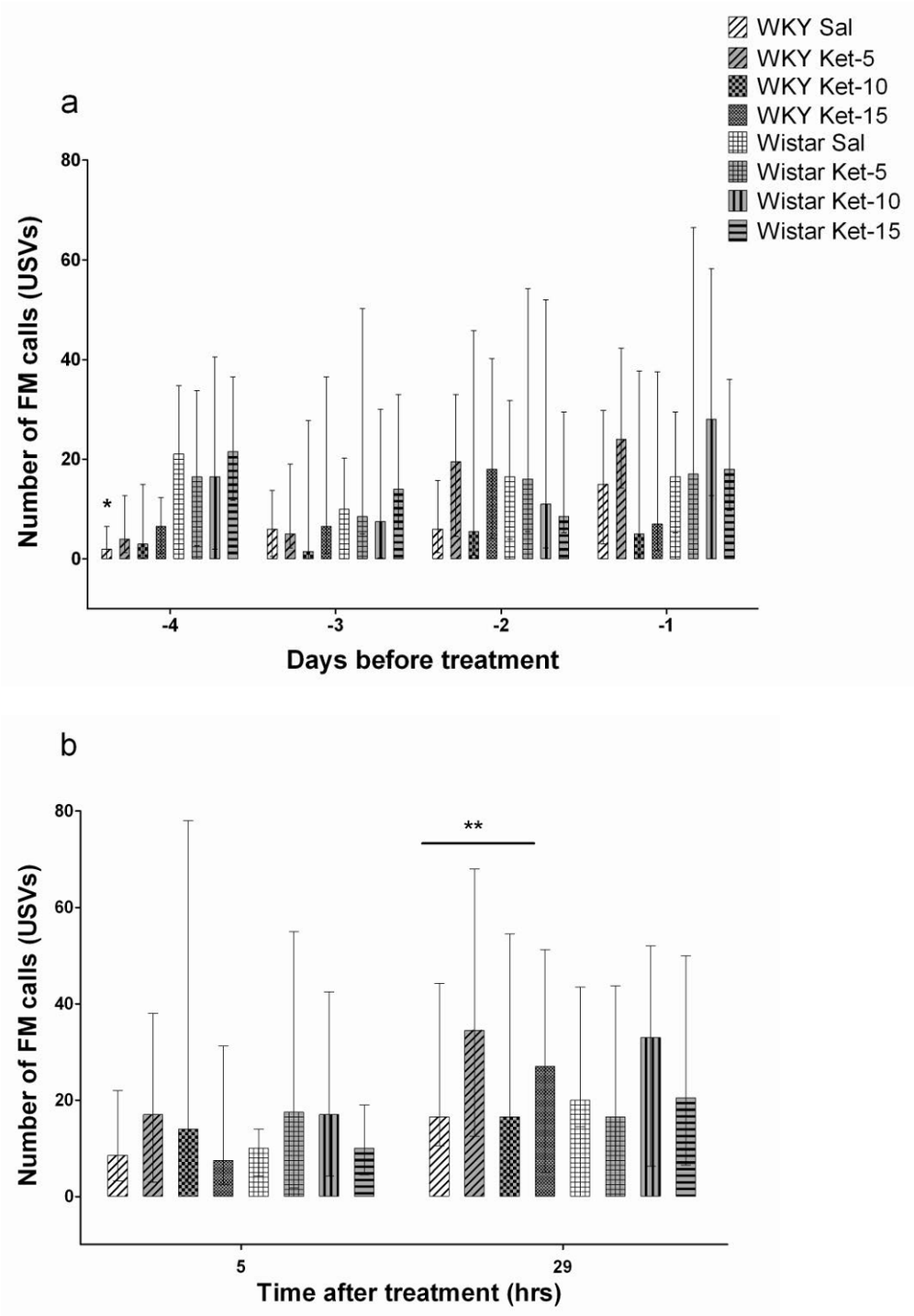


Figure 5.6: Number of FM USVs of WKY and Wistar rats treated with saline/ketamine. (a) WKY Sal vocalized less than Wistar Sal rats on day -4 and WKY rats before treatment on day -4 and day -3 vocalized significantly less than (b) WKY at 29 h after ketamine/saline injection. Ketamine treatment had no significant effect on the number of FM calls made by WKY and Wistar rats. * WKY Sal significantly different from Wistar Sal on day -4 before treatment, $p < 0.05$, ** WKY at 29 h after treatment significantly different from WKY on day -4 and day -3 before treatment; Dunn‟s multiple comparisons post-hoc test (n = 10 – 16/group). Data presented as median and interquartile range.

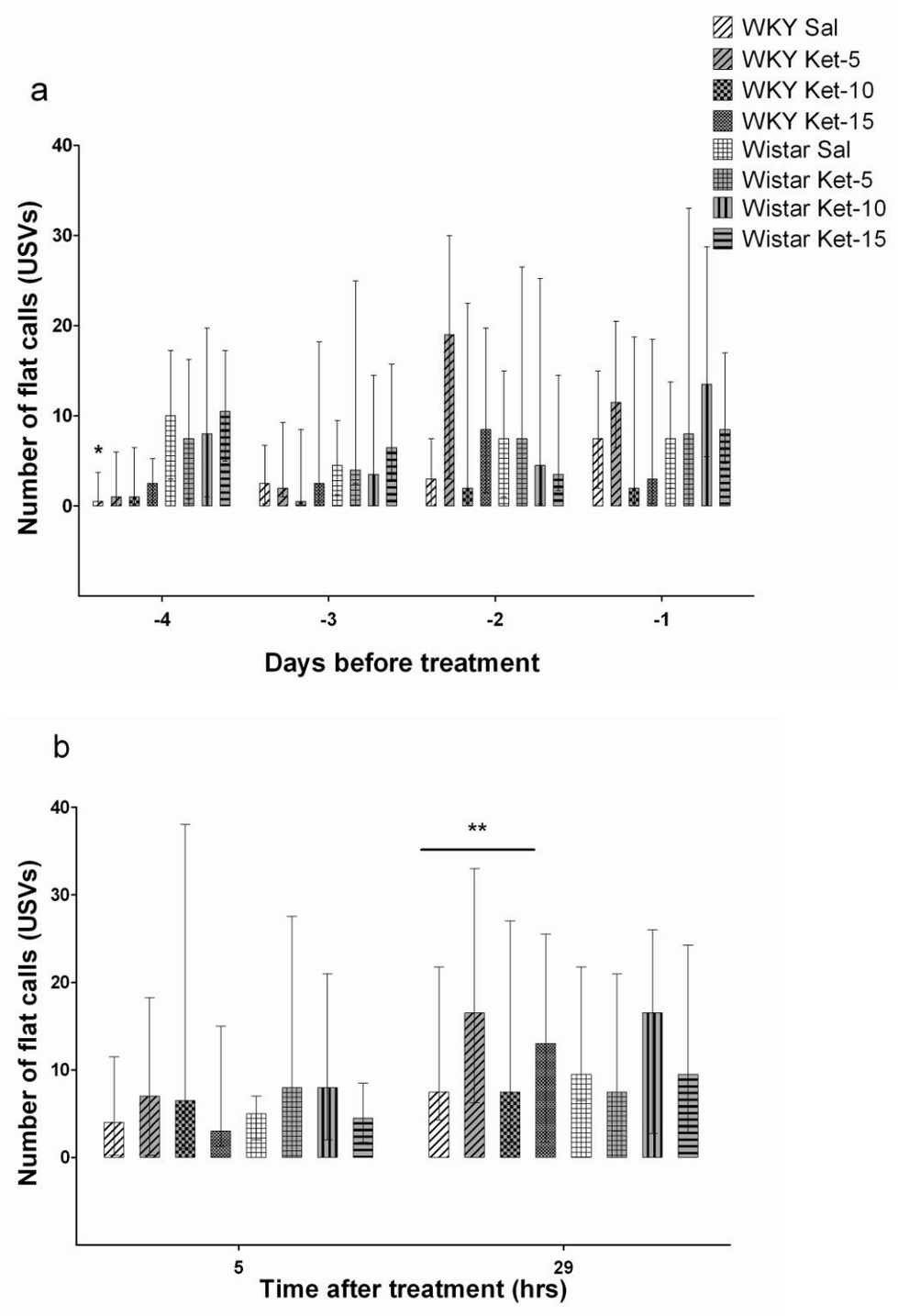


Figure 5.7: Number of flat USVs of WKY and Wistar rats treated with saline/ketamine. (a) WKY Sal vocalized less than Wistar Sal rats on day -4 and WKY rats before treatment on day -4 and day -3 vocalized less than (b) WKY at 29 h after ketamine/saline injection. Ketamine treatment had no significant effect on the number of flat calls made by WKY and Wistar rats. * WKY Sal significantly different from Wistar Sal on day -4 before treatment, $p < 0.05$, ** WKY at 29 h after treatment significantly different from WKY on day -4 and day -3 before treatment; Dunn‟s multiple comparisons post-hoc test (n = 10 – 16/group). Data presented as median and interquartile range.

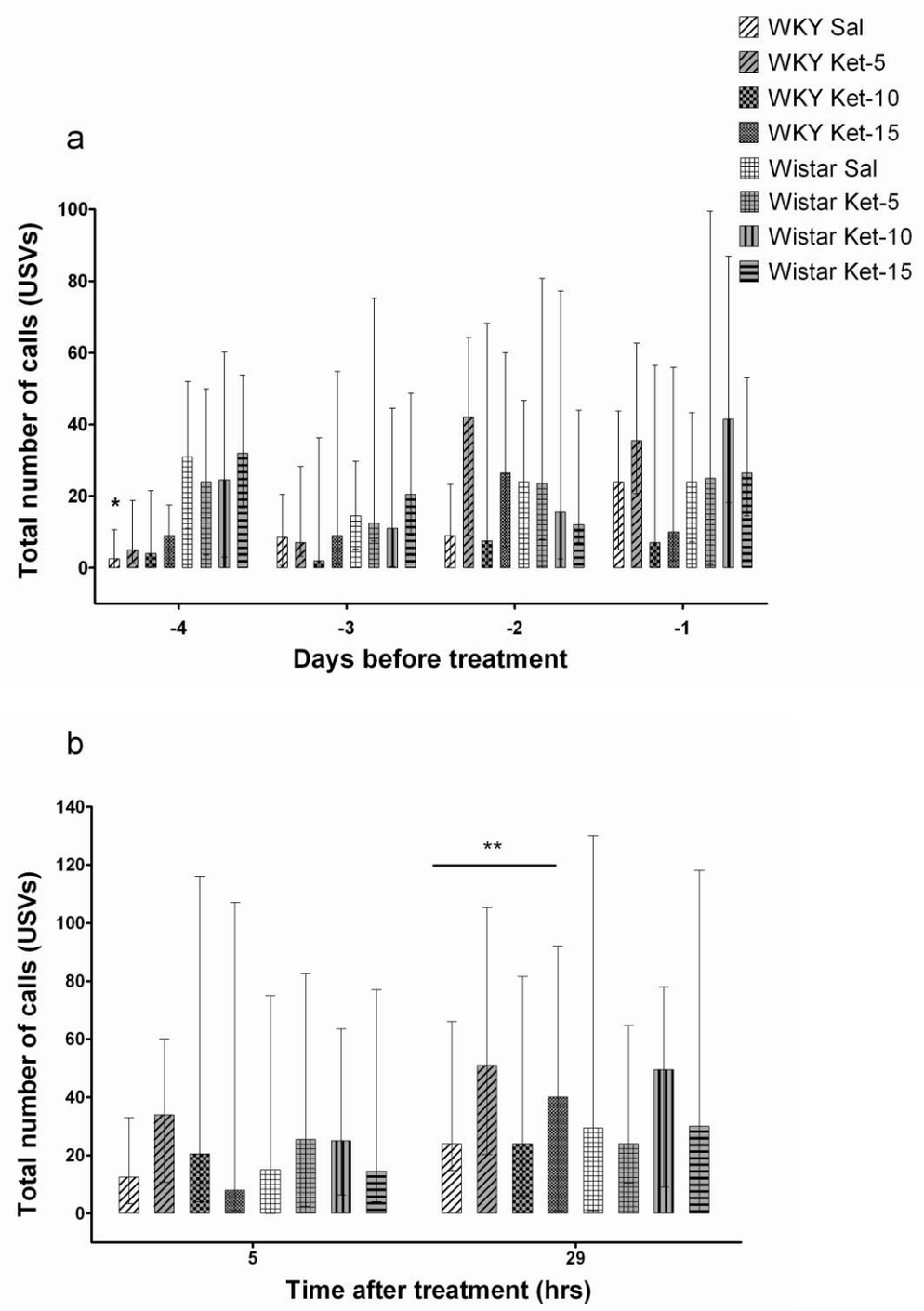


Figure 5.8: Total number of USVs of WKY and Wistar rats treated with saline/ketamine. (a) WKY Sal vocalized significantly less than Wistar Sal rats on day -4 and WKY rats before treatment on day -4 and day -3 vocalized significantly less than (b) WKY at 29 h after ketamine/saline injection. Ketamine treatment had no significant effect on the total number of calls made by WKY and Wistar rats. * WKY Sal significantly different from Wistar Sal on day -4 before treatment, $p < 0.05$, ** WKY at 29 h after treatment significantly different from WKY on day -4 and day -3 before treatment; Dunn‟s multiple comparisons post-hoc test (n = 10 – 16/group). Data presented as median and interquartile range.

5.3.2 Experiment 2: Rapid effect of acute ketamine in Wistar-Kyoto rats

5.3.2.1 Forced swim test

Immobility

Unpaired t-test showed no significant difference between WKY Sal and WKY Ket-10 rats in immobility in the FST (Fig. 5.9) 2 h after the ketamine/saline injection.

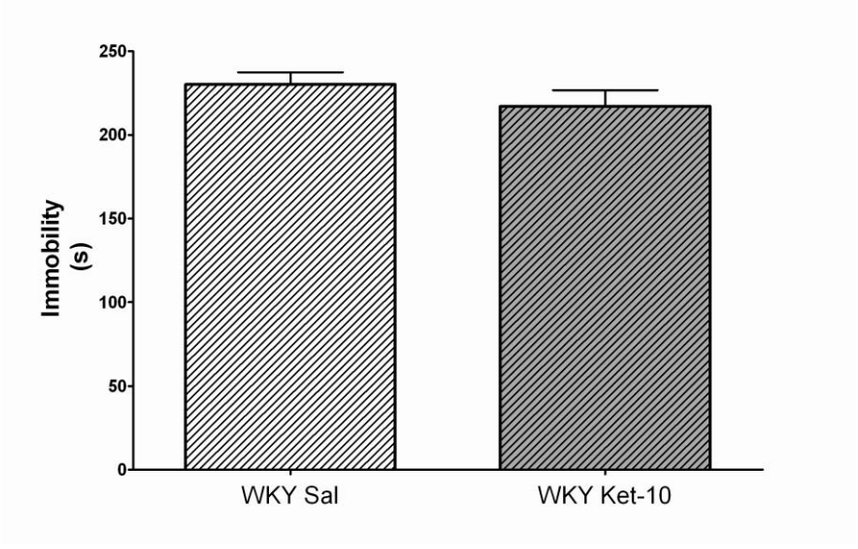


Figure 5.9: Immobility of WKY rats in the FST following treatment with saline/ketamine. Treatment with 10 mg/kg ketamine had no effect on immobility of WKY in the FST at 2 h after the ketamine/saline injection (WKY Sal, n = 13 and WKY Ket-10, n = 11). Data presented as mean ± SEM.

5.3.3 Experiment 3: Sustained effects of acute ketamine in non-maternally separated and maternally separated Sprague-Dawley rats

5.3.3.1 Open field test

Distance travelled

Two-way ANOVA (MS and drug as factors) showed no significant effect of MS or drug on the total distance travelled by nMS SD and MS SD rats in the open field at 22 h (Fig. 5.10a) and 46 h (Fig. 5.10b) after the ketamine/saline injection.

Since there was no difference between treated rat groups at 22 h and 46 h, the distance travelled data for ketamine/saline-treated rat groups at 22 h and 46 h after ketamine/saline

injection were combined (22 h + 46 h). Furthermore, a t-test showed no significant difference in distance travelled by these two groups of rats at the time points tested (22 h and 46 h) after the ketamine/saline injection. Two-way ANOVA revealed no significant MS or drug effect on distance travelled in the OFT (Fig. 5.10c).

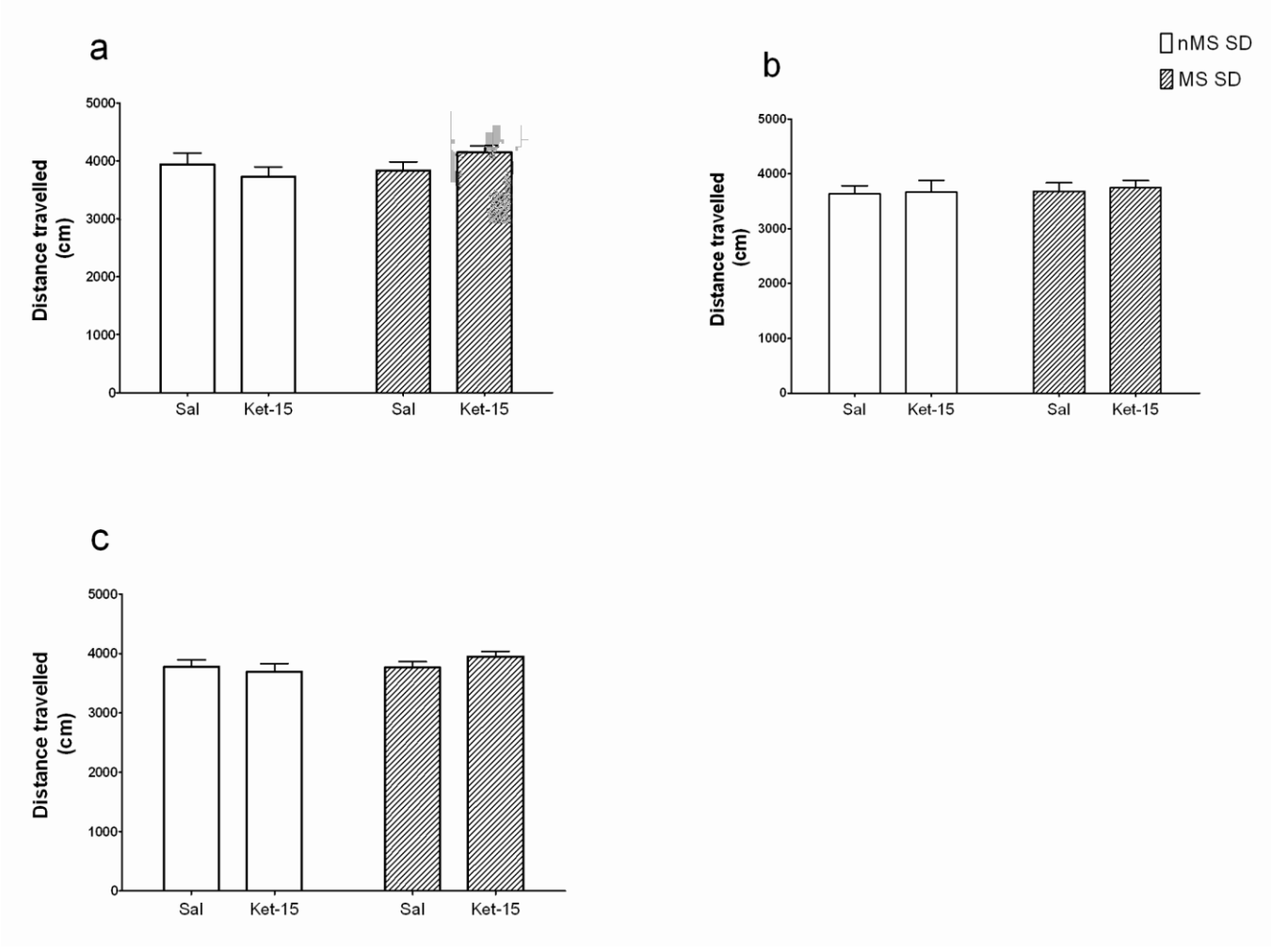


Figure 5.10: Distance travelled by non-maternally separated and MS SD rats in the FST following treatment with saline/ketamine. Treatment with 15 mg/kg ketamine had no effect on distance travelled at (a) 22 h (n = 15 – 16/group), (b) 46 h (n = 15 - 17/group) and (c) at the combined time points (22 h + 46 h) (n = 30 – 32/group) in nMS SD and MS SD rats. Data presented as mean ± SEM.

5.3.3.2 Forced swim test

Immobility

Two-way ANOVA (MS and drug as factors) showed no significant MS or drug effect on immobility in the FST at 48 h (analyzed by automated scoring, Fig. 5.11a and manual

scoring, Fig. 5.12a) and 72 h (analyzed by automated scoring, Fig. 5.11b and manual scoring, Fig. 5.12b) after the ketamine/saline injection.

Since there was no difference between treated rat groups at 48 h and 72 h, the immobility data for ketamine/saline-treated rat groups were also combined (48 h + 72 h). Furthermore a t-test showed no significant difference in immobility by the two treated rat groups at 48 h and 72 h after the ketamine/saline injection. Two-way ANOVA showed no significant MS or drug treatment effect in the combined data obtained at 48 h + 72 h after ketamine/saline injection for automated scoring (Fig. 5.11c) and manual scoring (Fig. 5.12c).

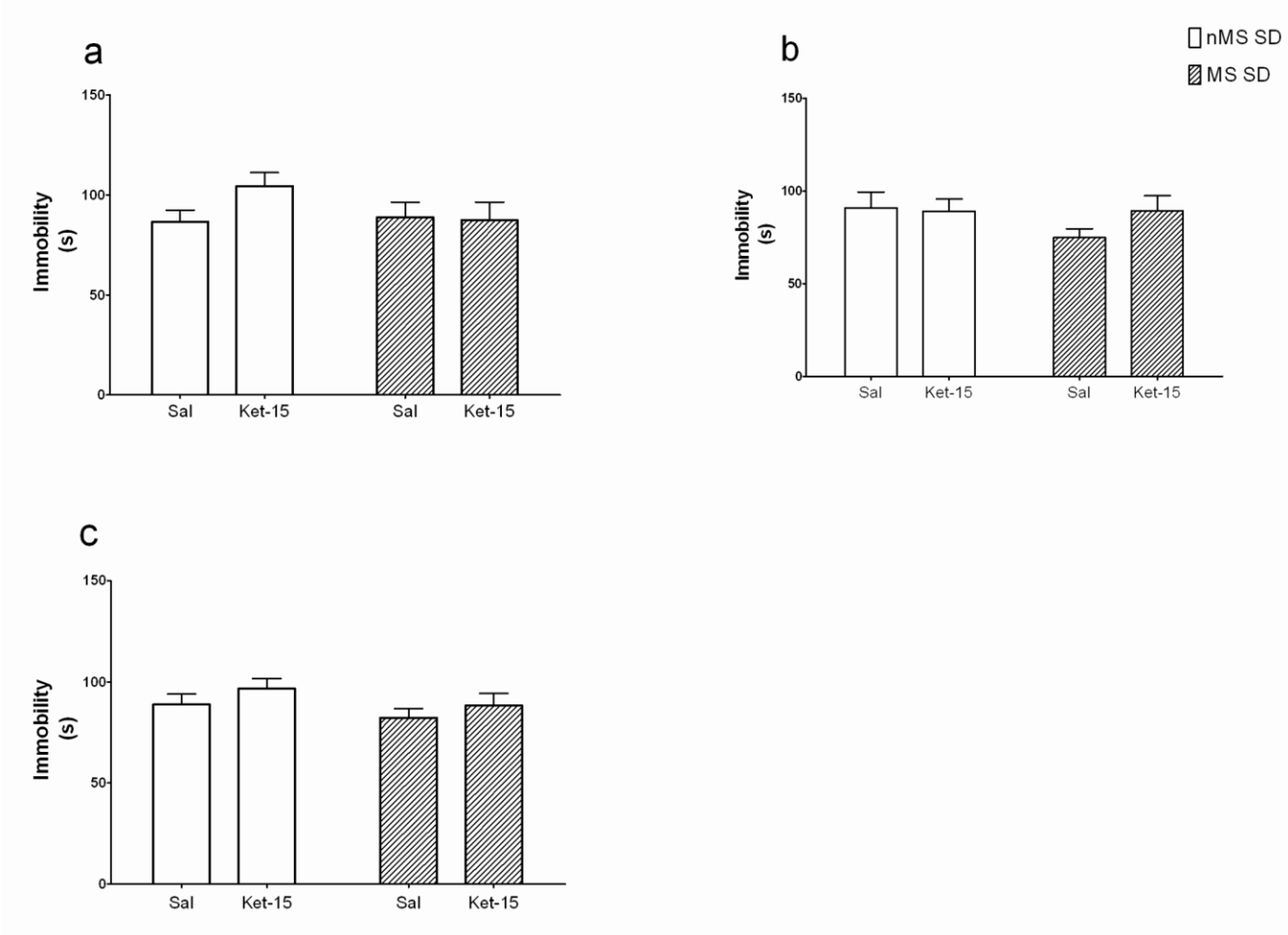


Figure 5.11: Immobility (automated scoring) of non-maternally separated and MS SD rats in the FST following treatment with saline/ketamine. Immobility in the FST obtained with ethovision (automated scoring). Treatment with 15 mg/kg ketamine showed no effect on immobility after (a) 48 h (n = 15 – 16/group) and (b) 72 h (n = 15 – 17/group) and (c) at the combined time points (48 h + 72 h) (n = 30 – 32/group) in nMS SD and MS SD rats. Data presented as mean ± SEM.

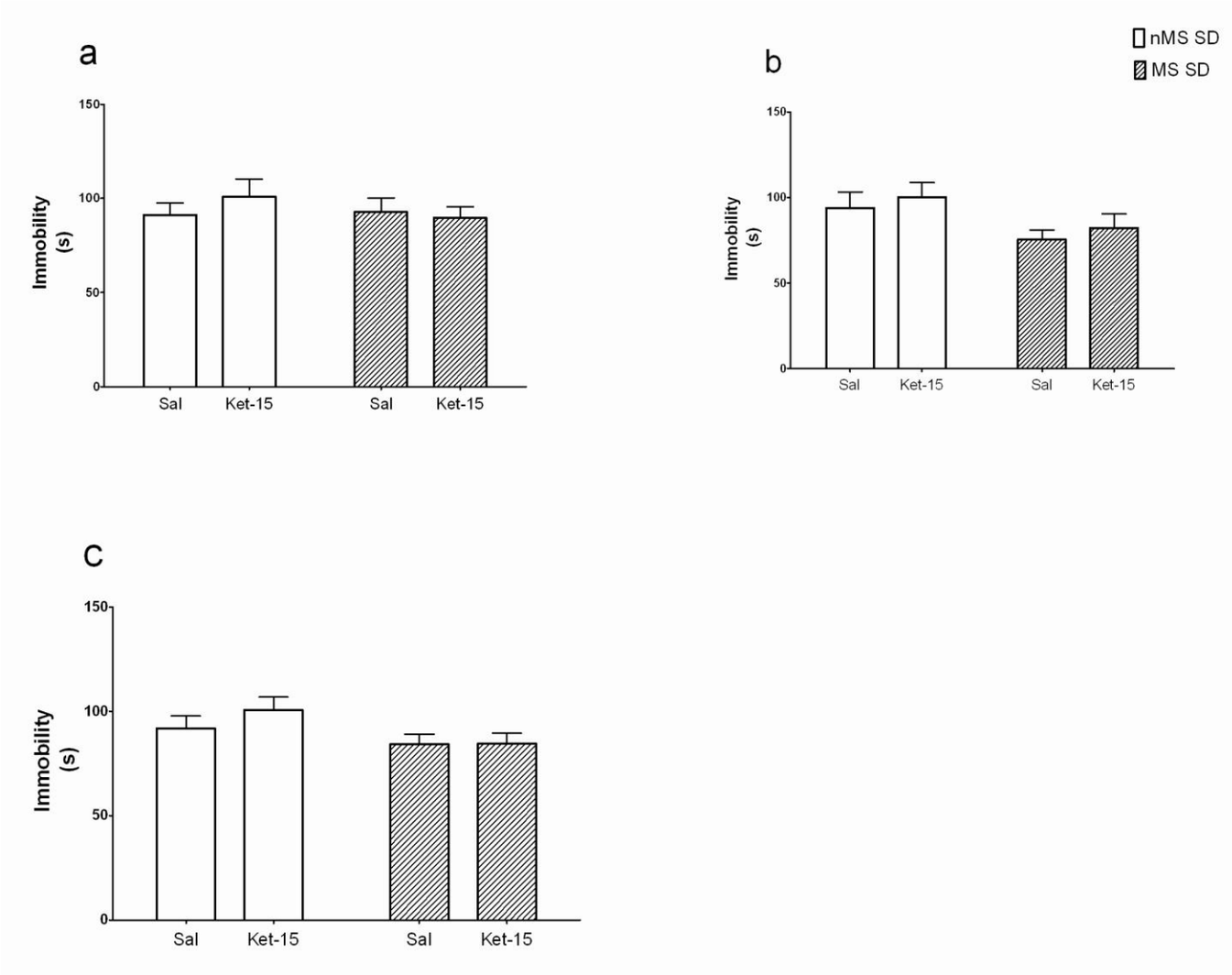


Figure 5.12: Immobility (manual scoring) of non-maternally separated and MS SD rats in the FST following treatment with saline/ketamine. Immobility in the FST obtained with manual scoring. Treatment with 15 mg/kg ketamine showed no effect on immobility after (a) 48 h (n = 15 – 16/group) and (b) 72 h (n = 15 – 17/group) and (c) at the combined time points (48 h + 72 h) (n = 30 – 32/group) in nMS SD and MS SD rats. Data presented as mean ± SEM.

5.3.3.3 Ultrasonic vocalizations

The Kruskal–Wallis ANOVA revealed no significant difference between ketamine/saline-treated rat groups in isolation-induced FM calls (Fig. 5.13), flat calls (Fig. 5.14) and total number of calls (Fig. 5.15) at any of the times before or after the ketamine/saline injection.

The Friedman test for repeated measures revealed no significant difference in FM calls, flat calls and total number of calls between nMS SD or MS SD rat groups at different time points before and after ketamine/saline injection.

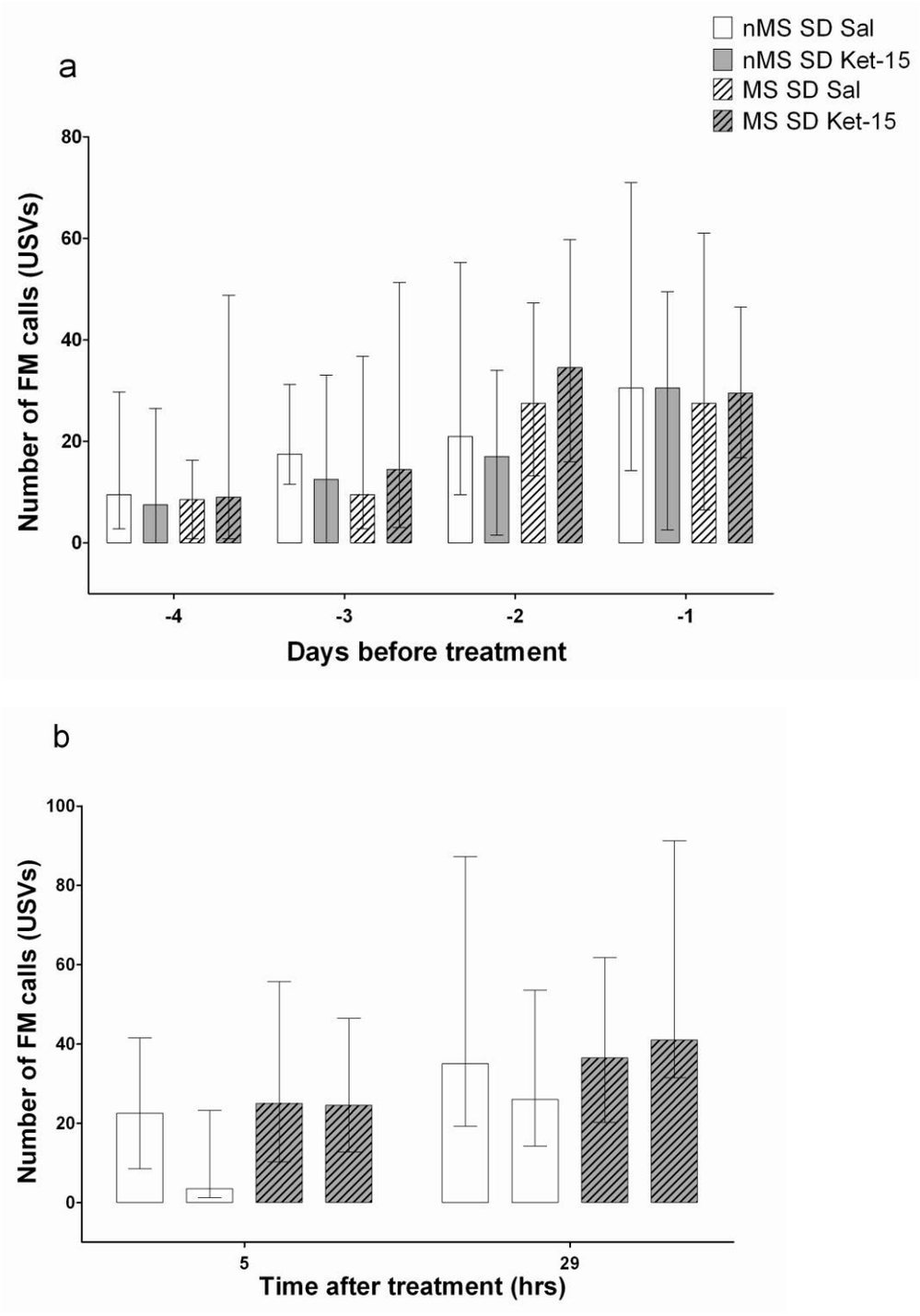


Figure 5.13: Number of FM USVs of non-maternally separated and MS SD rats treated with saline/ketamine. (a) No significant difference between ketamine/saline-treated rat groups with FM calls found before treatment. (b) Ketamine treatment had no significant effect on FM calls compared to saline treated nMS SD and MS SD rats (n = 10 – 14/group). Data presented as median and interquartile range.

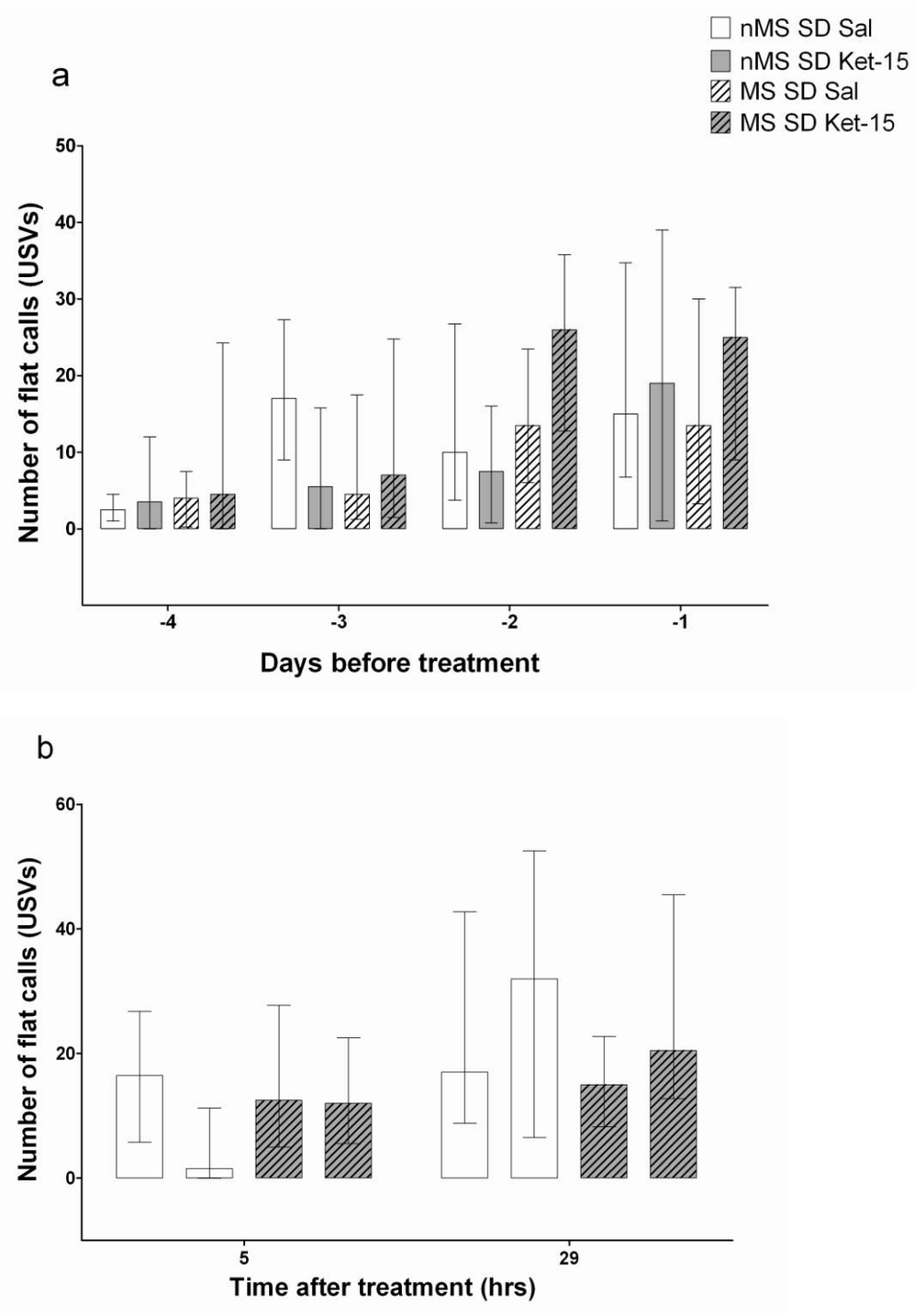


Figure 5.14: Number of flat USVs of non-maternally separated and MS SD rats treated with saline/ketamine. (a) No significant difference between ketamine/saline-treated rat groups with flat calls found before treatment. (b) Ketamine treatment had no significant effect on flat calls compared to saline treated nMS SD and MS SD rats (n = 10 – 14/group). Data presented as median and interquartile range.

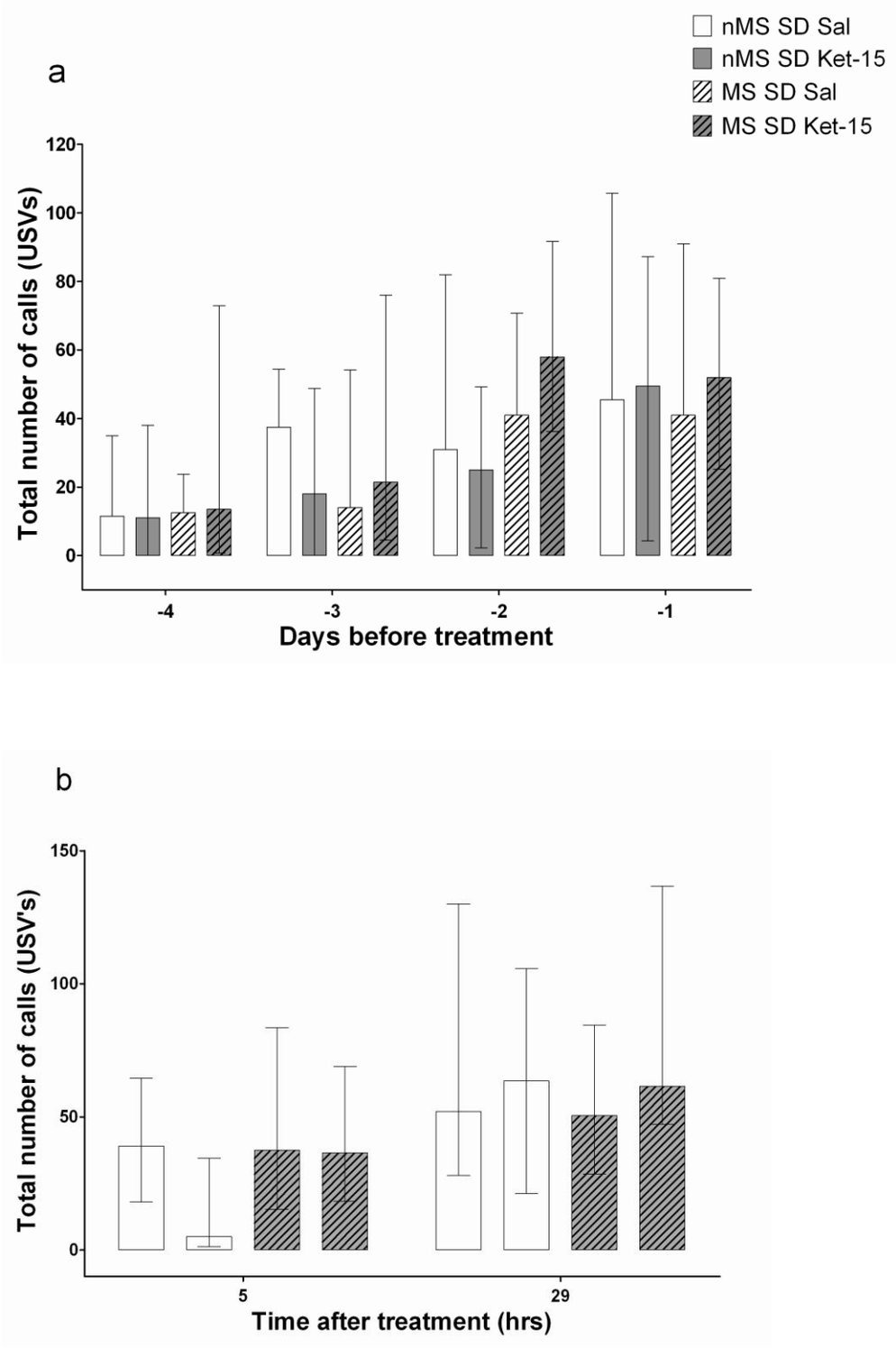


Figure 5.15: Total number of USVs of non-maternally separated and MS SD rats treated with saline/ketamine. (a) No significant differences between ketamine/saline-treated rat groups found with total number of calls (FM + flat calls) before treatment. (b) Ketamine treatment had no significant effect on total number of calls compared to saline treated nMS SD and MS SD rats (n = 10 – 14/group). Data presented as median and interquartile range.

5.4 Discussion

The primary findings on the effects of ketamine in the WKY and MS SD rat models of depression showed (1) enhanced depression-like behaviour in WKY at 48 h and 72 h after ketamine injection and no effect on Wistar or MS SD rats and (2) no change in isolation-induced USVs in WKY and MS SD rats.

The WKY rats travelled a shorter distance than Wistar rats in the OFT thereby confirming previous results in chapter 2 and 3 showing reduced activity of WKY rats in the OFT. However, the rats remained unaffected by acute treatment of ketamine. It was previously shown that ketamine produced hyperactivity immediately following acute treatment in rodents (Chatterjee et al. 2011; Hou et al. 2013; Imre et al. 2006). When rats were tested in the light phase, acute treatment with ketamine (16 mg/kg) significantly increased locomotor activity that lasted for 20 min and returned to baseline after 40 min (Imre et al. 2006). Also in mice, acute treatment with ketamine increased locomotor activity after 10 min at low subanaesthetic doses (5 – 25 mg/kg) (da Silva et al. 2010; Hou et al. 2013). However, most studies have reported no long-term effect of acute ketamine treatment on locomotor activity. These studies showed that a single injection of ketamine (0.25 – 15 mg/kg) had no effect on locomotor activity beyond 30 min after injection in rats (Akinfiresoye and Tizabi 2013; Garcia et al. 2008; Réus et al. 2011; Tizabi et al. 2012) or mice (Koike et al. 2011). The current study is therefore in agreement with most studies showing that a low subanaesthetic dose of ketamine had no effect on activity in the OFT in rats. However, taken together, these data highlight the importance of measuring the activity of rats following ketamine treatment in order to eliminate any possible false-positives in the behavioural response to ketamine. The effect of ketamine on immobility in the FST in WKY rats was therefore not a result of altered locomotor function.

The present study confirmed the depression-like behaviour of WKY rats in the FST as described in chapter 2 and 3. This was evidenced by increased immobility and decreased active swimming and struggling behaviours in the FST of WKY compared to Wistar rats. Furthermore, ketamine increased immobility and decreased active swimming behaviour of WKY rats in the FST without affecting Wistar rats. The results with ketamine treatment are in contrast to numerous studies which showed that acute treatment with ketamine at doses similar to those used in the present study produced rapid and sustained antidepressant-like effects in rodents (Autry et al. 2011; Carrier and Kabbaj 2013; Garcia et al. 2008; Li et al. 2010; Maeng et al. 2008; Tizabi et al. 2012). This was evidenced by decreased immobility in the FST at doses of 10 mg/kg and 15 mg/kg of ketamine at 1 h or 24 h after injection in Wistar and SD rats (Garcia et al. 2008; Li et al. 2010). The timing

between the drug administration and test swim may be important for the response of ketamine in the FST as previously suggested (Gigliucci et al. 2013). In these previous studies of SD and Wistar rats, ketamine was injected 1 h or 24 h before the test swim whereas in the current study ketamine was administered 48 h and 72 h before the 15-min pretest-swim. However, acute treatment with ketamine 2 h before the test swim in experiment 2 was also unable to reduce the immobility in the WKY rats although the rats used in these previous studies (Garcia et al. 2008; Li et al. 2010) were different from the current study. Indeed, the rat strain and dose of ketamine may also be an important consideration in the behavioural response to ketamine. At a lower dose of 5 mg/kg, a previous study by Tizabi et al. (2012) showed an immediate and sustained antidepressant effect of ketamine in female WKY rats as evidenced by decreased immobility in the FST at a dose that had no effect in Wistar rats. The decreased duration of immobility in the FST at a dose of 5 mg/kg lasted up to 1 week after injection of ketamine in female WKY rats (Tizabi et al. 2012). However, the current study was unable to show sustained antidepressant effects with a low dose of 5 mg/kg ketamine in male WKY rats in the FST. However, in male WKY rats, chronic treatment with ketamine for 10 days at a lower dose of 0.5 mg/kg reduced the immobility at 20 - 22 h after treatment (Akinfiresoye and Tizabi 2013). Therefore, the higher doses in the current study compared to the study by Akinfiresoye and Tizabi (2013) could have contributed in the lack of an antidepressant effect in the FST. Furthermore, the study by Tizabi et al. (2012) and Akinfiresoye and Tizabi (2013) followed a different methodology. For example, the behavioural experiments were performed in the rats‟ dark phase when they were more active. It was previously shown that rats displayed higher levels of immobility in the FST during their dark phase (Kelliher et al. 2000) which would allow for easier determination of reduction in immobility. Furthermore, the FST was performed without a pretest-swim session which could account for differences in antidepressant response (Borsini et al. 1989). Also, the acute ketamine study by Tizabi et al. (2012) was performed in female WKY rats. It has been previously shown that female rats were more sensitive to acute treatment with ketamine than male rats (Carrier and Kabbaj 2013). They showed that acute ketamine treatment at a low dose of 2.5 mg/kg reduced the duration of immobility in the FST in female SD rats but was found to be ineffective in male SD rats (Carrier and Kabbaj 2013). Therefore, the lack of ketamine response in male WKY rats in the current study may be further explained by the difference in the acute antidepressant-like response in the FST between male and female rats.

At high doses of 30 mg/kg – 100 mg/kg, sub-chronic treatment with ketamine for 5 days or more, increased duration of immobility in the FST in naïve rodents (Chatterjee et al.

2011; Chindo et al. 2012). In Wistar rats, sub-chronic treatment with ketamine for 5 days at a dose of 30 mg/kg or 50 mg/kg enhanced duration of immobility in the FST at 24 h after treatment (Chindo et al. 2012). In agreement with Chindo et al. (2012), acute treatment with ketamine in the current study had no effect on immobility in the FST in Wistar rats. However, the WKY rats in the current study appeared to be more sensitive to higher doses of ketamine-induced immobility in the FST. The enhanced immobility induced by ketamine may possibly be related to the neurochemical disturbances in the WKY rats. Previous studies showed that male WKY rats have reduced NMDA receptor function as measured by lower density of NMDA receptors in the substantia nigra, striatum, NAc, hippocampus, anterior cingulate cortex and PFC compared to Wistar rats (Lei and Tejani-Butt 2010; Lei et al. 2009). However, no difference in NMDA receptor density in the hippocampus was found in female WKY compared to Wistar rats (Tizabi et al. 2012). Furthermore, ketamine is a non-competitive antagonist of the NMDA receptor (Harrison and Simmonds 1985) and has been shown to regulate glutamate, dopamine and serotonin release as measured by microdialysis (Lindefors et al. 1997; Moghaddam et al. 1997; Westerink et al. 1998). Therefore, dysfunction of this receptor may further alter neurotransmission. It is possible that together with the reduced NMDA receptor function in the various brain areas in WKY rats, ketamine may have further blocked NMDA receptor function and therefore enhanced the duration of immobility as previously found with a chronic high dose of ketamine in naïve rats.

High frequency USVs have been postulated as an index of the rat‟s positive emotional state (reviewed in Burgdorf et al. 2011; Knutson et al. 2002) as well as providing a way of communication during social interaction or anticipation of interaction (Brudzynski and Pniak 2002; Willey et al. 2009). However, these two functions are not necessarily unrelated since USVs can also convey specific information associated with the emotional state of the rat (Brudzynski and Pniak 2002; Wohr et al. 2008). In the current study, the isolation-induced USVs in WKY rats were lower at -4 days and -3 days before treatment compared to 29 h after treatment. However, the number of USVs in Wistar rats remained unaffected before and after treatment. Previous studies showed that rats required habituation to the experimental conditions before emitting stable levels of USVs (Knutson et al. 1998; Mällo et al. 2007). It therefore appeared that the WKY rats became more gradually accustomed to the USV procedure than Wistar rats before treatment so that differences in USVs between WKY and Wistar rats were shown on the first day of USV testing. Another possibility may originate from the fact that rats were immediately rejoined with the cage mate(s) following isolation-induced USV testing. It is therefore plausible that WKY rats might have learned over time to anticipate reconnection with the

cage mate(s) after USV testing. A recent study showed that rats emitted 50-kHz USVs (FM and flat calls) during food cue presentation and that the number of USVs increased across days in response to the food cues (Buck et al. 2014). Similarly, other studies also reported increased number of 50-kHz USVs across days in response to rewarding cues such as olfaction from other rats (Bialy et al. 2000; Brudzynski and Pniak 2002; Burgdorf et al. 2000). Therefore, the increased number of isolation-induced USVs (FM, flat and total number of calls) from baseline (-4 and -3 days before treatment) to treatment (at 29 h after treatment) in WKY rats may have resulted from cues of olfaction from the home cage to anticipate reconnection with the cage mate(s).

Ketamine treatment had no effect on the FM calls, flat calls and total number of calls in WKY and Wistar rats. Previous studies showed that NMDA antagonists such as ketamine and MK-801 decreased 50-kHz USVs therefore suggesting a role of glutamate in the production of USVs during social signalling (Bialy et al. 2000; Brudzynski and Pniak 2002; Nikiforuk et al. 2013). This was evidenced by a study showing that acute treatment with ketamine at a dose of 20 mg/kg decreased 50-kHz USVs during social interaction 30 min after treatment (Nikiforuk et al. 2013). Furthermore, the NMDA antagonist, MK-801 (0.1 mg/kg intraperitoneal or 0.6 µg intracerebral), reduced 50-kHz USVs that were emitted by a rat during repeated exposure to a cage that was previously visited by other rats or before introduction of a female rat (Bialy et al. 2000; Brudzynski and Pniak 2002). However, USVs in the current study were measured long after treatment (5 h and 29 h) at lower doses (5 mg/kg – 15 mg/kg) compared to the previous study by Nikiforuk et al. (2013). Also, the context in which USVs were recorded was different, since the USVs recorded from their study were measured during social interaction whereas USVs in the current study were measured during social isolation, which could account for the difference in findings between the studies.

The aim of experiment 3 was to further investigate the effects of ketamine in the MS rat model of depression. MS showed no effect on immobility in the FST in SD rats. Previous studies reported that MS in early life produced depression-like behaviour in adult rats as evidenced by increased immobility in the FST (Dimatelis et al. 2012b; Lambás-Señas et al. 2009; Lee et al. 2007; Ryu et al. 2009). The results of the current study are therefore in contrast to these studies. However, some studies also reported no effect of MS during early life on immobility in the FST (Marais et al. 2008; Nam et al. 2014; Piubelli et al. 2011) or a decreased duration of immobility in the FST (Kwak et al. 2009). These inconsistencies reported may be related to methodological differences between studies. Indeed, there is an ongoing discussion on the various factors leading to the

inconsistencies of data reported between previous studies (Lehmann and Feldon 2000; Vetulani 2013). For example, the most common differences between studies are the duration of MS procedure, frequency of separations, time point of MS exposure during development and choice of control group. However, a previous study using the exact same MS procedure, showed that MS on P2 until P14 for 3 h daily, increased the duration of immobility in the FST at P67 (Dimatelis et al. 2012b). In the current study, rats were handled from P60 - P73 during USV testing and injections that may have reduced their depression-like behaviour. Repeated handling has previously been shown to result in rats being less susceptible to stress-induced depression-like behaviour in later life (Hsu et al. 2003; Silveira et al. 2011) as well as reducing their anxiety (Costa et al. 2012; Schmitt and Hiemke 1998).

Furthermore, ketamine showed no effect on immobility in the FST of MS rats. These results are in contrast to a previous study showing that the immobility of MS Wistar rats in the FST was reversed by chronic treatment with a subanaesthetic dose of ketamine (15 mg/kg) at 1 h after treatment (Réus et al. 2013). Although ketamine was administered at a similar dose, rats in the current study were treated acutely and the FST was conducted much later (48 h and 72 h) which might explain the differences in behavioural findings. Also, the study by Réus et al. (2013) showed that ketamine did not affect immobility of control rats in the FST. Since the immobility of the MS rats was similar to the control rats, it is therefore possible that ketamine treatment was unable to further reduce the immobility in SD rats.

Similar to WKY and Wistar rats, ketamine had no effect on the number of USVs (FM, flat and total number of calls) in SD rats. However, the results in this study are in contrast to the previous study that measured USVs at 30 min after ketamine treatment during social interaction in SD rats (Nikiforuk et al. 2013). Although the dose of 15 mg/kg ketamine used in the current study (experiment 3) was very similar to the study by Nikiforuk et al. (2013), USV testing was performed at 5 h and 29 h after ketamine treatment. Therefore, similar to experiment 1, USV testing in SD rats in the current study was measured at a longer time after treatment as well as during social isolation opposed to social interaction which may explain the different results obtained in the studies.

In conclusion, WKY rats were unresponsive to the rapid and sustained antidepressant effects of an acute subanaesthetic dose of ketamine but instead more sensitive to the ketamine-induced immobility at a higher dose possibly because the NMDA receptor function was already reduced. Therefore, the WKY rat may be a useful model to study treatment-resistant depression. Furthermore, ketamine had no antidepressant-like effect in

MS SD and Wistar rats since these strains did not represent a model for depression. Therefore, the rat strain or model as well as the dose and duration of ketamine treatment may all have important implications for the effects of ketamine.

Chapter 6

General discussion

In this chapter, the key findings of the study will be summarized and novel findings further discussed.

6.1 Summary of results

Since the results of the current study are presented in different chapters, a summary of the main findings will be provided and their implications discussed. This study successfully achieved all the aims through multiple objectives in order to establish a more robust WKY and MS model of depression as outlined in Chapter 1, section 1.6, as follows:

- Chapter 2: When comparing depression-/anxiety-like behaviour in WKY substrains, the WKY/NCrl rats displayed higher immobility levels in the FST and less activity in the OFT compared to the WKY/NHsd substrain and Wistar control strain, which is consistant with its use as a model of depression. Furthermore, chronic desipramine treatment (15 mg/kg) of WKY/NCrl rats attenuated the depression-like behaviour as evidenced by decreased immobility and increased active struggling behaviour in the FST. However, desipramine had no effect on opioid receptors (MOR and KOR) and TH in the NAc or PFC in WKY/NCrl rats.
- Chapter 3: Similar to normally reared WKY/NCrl rats, MS WKY/NCrl rats displayed reduced activity in the FST and OFT and spent more time in the center zone of the EPM. Furthermore, MS WKY/NCrl rats subjected to early-life MS, displayed anxiety-like behaviour as presented by increased time spent in the closed arms of the EPM compared to normally reared WKY/NCrl rats. Moreover, both MS WKY/NCrl and WKY/NCrl rats emitted more USVs than Wistar rats following separation from the cage mate(s). Chronic desipramine treatment not only decreased depression-like behaviour of WKY rats, but also increased the time spent in the open arms of the EPM by MS WKY/NCrl, indicative of an anxiolytic effect. Furthermore, desipramine treatment decreased FM USVs in both MS WKY/NCrl and WKY/NCrl rats. Biochemical studies showed no effect of desipramine on serotonin levels and p-ERK in the PFC or dopamine concentration and opioid receptors in the NAc of normally reared WKY/NCrl and MS WKY/NCrl rats. However, desipramine treatment increased p-GSK3β in the PFC of

WKY/NCrl rats but not in MS WKY/NCrl rats, therefore indicating that this effect of desipramine was blocked by MS.

- Chapter 4: The stress of MS increased depression-like behaviour in SD rats as evidenced by increased immobility and decreased active swimming behaviour in the FST. Restraint stress did not exaggerate the effect of MS on depression-/anxiety-like behavior of MS rats in the FST. Further biochemical studies to determine changes in the brain associated with the depression-like behaviour of MS SD rats subjected to restraint stress, revealed no effect of MS, restraint stress, or MS together with restraint stress, on levels of BDNF in the ventral hippocampus. However, proteomic analysis of the PFC revealed a decrease in structural proteins (actin-related) in MS rats and non-separated rats subjected to restraint stress as well as a decrease in mitochondrial energy-related proteins in MS rats with or without subsequent exposure to restraint stress and non-separated rats subjected to restraint stress. Furthermore, rats subjected to both MS and restraint stress displayed a decrease in proteins involved in protein synthesis and an increase in proteins involved in protein degradation
- The study in Chapter 5 provides novel evidence in that it shows that 10 mg/kg of ketamine increased immobility of WKY/NCrl rats in the FST at 48 h and 72 h after treatment which may be indicative of a disturbance in glutamate function. Ketamine had no effect on the MS SD rats and Wistar control rats.

6.2 Novel findings and conclusion

The novel key findings related to the rat models of depression (normally reared WKY/NCrl, MS WKY/NCrl, MS SD rats and MS SD rats subjected to restraint stress) will be further discussed.

6.2.1 Comparison of WKY/ NCrl and maternally separated WKY/NCrl as rat models of depression/anxiety

WKY/NCrl

This is the first study that measured depression- and anxiety-like behaviour in WKY substrains in order to characterize the WKY as a rat model of depression/anxiety (Chapter 2). The WKY/NCrl substrain was selected as the most suitable model of depression-like behaviour according to its high levels of immobility in the FST and reduced activity in the OFT. WKY/NCrl rats displayed increased immobility in the FST and reduced activity in the OFT compared to Wistar rats, similar to results reported in Chapter 3 and Chapter 5.

However, neither of the WKY substrains displayed anxiety-like behaviour in the EPM. A previous study comparing WKY substrains as suitable controls for the spontaneously hyperactive rat (SHR) model of attention-deficit/hyperactivity disorder (ADHD), showed that WKY/NCrl displayed no overactivity, compared to WKY/NHsd in a visual discrimination task, which suggested that WKY/NCrl may model an inattentive subtype of ADHD (Sagvolden et al. 2008). In addition, most of the studies that specified the substrain of WKY, showed depression-like behaviour in the FST and OFT in WKY obtained from Charles River Laboratories (Rubalcava and Lucki 2000; Tejani-Butt et al. 2003; Nam et al. 2014; Lahmame and Armario 1996). Therefore, this study provides novel evidence for the WKY/NCrl as a more appropriate substrain than the WKY/NHsd to model the depression-like behaviour.

In further characterization of the WKY/NCrl as a model of depression, its response to desipramine treatment in terms of immobility in the FST was determined (Chapter 2). This study provides novel evidence to show that a 15-min pretest-swim is necessary for desipramine (15 mg/kg) to reverse the depression-like behaviour of WKY/NCrl in the FST. Since WKY rats exhibit spontaneous immobility in the FST, it was previously considered that no pretest-swim is necessary to induce such behaviour (Tizabi et al. 2012). However, the dose of antidepressant drug, duration of treatment (sub-chronic or chronic) and protocol of FST (inclusion of a pretest-swim or single swim session) are all important factors to consider when determining the response of WKY/NCrl rats to desipramine in the FST. Previous studies that measured the antidepressant effect of desipramine in WKY/NCrl rats, showed that desipramine had no effect on immobility in a single FST session at a sub-chronic dose (5 mg/kg – 15 mg/kg) but decreased immobility in the FST at a higher dose of 25 mg/kg (Lahmame and Armario 1996). However, when WKY/NCrl rats were habituated to a pretest-swim in the FST, sub-chronic or chronic treatment with desipramine (5 mg/kg – 20 mg/kg) was effective in reversing the immobility (Lopez-Rubalcava and Lucki 2000; Tejani-Butt et al. 2003). Since learning and memory consolidation has been suggested to be required for immobility during the 5-min test swim (Campus et al. 2015), it may be speculated that the WKY/NCrl rats have impaired memory consolidation because of their reduced NMDA receptor density in the striatum, substantia nigra, NAc, hippocampus (CA1 region) and cingulate cortex (Lei and Tejani-Butt 2010; Lei et al. 2009). The immobility in the WKY rats may also reflect an increased stress response as evidenced by a previous study showing that increased immobility of WKY rats in the FST was accompanied by increased ACTH and corticosterone secretion (Rittenhouse et al. 2002).

Chronic desipramine treatment had no effect on TH, dopamine and serotonin levels, opioid receptors (KOR and MOR) or p-ERK in the NAc or PFC of WKY/NCrl rats (Chapter 2 and 3). However, desipramine treatment increased p-GSK3β in the PFC of WKY/NCrl rats (Chapter 3). The latter provides novel evidence for the mechanism of action of desipramine and the role of p-GSK3β (inactive form of GSK3β) in possibly mediating the decrease in depression-like behaviour in WKY/NCrl rats. GSK3β play a key role as regulator of cell survival and apoptosis and over-expression of GSK3β has been linked to apoptotic cell death in neuronal cell cultures (Beurel and Jope 2006; Crowder and Freeman 2000; Liu 2014). The mechanism by which desipramine may affect p-GSK3β is still unknown and needs to be further explored. GSK3β is regulated by several mehanisms that include direct or indirect modulation by the serotonergic, dopaminergic neuropsychiatric drugs and lithium, the Wingless (Wnt) signalling pathway, protein kinase A, protein kinase C and Akt (Crowder and Freeman 2000; Polter and Li 2011; Sutton and Rushlow 2011). Neuropsychiatric classes of drugs such as antipsychotics (haloperidol and clozapine), mood stabilizers (lithium and valproic acid) and antidepressant drugs (imipramine and fluoxetine) regulate GSK3β activity by increasing Akt signalling (increased phosphorylated Akt and p-GSK3) in the PFC and striatum (Sutton and Rushlow 2011). However, regulation of the Wnt pathway is specific to antipsychotics (Sutton and Rushlow 2011). It is well recognized that chronic antidepressant drug treatment, including desipramine treatment, enhance BDNF neurotransmission (Castrén et al. 2007; Nibuya et al. 1995) and consequently may increase phosphorylation of GSK3β through increasing activity in the phosphatidylinositol-3 kinase (PI3K)/Akt pathway (Johnson-Farley et al. 2006; Smillie et al. 2013). However, further studies are needed to determine the involvement of the PI3K/Akt pathway and other components such as the mammalian target of rapamycin (mTOR), in order to study the role of GSK3β in the depression phenotype of WKY/NCrl rats and antidepressant mechanism of action of desipramine treatment. Studies to show the involvement of p-GSK3β may include using GSK3 knock-in mice where phosphorylatioin of GSK3β is blocked, This is achieved by altering the phosphorylation site (Ser9 replaced by mutant protein) on GSK3β. GSK3 knock-in may diminish the reduced immobility in the FST following desipramine treatment as previously shown with other antidepressant drugs (Polter et al. 2012). This may reveal whether p-GSK3β plays a role in the mechanism of action of desipramine.

Maternally separated WKY/NCrl

In addition to the depression-like behaviour, WKY/NCrl rats subjected to MS enhanced the potential of WKY rats to serve as a model of anxiety-like behaviour (Chapter 3).

Previous studies showed that WKY rats displayed anxiety-like behaviour only in some of the behavioural tests such as a longer latency to feed in the novelty-supressed feeding test and increased freezing in the OFT (Braw et al. 2006; Malkesman et al. 2005; Yamada et al. 2013). However, they did not exhibit any anxiety-like behaviour in the EPM (Braw et al. 2006; Getachew et al. 2008; Nam et al. 2014; Pardon et al. 2002). Therefore, this study provides novel evidence in showing that the WKY/NCrl rats subjected to MS may be useful as a model for depression- and anxiety-like behaviour.

In line with the efficacy of desipramine to treat symptoms of depression and anxiety, MS WKY/NCrl rats responded to the antidepressant-like effects and anxiolytic effect of desipramine in the FST and EPM (Chapter 3). Furthermore, desipramine treatment decreased the duration that MS WKY/NCrl rats spent in the center area of the EPM, indicative of reduced indecisiveness; an effect that was not observed in WKY/NCrl rats. Therefore, WKY/NCrl rats subjected to MS and treated with desipramine provide more evidence for WKY/NCrl rats subjected to MS to model depression/anxiety. Desipramine had no effect on dopamine and serotonin concentration, opioid receptors (KOR and MOR), p-ERK or p-GSK3β in the NAc or PFC of MS WKY/NCrl rats (Chapter 3). Since desipramine treatment increased p-GSK3β in normally reared WKY/NCrl rats and not in MS WKY/NCrl rats, it may be suggested that MS blocked this effect and therefore suggests that other pathways or brain areas may be involved in the depression-/anxiety-like phenotype of MS WKY/NCrl rats.

Ultrasonic vocalizations

High frequency USVs in adult rats may indicate a positive affective state and/or may function as a social signal (Brudzynski and Pniak 2002; Mällo et al. 2007). In chapter 3, rats emitted high frequency USVs during a situation considered stressful (social isolation). Furthermore, the number of USVs was greater in both WKY/NCrl and MS WKY/NCrl rats compared to Wistar rats. Therefore, the 50-kHz USVs emitted in response to removal of the cage mate(s) in the present study, are in contrast to the positive emotion frequently associated with 50-kHz USVs (Burgdorf et al. 2007; Burgdorf et al. 2008; Panksepp and Burgdorf 2000). However, high frequency USVs have also been found in situations generally not seen as appetitive such when rats were singly tested in a test arena previously visited by other rats, when rats were reunited following separation or when isolated from the cage mate(s), indicative of a communicational function (Brudzynski and Pniak 2002; Wohr et al. 2008). Therefore, it may be suggested that the USVs in this study function to serve a communicational role in signalling the need to re-connect with the cage mate(s). Since FM calls play a role in emotion (Burgdorf et al. 2008) whereas flat

calls have a communicational function in rats (Wohr et al. 2008), the FM calls that were affected by the social isolation in the present study may also convey information regarding the emotional state of the rats. Furthermore, desipramine treatment decreased FM calls in WKY rats which further supports an emotional function. In the current context of the study, USVs may therefore function to signal emotional information to the cage mate(s) during social isolation regarding the need to re-connect. Furthermore, the high number of USVs in WKY rats may be an additional marker for their depression phenotype although further USV studies are needed in different experimental conditions (e.g. other stressors) to provide further support.

It should be noted that the strain differences (WKY and Wistar rats) in USVs in Chapter 3 were only evident while they were being treated with desipramine (after 18 days of habituation). It could be that the WKY and Wistar rats needed time to habituate to the handling and testing conditions before differences between WKY and Wistar rats became apparent. However, no significant difference in the number of USVs was found between WKY and Wistar rats when they were habituated and baseline USVs were measured at an older age (P60-P69 in Chapter 5, compared to rats at P52-P59 in Chapter 3). Also, rats in Chapter 5 were handled for a shorter period before USVs were measured during treatment (from 10 days after habituation started). Therefore, the USVs may be influenced by the experimental procedure.

6.2.2 Maternally separated Sprague-Dawley rats subjected to restraint stress as a model of depression

It is well recognized that early life trauma results in hyperactivity of the HPA axis and symptoms of depression when individuals are confronted with stressful life events (Juruena 2014; Shapero et al. 2014; Slavich et al. 2011). Similarly, animal studies have shown that the stress of MS in early life potentiates the HPA axis response to various types of stressors and is associated with the development of depression-/anxiety-like behaviour in adulthood (Eiland and McEwen 2012; Marais et al. 2008; Uchida et al. 2010). MS SD rats were chosen for BDNF and proteomic analysis (Chapter 4) since they represent a more general widely accepted rat model of depression and it was important to gain further insight into the molecular changes in this model. Consistent with its use as a model of depression, SD rats subjected to MS displayed depression-like behaviour in the FST. However, in agreement with the previously reported variability of the effects of MS, the study in Chapter 5 did not reveal any effect of MS on depression-like behaviour in the FST. The variability of this model has been extensively reviewed with difference in frequency and timing of separation, gender differences and choice of control that all

contribute in the variability of results between studies (Vetulani 2013). Although a similar MS protocol was followed in the studies in Chapter 4 and 5, rats were handled significantly more in the study in Chapter 5 due to USV testing. This may have had an effect on their emotionality which may have attenuated difference between the MS and non-maternally separated rats.

Restraint stress did not alter depression-like behaviour of MS rats in the FST, nor did it induce anxiety-like behaviour in the EPM (Chapter 4). This is in contrast to previous studies that showed that additional stress in adulthood in MS rats potentiated their depression-/anxiety-like behaviour (Eiland and McEwen 2012; Marais et al. 2008; Uchida et al. 2010). However, these studies showed that MS on its own did not evoke depression-/anxiety-like behaviour but MS rats were more susceptible to developing depression-/anxiety-like behaviour when subjected to chronic stress in adulthood (Marais et al. 2008). Since MS on its own produced depression-like behaviour in the current study (Chapter 4), it may be suggested that restraint stress, that has similar effects on the HPA axis and acts on the same signalling pathways involving BDNF, was prevented from exerting its depression-like effects.

A proteomic analysis of the PFC (Chapter 4) revealed that MS and restraint stress affected a number of proteins from various functional groups. The proteins affected were related to structure, energy metabolism, neurotransmission, protein synthesis and degradation. Rats subjected to MS or restraint stress had decreased structural proteins associated with actin (actin related protein 2/3 complex, alpha II spectrin, ARP10 actin-related protein). Furthermore, a decrease in energy-related proteins was found in MS rats, restraint stressed rats and MS rats subjected to restraint stress. This is in line with a previous study that found reduced structure- and energy-related proteins in the PFC in response to MS stress (Dimatelis et al. 2013). The reduced energy-related proteins in the PFC found in this study and increased energy-related proteins in the ventral hippocampus (Marais et al. 2009) may possibly be related to clinical studies of depression that showed hypometabolism in the PFC and hypermetabolism in limbic brain areas (Salerian and Altar 2012). In addition, the current study showed that MS rats subjected to restraint stress had a decrease in proteins involved in protein synthesis (Protein Tardbp, 40S ribosomal protein S12, RCG45615 and Tyrosine--tRNA ligase) and an increase in proteins involved in protein degradation (F-box only protein 2 and COP9). However, the current study did not reveal similarity in the protein profile between MS and MS rats subjected to restraint stress as well as differences between MS rats with or without stress and non-maternally separated rats subjected to restraint stress. Therefore, the changes in proteins that were identified in the PFC of MS rats with or without stress may not be

related to their depression-like behaviour. For example, the structural proteins affected in MS rats were not affected in MS rats subjected to restraint stress. However, the energy-related proteins were similarly reduced in MS rats with or without restraint stress but were also in non-maternally separated rats subjected to restraint stress and therefore may rather indicate an effect of stress on the PFC. The lack of similarity in the protein profile between MS rats and MS rats subjected to restraint stress may be due to the fact that they were analysed separately (8-plex vs 4-plex) which accounted for different proteins being identified.

In conclusion, the effect of MS on depression-like behaviour was found to be inconsistent between studies in Chapter 4 and 5, possibly due to increased handling of the rats in Chapter 5, highlighting the need for the MS protocol to be standardized across studies. However, proteomic results in the MS rats with or without restraint stress revealed important proteins affected that are related to structure, energy metabolism and proteins involved in protein synthesis and degradation, which may be the effect of stress on the PFC and not related to their depression-like behaviour.

6.2.3 The effect of ketamine in WKY/NCrl and maternally separated Sprague-Dawley models of depression

Ketamine at subanaesthetic doses has shown rapid and sustained antidepressant effects in patients with depression (Larkin and Beautrais 2011; Zarate et al. 2006) as well as in animal models of depression at doses of 5 mg/kg - 15 mg/kg (Koike et al. 2011; Tizabi et al. 2012). However, chronic ketamine treatment for 5 days at higher doses (30 mg/kg – 100 mg/kg) induces depression-like behaviour in naïve rats in the FST (Chatterjee et al. 2011; Chindo et al. 2012; Hou et al. 2013). In the current study (Chapter 5), acute ketamine increased immobility in the FST at 48 h and 72 h after treatment at a dose (10 mg/kg) that normally showed antidepressant effects in other rat models of depression (Garcia et al. 2008; Koike et al. 2011; Li et al. 2011). Therefore, this study provides novel evidence showing that ketamine treatment in WKY rats at a dose of 10 mg/kg potentiates their depression-like behaviour in the FST. This is the first study that measured the effect of acute treatment of male WKY rats with ketamine. A previous study in female WKY rats showed that acute ketamine treatment had a rapid and sustained antidepressant effect at a lower dose of 5 mg/kg, measured as reduced immobility in the FST. However, 5 mg/kg of ketamine in the current study had no effect on male WKY rats in the FST at 48 h or 72 h after injection. Therefore, the current study provides evidence of ketamine-induced immobility in male WKY rats which may possibly be related to their disrupted glutamate neurochemistry. This is supported by studies showing reduced NMDA receptor

density in male WKY rats at basal levels in various brain areas such as the substantia nigra, striatum, NAc, hippocampus, anterior cingulate cortex and PFC compared to Wistar rats (Lei and Tejani-Butt 2010; Lei et al. 2009). However, no difference in NMDA receptor density was reported in female WKY rats in the hippocampus (Tizabi et al. 2012). It may therefore be concluded that by further blocking the NMDA receptor in WKY rats, with reduced NMDA receptor density, ketamine treatment may ultimately lead to effects that are similar to the effects exhibited by naïve rats treated with high doses of ketamine.

This study further supports suggestions that WKY rats are selective in their response to antidepressant drug treatment and only responsive to the antidepressant effects of desipramine in the current study (Chapter 2 and 3). The response to the antidepressant effects of desipramine would suggest that a noradrenergic mechanism is involved in the depression-like behaviour of WKY rats. Indeed, consistent with the monoamine hypothesis, previous studies showed reduced noradrenaline levels in several brain areas of WKY rats (ventral hippocampus, NAc, dorsal raphe nuclei, lateral hypothalamus and locus coeruleus) compared to Wistar or SD rats (Scholl et al. 2010) that may be implicated in their depression phenotype.

6.2.4 Conclusion

In summary, this study provides novel evidence showing that WKY/NCrl is a more appropriate WKY substrain to model depression and when subjected to MS provides a more robust model of depression/anxiety that responds to the antidepressant and anxiolytic effects of desipramine in the FST and EPM. Furthermore, desipramine treatment increased p-GSK3β in the PFC of normally reared WKY/NCrl rats, an effect blocked by MS which provides novel evidence for the mechanism of action of desipramine and role of p-GSK3 β in possibly mediating the decrease in depression-/anxiety-like behaviour of WKY/NCrl rats. The USVs in WKY rats appeared to reflect signalling to establish social contact with the cage mate(s) and the high number of isolation-induced USVs in WKY compared to Wistar rats may reflect a marker for their depression phenotype that was attenuated by desipramine treatment. The MS SD as a comparator model provides evidence for depression-like behaviour in the FST, but findings were inconsistent between experiments, possibly due to increased handling of the rats during repeated recording of isolation-induced vocalizations. In addition, restraint stress showed no effect on the depression-like behaviour of MS rats in the FST but revealed important proteins related to energy metabolism and protein synthesis and degradation that were affected. The WKY rats were selective in their response to

antidepressant drug treatment, since ketamine showed no antidepressant effects in the FST but increased their immobility which may be related to a dysfunction of NMDA receptors.

The above findings could be extended into further experiments of *in vivo* chronoamperometric measurements of drug-induced changes of glutamate, noradrenaline and dopamine in the NAc, PFC or hippocampus in order to further elucidate the mechanisms underlying the depression-like behaviour. Furthermore, electrochemical measurements of these neurotransmitters in different subareas of the PFC, that suggest disparate functionality, may provide more insight in the neuropathology of depression. For example, the ventromedial PFC has been associated with cognitive or executive functions whereas the dorsolateral PFC with emotional functions (Koenigs and Grafman 2009). Proteomic analysis could also be followed up with a more targeted analysis of specific molecules of interest in different brain regions such as the hippocampus and nucleus accumbens. Future behavioural and neurochemical studies could also compare male and female rats. Ketamine showed no antidepressant effect in the FST of male WKY rats. It may therefore be useful to replicate the antidepressant effect of ketamine in female WKY rats and to include a dose response study before continuing this line of investigation.

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Appendix A

Additional proteomic results

Table A1 (8-plex) and A2 (4-plex) contains all the proteins identified in Ctr, RS, MS and MS+RS rat groups in Chapter 4. Proteins are divided in different functional groups (cytoskeletal, energy metabolism, neurotransmission, proteins synthesis, proteins degradation and other) and further subdivided according to their specific function in that group. Values for duplicate rat groups were averaged and MS, RS and MS+RS rat groups expressed as fold difference relative to the Ctr group. Data of proteins highlighted in turquoise were considered to be increased or decreased when they were ± 0.2 fold different from Ctr rats (reported and discussed in Chapter 4).

Table A 1: Proteomic (8-plex) profile of the PFC of Ctr, MS and restraint stressed (RS) rats. Data presented as a ratio to Ctr 1. The average Ctr ratio (Avg) was calculated and normalized to 1.0 (blue). Data in red differed from the normalized Ctr/Ctr by more than 20% (1.2-fold increase or decrease).

Protein / function	Accession no	Ctr 1	Ctr 2	Avg	Ctr/ Ctr	MS 1	MS 2	Avg	MS/ Ctr	RS 1	RS 2	Avg	RS/ Ctr
Cytoskeletal/Structural													
Actin-binding													
Actin related protein 2/3 complex, subunit 3	tr | B2GV73 |	1	1.20	1.10	1.0	0.80	0.80	0.80	**0.73**	1.20	1.20	1.20	1.09
Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	tr | B2RZ72 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	1.00	0.95	0.95
Actin, alpha cardiac muscle 1	sp | P68035 |	1	1.20	1.10	1.0	1.00	1.00	1.00	0.91	1.20	1.20	1.20	1.09
Actin-related protein 2	sp | Q5M7U6 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Actin-related protein 2/3 complex subunit 1A	sp | Q99PD4 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
Actin-related protein 2/3 complex subunit 2	sp | P85970 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actin-related protein 2/3 complex subunit 5	sp | Q4KLF8 |	1	1.00	1.00	1.0	1.20	1.00	1.10	1.10	1.00	1.00	1.00	1.00
Actin-related protein 2/3 complex subunit 5-like protein	sp | A1L108 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Actin-related protein 3	sp | Q4V7C7 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	0.90	1.00	0.95	0.90
Actin, cytoplasmic 1	sp | P60711 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actn1 protein	tr | Q6GMN8 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00
Spectrin alpha chain, non-erythrocytic 1	sp | P16086 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Alpha II spectrin	tr | C9EH87 |	1	1.10	1.05	1.0	0.80	0.70	0.75	**0.71**	0.90	1.30	1.10	1.05
Spectrin beta 3	tr | F1MA36 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
F-actin-capping protein subunit alpha-1	sp | B2GUZ5 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
F-actin-capping protein subunit alpha-2	sp | Q3T1K5 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
F-actin-capping protein subunit beta	sp | Q5XI32 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Vinculin	tr | R9PXU6 |	1	1.70	1.35	1.0	1.80	1.30	1.55	1.15	1.40	1.50	1.45	1.07
ARP10 actin-related protein 10 homolog (S. cerevisiae)	tr | Q5M9F7 |	1	1.00	1.00	1.0	1.10	0.70	0.90	0.90	0.80	0.80	0.80	**0.80**
Dynactin 1, isoform CRA_a	tr | D4A8U7 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Dynactin subunit 2	sp | Q6AYH5 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha-actinin-4	sp | Q9QXQ0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90

Tropomodulin-2	sp | P70566 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Tropomyosin 5	tr | P97726 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Tropomyosin alpha isoform	tr | Q91XN7 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Myl6 protein	tr | B2GV99 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Myosin regulatory light chain	tr | Q63781 |	1	1.00	1.00	1.0	1.20	1.20	1.20	**1.20**	1.00	1.10	1.05	1.05
Myosin, heavy polypeptide 10, non-muscle, isoform CRA_b	tr | G3V9Y1 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Myosin, heavy polypeptide 9, non-muscle	tr | G3V6P7 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
Protein Twf2	tr | B0BMY7 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.10	1.05	1.05
Plectin 7	tr | Q6S399 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Fascin	sp | P85845 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Destrin	sp | Q7M0E3 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
Drebrin E	tr | C6L8E0 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Drebrin-like protein	sp | Q9JHL4 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.10	1.05	1.05
Adducin 3 (Gamma), isoform CRA_b	tr | D3ZCH7 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Alpha-adducin	sp | Q63028 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Beta-adducin	tr | F8WFS9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Band 4.1-like protein 1	tr | D3ZMI4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Calponin-3	sp | P37397 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	0.80	0.90	0.85	0.85
Cofilin-1	sp | P45592 |	1	1.20	1.10	1.0	1.00	1.00	1.00	0.91	1.10	1.20	1.15	1.05
Coronin (Fragment)	tr | F1LMV9 |	1	0.90	0.95	1.0	0.90	1.10	1.00	1.05	1.10	0.90	1.00	1.05
Coronin	tr | G3V940 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.80	0.85	0.89
Coronin-1A	sp | Q91ZN1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cytoplasmic FMR1 interacting protein 1 (Predicted)	tr | D4A8H8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Cytoplasmic FMR1 interacting protein 2 (Predicted)	tr | D3ZX82 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Erythrocyte protein band 4.1-like 3, isoform CRA_d	tr | Q9JMB3 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Profilin	tr | D3ZDU5 |	1	0.90	0.95	1.0	1.10	1.10	1.10	1.16	0.90	0.90	0.90	0.95
Profilin-1	sp | P62963 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Ank2 (Fragment)	tr | F1M9N9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Synaptopodin	tr | B1VKB4 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Ezrin	sp | P31977 |	1	1.00	1.00	1.0	0.80	0.90	0.85	0.85	1.10	1.00	1.05	1.05

NCK interacting protein with SH3 domain (Predicted), isoform CRA_a	tr | D3ZWX4 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.20	1.10	1.15	1.10
Nck-associated protein 1	sp | P55161 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Actbl2	tr | D3ZRN3 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Protein Sptbn1	tr | G3V6S0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Twinfilin-1	sp | Q5RJR2 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	0.90	0.90	0.90	0.90
Alpha-centractin	sp | P85515 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adenylyl cyclase-associated protein 1	sp | Q08163 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adenylyl cyclase-associated protein 2	sp | P52481 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	1.00	1.00	1.00	0.95
Myristoylated alanine-rich C-kinase substrate	sp | P30009 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Tubulin-binding													
Tubulin alpha-1A chain	sp | P68370 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Tubulin alpha-4A chain	sp | Q5XIF6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tubulin beta-2A chain	sp | P85108 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
Tubulin beta-3 chain	sp | Q4QRB4 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Tubulin beta-5 chain	sp | P69897 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Microtubule-actin cross-linking factor 1	tr | M9MMM9 |	1	1.30	1.15	1.0	1.10	1.00	1.05	0.91	1.10	1.20	1.15	1.00
Microtubule-associated protein 1 A, isoform CRA_c	tr | G3V7U2 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.00	1.00	0.95
Microtubule-associated protein 1B	tr | F1LRL9 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Microtubule-associated protein 4	sp | Q5M7W5 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	0.90	0.95	0.95
Microtubule-associated protein 6	sp | Q63560 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Microtubule-associated protein	tr | F1MAQ5 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Microtubule-associated protein RP/EB family member 3	sp | Q5XIT1 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Microtubule-associated proteins 1A/1B light chain 3A	sp | Q6XVN8 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Protein Tppp	tr D3ZQL7 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Protein Tubb4a	tr | B4F7C2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Tubb6	tr | Q4QQV0 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Protein Tubal3	tr | F1LUM5 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
HMW-MAP2 (Fragment)	tr | P70652 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	0.90	1.10	1.00	0.95
CAP-Gly domain-containing linker protein 2	tr | G3V949 |	1	1.20	1.10	1.0	1.30	0.90	1.10	1.00	1.10	1.00	1.05	0.95
2',3'-cyclic-nucleotide 3'-phosphodiesterase	sp | P13233 |	1	0.70	0.85	1.0	0.90	0.80	0.85	1.00	1.00	0.80	0.90	1.06

Gephyrin	sp | Q03555 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	0.90	1.00	0.95	0.90
Echinoderm microtubule associated protein like 4 (Predicted), isoform CRA_a	tr | F1LT71 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	0.90	0.95	0.95
Intermediated filament binding													
Vimentin	sp | P31000 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Lamin A, isoform CRA_b	tr | G3V8L3 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha-internexin	sp | P23565 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Neurofilament heavy polypeptide	tr | F1LRZ7 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Neurofilament light polypeptide	sp | P19527 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.10	1.10	1.10	1.10
Neurofilament medium polypeptide	sp | P12839 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Glial fibrillary acidic protein	sp | P47819 |	1	0.90	0.95	1.0	1.10	1.00	1.05	1.11	0.90	0.90	0.90	0.95
sp|P30427|PLEC_RAT-DECOY Plectin	sp | P30427 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Synm	tr | G3V9G5 |	1	1.20	1.10	1.0	1.20	1.00	1.10	1.00	1.00	1.10	1.05	0.95
Other													
Paralemmin-1	sp | Q920Q0 |	1	1.20	1.10	1.0	1.30	1.00	1.15	1.05	1.10	1.00	1.05	0.95
Brevican, isoform CRA_a	tr | G3V8G4 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	0.90	1.00	0.95	0.90
Hyaluronan and proteoglycan link protein 1	sp | P03994 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Myelin basic protein transcript variant 1	tr | I7FKL4 |	1	0.50	0.75	1.0	0.90	0.80	0.85	1.13	1.20	1.00	1.10	**1.47**
Myelin proteolipid protein	sp | P60203 |	1	0.70	0.85	1.0	0.70	0.70	0.70	0.82	0.90	0.70	0.80	0.94
Catenin beta-1	sp | Q9WU82 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.20	1.10	1.15	1.10
Laminin, alpha 5, isoform CRA_a	tr | F1MAN8 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.00	0.90	0.95	0.86
Keratin, type I cytoskeletal 10	sp | Q6IFW6 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.10	0.90	1.00	1.00
Keratin, type II cytoskeletal 1	sp | Q6IMF3 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00
Dihydropyrimidinase-related protein 1	sp | Q62950 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydropyrimidinase-related protein 2	sp | P47942 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydropyrimidinase-related protein 3	sp | Q62952 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Dihydropyrimidinase-related protein 4 (Fragment)	sp | Q62951 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydropyrimidinase-related protein 5	sp | Q9JHU0 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00

Energy metabolism

Glycolysis													
Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	tr | F7FKI5 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	sp | P49432 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Pyruvate carboxylase, mitochondrial	sp | P52873 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00
Pyruvate kinase	tr | Q6P7S0 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.20	1.15	1.10
Pyruvate kinase PKM	sp | P11980 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gamma-enolase	sp | P07323 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Citric Acid Cycle													
2-oxoglutarate dehydrogenase, mitochondrial	sp | Q5XI78 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
Fumarate hydratase, mitochondrial	sp | P14408 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Aconitate hydratase, mitochondrial	sp | Q9ER34 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
ATP-citrate synthase	sp | P16638 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Citrate synthase	tr | G3V936 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex), isoform CRA_a	tr | G3V6P2 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.80	0.80	0.80	0.84
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	sp | P08461 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	0.90	0.95	0.95
Glutamate dehydrogenase 1, mitochondrial	sp | P10860 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Malate dehydrogenase, cytoplasmic	sp | O88989 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Malate dehydrogenase, mitochondrial	sp | P04636 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Malic enzyme (Fragment)	tr | F1LQQ1 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.10	1.10	1.00
Malic enzyme	tr | F1M5N4 |	1	1.20	1.10	1.0	1.30	1.10	1.20	1.09	0.90	0.90	0.90	0.82
Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	sp | P13086 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Trifunctional enzyme subunit alpha, mitochondrial	sp | Q64428 |	1	1.10	1.05	1.0	0.40	1.00	0.70	**0.67**	0.90	0.70	0.80	**0.76**
Protein Sucla2	tr | F1LM47 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Oxidative phosphorylation													
Cytochrome b-c1 complex subunit 2, mitochondrial	sp | P32551 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.80	0.90	0.85	0.89

Cytochrome c oxidase subunit 2	sp | P00406 |	1	0.60	0.80	1.0	0.60	0.70	0.65	0.81	0.70	0.70	0.70	0.88
Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	sp | P10888 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.80	0.70	0.75	0.88
Cytochrome c oxidase subunit 5B, mitochondrial	sp | P12075 |	1	0.80	0.90	1.0	0.70	0.80	0.75	0.83	0.90	0.70	0.80	0.89
Cytochrome c, somatic	sp | P62898 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.20	1.15	1.10
Cox7a2 protein	tr | B2RYS0 |	1	0.80	0.90	1.0	0.80	0.80	0.80	0.89	0.90	0.80	0.85	0.94
Ndufa4 protein	tr | B2RZD6 |	1	0.70	0.85	1.0	0.70	0.80	0.75	0.88	0.80	0.70	0.75	0.88
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	tr | F1LNF7 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	sp | Q68FX0 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
Isocitrate dehydrogenase [NAD] subunit gamma 1, mitochondrial	sp | P41565 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Isocitrate dehydrogenase [NADP] cytoplasmic	sp | P41562 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00
Isocitrate dehydrogenase [NADP], mitochondrial	sp | P56574 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	sp | Q561S0 |	1	0.70	0.85	1.0	0.50	0.70	0.60	**0.71**	0.70	0.70	0.70	0.82
NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	sp | Q66HF1 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Succinate dehydrogenase complex subunit A (Fragment)	tr | Q0QF18 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.80	0.90	0.95
ATP synthase F(0) complex subunit B1, mitochondrial	sp | P19511 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.80	0.70	0.75	0.88
ATP synthase subunit alpha, mitochondrial	sp | P15999 |	1	0.70	0.85	1.0	0.70	0.80	0.75	0.88	0.80	0.70	0.75	0.88
ATP synthase subunit beta, mitochondrial	sp | P10719 |	1	0.70	0.85	1.0	0.70	0.90	0.80	0.94	0.80	0.70	0.75	0.88
ATP synthase subunit delta, mitochondrial	sp | P35434 |	1	0.80	0.90	1.0	0.80	0.90	0.85	0.94	0.80	0.80	0.80	0.89
ATP synthase subunit gamma, mitochondrial	sp | P35435 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.80	0.70	0.75	0.88
ATP synthase subunit O, mitochondrial	sp | Q06647 |	1	0.90	0.95	1.0	0.80	0.80	0.80	0.84	0.80	0.70	0.75	**0.79**
ADP/ATP translocase 1	sp | Q05962 |	1	0.70	0.85	1.0	0.60	0.70	0.65	**0.76**	0.80	0.70	0.75	0.88
ADP/ATP translocase 2	sp | Q09073 |	1	0.70	0.85	1.0	0.60	0.80	0.70	0.82	0.80	0.70	0.75	0.88
Phosphate carrier protein, mitochondrial	tr | G3V741 |	1	0.60	0.80	1.0	0.60	0.80	0.70	0.88	0.70	0.60	0.65	0.81
Phosphagen System													
Creatine kinase B-type	sp | P07335 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Creatine kinase, mitochondrial 1, ubiquitous	tr | Q5BJT9 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.80	0.85	0.89

Other													
3'(2'),5'-bisphosphate nucleotidase 1	sp | Q9Z1N4 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95

Neurotransmission/signalling

Cell Adhesion													
Neural cell adhesion molecule 1 (Fragment)	tr | F1LNY3 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	0.90	0.95	1.00
Limbic system-associated membrane protein	sp | Q62813 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.80	0.85	0.89
Neurocan core protein	tr | G3V8R2 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Neurofascin	sp | P97685 |	1	1.10	1.05	1.0	0.90	1.10	1.00	0.95	1.10	1.10	1.10	1.05
Neuronal growth regulator 1	sp | Q9Z0J8 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.10	1.00	1.05	1.11
Neurotrimin	tr | G3V964 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Obg-like ATPase 1	sp | A0JPJ7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Opioid-binding protein/cell adhesion molecule	sp | P32736 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	0.90	0.95	1.00
Peripheral plasma membrane protein CASK	sp | Q62915 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Protein Cdh13 (Fragment)	tr | F1M7X3 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	0.90	0.80	0.85	0.85
Protein Cttn	tr | D3ZGE6 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	0.90	0.95	0.90
Synaptosomal-associated protein 25	sp | P60881 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Tenascin-R	sp | Q05546 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Versican core protein (Fragments)	sp | Q9ERB4 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Thy-1 membrane glycoprotein	sp | P01830 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.80	0.85	0.94
Neuroplastin	tr | D3ZDF0 |	1	0.80	0.90	1.0	0.90	1.00	0.95	1.06	0.80	0.80	0.80	0.89
Protein Omg	tr | F7EYB9 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	0.90	0.95	0.95
Protein Icam5	tr | D4A435 |	1	1.10	1.05	1.0	1.10	1.20	1.15	1.10	1.00	1.00	1.00	0.95
Vesicle Regulation													
Vesicle-associated membrane protein 1 (Fragment)	tr | F1M9V2 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.80	0.70	0.75	0.88
Vesicle associated membrane protein 2B	tr | Q9WUW2 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Vesicle-associated membrane protein-associated protein A	sp | Q9Z270 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	0.90	1.00	0.95	0.95
Vesicle-fusing ATPase	sp | Q9QUL6 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Vesicle-trafficking protein SEC22b	sp | Q4KM74 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00

Vesicular glutamate transporter 1	sp | Q62634 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Adaptin ear-binding coat-associated protein 1	sp | P69682 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Adaptor protein complex AP-1, sigma 1 (Predicted), isoform CRA_b	tr | B5DFI3 |	1	1.00	1.00	1.0	1.10	1.30	1.20	**1.20**	1.10	1.00	1.05	1.05
Adaptor protein complex AP-2, alpha 1 subunit (Predicted)	tr | D3ZUY8 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Adaptor-related protein complex 1, gamma 1 subunit, isoform CRA_b	tr | B2RYN6 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	0.90	0.95	0.95
Adaptor-related protein complex 3, delta 1 subunit, isoform CRA_b	tr | B5DFK6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.00	1.05	1.05
AP-1 complex subunit beta-1	tr | G3V9N8 |	1	1.20	1.10	1.0	1.10	1.00	1.05	0.95	1.10	1.20	1.15	1.05
AP-2 complex subunit alpha-2	sp | P18484 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
AP-2 complex subunit beta	sp | P62944 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AP-2 complex subunit mu	sp | P84092 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
AP-2 complex subunit sigma	sp | P62744 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
AP2-associated protein kinase 1	sp | P0C1X8 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
AP-3 complex subunit mu-2	sp | P53678 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	0.90	0.95	0.95
Alpha-soluble NSF attachment protein	sp | P54921 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00
Beta-soluble NSF attachment protein	tr | F8WFM2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Calnexin	sp | P35565 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Clathrin coat assembly protein AP180	tr | F1LRK0 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Clathrin heavy chain	tr | F1M779 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Clathrin light chain A	sp | P08081 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Clathrin light chain B	sp | P08082 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Coatomer subunit delta	sp | Q66H80 |	1	1.10	1.05	1.0	1.20	0.90	1.05	1.00	1.10	1.00	1.05	1.00
Complexin-2	sp | P84087 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	1.10	1.10	1.10	1.05
Copa protein	tr | B5DFK1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.80	0.90	0.85	0.85
EH domain-containing protein 1	sp | Q641Z6 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.10	0.90	1.00	1.05
EH domain-containing protein 3	sp | Q8R491 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Endophilin-A1 (Fragment)	tr | F1LQ05 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Endophilin-A2	sp | O35964 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.20	1.15	1.10
Endophilin-B2	tr | D4A7V1 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.10	1.05	1.05
Myc box-dependent-interacting protein 1	sp | O08839 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Protein Psd3	tr | D3ZUW0 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95

Protein Snx2	tr | B2RYP4 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	0.80	0.90	0.85	0.89
Reticulon	tr | F1LQN3 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Reticulon-1	sp | Q64548 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Reticulon-3	sp | Q6RJR6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Secernin-1	sp | Q6AY84 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Secretory carrier-associated membrane protein 5 (Fragment)	tr | F1M882 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.80	0.80	0.80	0.89
SH3 domain-binding glutamic acid-rich-like protein	tr | B5DFD8 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	0.90	0.90	0.90
SH3-containing GRB2-like protein 3-interacting protein 1	sp | P0DJJ3 |	1	1.00	1.00	1.0	1.20	1.30	1.25	**1.25**	1.20	1.10	1.15	1.15
SRC kinase signaling inhibitor 1	sp | Q9QXY2 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	1.00	1.10	1.05	1.05
Synapsin-1	sp | P09951 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Synapsin-2	sp | Q63537 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Synaptogyrin-1	sp | Q62876 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	0.90	0.90	0.90	0.95
Synaptic vesicle glycoprotein 2A	sp | Q02563 |	1	0.80	0.90	1.0	1.00	0.90	0.95	1.06	0.90	0.90	0.90	1.00
Synaptic vesicle glycoprotein 2B	sp | Q63564 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
Synaptobrevin homolog YKT6	sp | Q5EGY4 |	1	1.20	1.10	1.0	1.10	1.20	1.15	1.05	1.00	1.00	1.00	0.91
Synaptojanin-1	sp | Q62910 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Synaptophysin	sp | P07825 |	1	0.90	0.95	1.0	0.80	0.90	0.85	0.89	0.90	1.00	0.95	1.00
Syntaxin 1A	tr | Q9QXG3 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Syntaxin-1B	sp | P61265 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Syntaxin-binding protein 1	sp | P61765 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Unconventional myosin-Va	sp | Q9QYF3 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Amphiphysin	sp | O08838 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein kinase C and casein kinase substrate in neurons protein 1	tr | F1LPP3 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Dynamin-1	sp | P21575 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dynamin-1-like protein	sp | O35303 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Dynamin-3	sp | Q08877 |	1	1.50	1.25	1.0	1.20	1.30	1.25	1.00	1.20	1.70	1.45	1.16
Protein Syngr3	tr | D4ABK1 |	1	0.90	0.95	1.0	0.80	1.00	0.90	0.95	0.80	0.80	0.80	0.84
Synaptotagmin-1	sp | P21707 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Calcium-dependent secretion activator 1	sp | Q62717 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Transport

Excitatory amino acid transporter 1	sp | P24942 |	1	0.70	0.85	1.0	1.00	0.90	0.95	1.12	0.80	0.70	0.75	0.88
Excitatory amino acid transporter 2	sp | P31596 |	1	0.70	0.85	1.0	0.80	0.70	0.75	0.88	0.80	0.70	0.75	0.88
Acyl-CoA-binding protein	tr | Q6TXF3 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Disks large homolog 3	sp | Q62936 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	1.00	0.95	1.00
ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1	tr | Q5M7T6 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	1.00	0.95	1.00
ATPase, H+ transporting, V1 subunit D, isoform CRA_c	tr | Q6P503 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
ATPase, H+ transporting, V1 subunit E isoform 1, isoform CRA_a	tr | G3V7L8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ATPase, H+ transporting, V1 subunit G isoform 2	tr | Q8R2H0 |	1	0.90	0.95	1.0	1.20	1.00	1.10	1.16	0.90	1.10	1.00	1.05
V-H+ATPase subunit a1-III	tr | Q2I6B2 |	1	0.80	0.90	1.0	0.90	1.00	0.95	1.06	0.90	0.90	0.90	1.00
V-type proton ATPase subunit B, brain isoform	sp | P62815 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
V-type proton ATPase subunit C 1	sp | Q5FVI6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
V-type proton ATPase subunit F	sp | P50408 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Protein Atp6v1a	tr | D4A133 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Protein Atp6v1h	tr | E9PTI1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Voltage-dependent anion-selective channel protein 1	sp | Q9Z2L0 |	1	0.80	0.90	1.0	0.70	0.90	0.80	0.89	0.80	0.80	0.80	0.89
Voltage-dependent anion-selective channel protein 2	sp | P81155 |	1	0.80	0.90	1.0	0.70	0.90	0.80	0.89	0.80	0.80	0.80	0.89
Voltage-dependent calcium channel subunit alpha-2/delta-1	tr | D3ZKP9 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.30	1.20	1.25	1.14
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	sp | P11507 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	1.00	0.95	1.00
Plasma membrane calcium-transporting ATPase 1	sp | P11505 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Plasma membrane calcium-transporting ATPase 2	tr | D3ZCE9 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Plasma membrane calcium-transporting ATPase 4	tr | D3ZH00 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.80	0.85	0.94
Sodium/potassium-transporting ATPase subunit alpha-1	sp | P06685 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.90	0.80	0.85	1.00
Sodium/potassium-transporting ATPase subunit alpha-2	sp | P06686 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Sodium/potassium-transporting ATPase subunit alpha-3	sp | P06687 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.90	0.80	0.85	1.00

Sodium/potassium-transporting ATPase subunit beta-1	sp | P07340 |	1	0.70	0.85	1.0	0.80	0.80	0.80	0.94	0.90	0.90	0.90	1.06
Sodium-dependent neutral amino acid transporter SLC6A17	sp | P31662 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha-endosulfine	sp | P60841 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	1.00	1.00	1.00	1.00
Solute carrier family 12 member 5	sp | Q63633 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Astrocytic phosphoprotein PEA-15	sp | Q5U318 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cytoplasmic dynein 1 heavy chain 1	tr | M0R9X8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cytoplasmic dynein 1 light intermediate chain 1	sp | Q9QXU8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Cytoplasmic dynein 1 light intermediate chain 2	sp | Q62698 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	0.90	0.90	0.90
Dynein light chain 2, cytoplasmic	sp | Q78P75 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Dynein light chain roadblock-type 1	sp | P62628 |	1	1.20	1.10	1.0	1.30	1.10	1.20	1.09	1.20	1.10	1.15	1.05
Fatty acid-binding protein, epidermal	sp | P55053 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
Fatty acid-binding protein	sp | P07483 |	1	1.20	1.10	1.0	1.20	1.20	1.20	1.09	1.10	1.20	1.15	1.05
Importin 7 (Predicted), isoform CRA_c	tr | D4AE96 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
Importin subunit beta-1	tr | F2Z3Q8 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00
LIM and SH3 domain protein 1	sp | Q99MZ8 |	1	0.90	0.95	1.0	1.10	1.10	1.10	1.16	1.00	1.00	1.00	1.05
Mitochondrial import receptor subunit TOM34	sp | Q3KRD5 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Phosphofurin acidic cluster sorting protein 1	tr | F1LPG3 |	1	1.10	1.05	1.0	1.30	1.10	1.20	1.14	1.00	1.00	1.00	0.95
Protein Napg	tr | D4A0E2 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Protein Ranbp1	tr | D4A2G9 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Protein Tom1l2	tr D4A6C9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Rabphilin-3A	tr | F1LPB9 |	1	1.00	1.00	1.0	1.20	1.00	1.10	1.10	0.90	1.00	0.95	0.95
Vacuolar protein sorting-associated protein 29	sp | B2RZ78 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.80	0.85	0.89
Vacuolar protein sorting-associated protein 35	tr | G3V8A5 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Vps53	tr | D3ZPE5 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	0.90	1.10	1.00	0.95
Protein Ipo5	tr | D4A781 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.10	0.80	0.95	1.00
GTPase Signalling													
CB1 cannabinoid receptor-interacting protein 1	sp | Q5M7A7 |	1	1.30	1.15	1.0	1.20	1.10	1.15	1.00	1.10	1.10	1.10	0.96
GTP-binding nuclear protein Ran	sp | P62828 |	1	1.10	1.05	1.0	1.10	0.90	1.00	0.95	1.00	1.10	1.05	1.00
Septin 5, isoform CRA_d	tr | D3ZDH8 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Septin 7	tr | A2VCW8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Septin 8 (Predicted)	tr | G3V9Z6 |	1	0.90	0.95	1.0	0.90	1.10	1.00	1.05	1.00	0.90	0.95	1.00

Septin-11	sp | B3GNI6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Septin-2	sp | Q91Y81 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.10	1.05	1.05
Septin-9	sp | Q9QZR6 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Neuronal-specific septin-3	tr | D3ZPP8 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Protein Diras2	tr | D3ZHX3 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
ADP-ribosylation factor 3	sp | P61206 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.20	1.15	1.10
ADP-ribosylation factor 5	sp | P84083 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
ADP-ribosylation factor-like protein 3	sp | P37996 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	1.10	1.10	1.10	1.05
Cell division control protein 42 homolog	sp | Q8CFN2 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Guanine nucleotide binding protein, alpha q polypeptide, isoform CRA_a	tr | D4AE68 |	1	0.80	0.90	1.0	1.00	1.00	1.00	1.11	0.90	1.00	0.95	1.06
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	sp | P54311 |	1	0.80	0.90	1.0	1.00	1.00	1.00	1.11	1.00	0.90	0.95	1.06
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	sp | P54313 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Guanine nucleotide-binding protein G(k) subunit alpha	sp | P08753 |	1	0.70	0.85	1.0	1.10	1.10	1.10	**1.29**	0.80	0.90	0.85	1.00
Guanine nucleotide-binding protein G(o) subunit alpha	sp | P59215 |	1	0.70	0.85	1.0	0.90	0.80	0.85	1.00	0.90	0.90	0.90	1.06
Guanine nucleotide-binding protein subunit beta-2-like 1	sp | P63245 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.00	1.10	1.05	0.95
Guanine nucleotide-binding protein subunit beta-5	sp | P62882 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Guanine nucleotide-binding protein subunit gamma	tr | G3V8K2 |	1	0.60	0.80	1.0	0.80	1.00	0.90	1.13	0.80	0.80	0.80	1.00
Neuronal guanine nucleotide exchange factor (Predicted)	tr | G3V856 |	1	1.10	1.05	1.0	1.00	0.90	0.95	0.90	0.90	0.90	0.90	0.86
Cytohesin-2	sp | P63035 |	1	1.10	1.05	1.0	0.90	1.30	1.10	1.05	1.00	1.00	1.00	0.95
Ras-related C3 botulinum toxin substrate 1	sp | Q6RUV5 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.90	0.90	0.90	1.00
Ras-related protein Rab-11B	sp | O35509 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ras-related protein Rab-18	sp | Q5EB77 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	1.10	0.90	1.00	1.00
Ras-related protein Rab-1A	sp | Q6NYB7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ras-related protein Rab-2A	sp | P05712 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	0.90	0.90	0.90	0.90
Ras-related protein Rab-3A	sp | P63012 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	1.00	1.00	1.05
Ras-related protein Rab-3C	sp | P62824 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Ras-related protein Rab-6A	sp | Q9WVB1 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	1.00	0.95	1.00

Ras-related protein Rab-7a	sp | P09527 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Ras-related protein Ral-A	sp | P63322 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.80	0.90	0.85	0.89
Ras-related protein Rap-1A	sp | P62836 |	1	0.80	0.90	1.0	1.00	0.90	0.95	1.06	0.90	1.00	0.95	1.06
Protein Rab5c	tr | B0BNK1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Rab6b	tr | F1LVC3 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Protein Rab5b	tr | A1L1J8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
GTPase NRas	sp | Q04970 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Rho GDP-dissociation inhibitor 1	sp | Q5XI73 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Transforming protein RhoA	sp | P61589 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
SLIT-ROBO Rho GTPase-activating protein 2	tr | B5DEJ1 |	1	0.80	0.90	1.0	1.00	0.90	0.95	1.06	0.90	1.00	0.95	1.06
Protein Rap1gds1	tr | F1M7Y3 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Rab GDP dissociation inhibitor alpha	sp | P50398 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Rab GDP dissociation inhibitor beta	sp | P50399 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
RAB14, member RAS oncogene family	tr | B0BMW0 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
RAB10, member RAS oncogene family	tr | Q5RKJ9 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
RAB5A, member RAS oncogene family	tr | O88565 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	1.10	1.00	1.05	1.00
Protein Sbf1	tr | D3ZNN0 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	0.90	0.95	0.95
Protein Abi2	tr | F1LYA6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Arhgap1	tr | D4A6C5 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
Regulator of G-protein signaling 7	sp | P49803 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Protein Pkn3	tr | D3ZC07 |	1	1.00	1.00	1.0	1.20	1.00	1.10	1.10	1.00	1.00	1.00	1.00
Serine/threonine-protein kinase BRSK1	sp | B2DD29 |	1	1.30	1.15	1.0	1.20	1.00	1.10	0.96	1.20	1.30	1.25	1.09
Serine/threonine-protein kinase DCLK1	sp | O08875 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Serine/threonine-protein kinase PAK 1	sp | P35465 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	sp | P36876 |	1	1.10	1.05	1.0	1.80	1.00	1.40	**1.33**	1.00	1.10	1.05	1.00
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	sp | P63331 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	sp | P63329 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine/threonine-protein phosphatase 2B catalytic subunit beta isoform	sp | P20651 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	1.00	0.95	0.95
Serine/threonine-protein phosphatase 5	sp | P53042 |	1	1.10	1.05	1.0	1.00	0.90	0.95	0.90	1.00	0.90	0.95	0.90
Serine/threonine-protein phosphatase PP1-alpha	sp | P62138 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05

catalytic subunit													
Serine/threonine-protein phosphatase PP1-beta catalytic subunit	sp | P62142 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Ppp2r1a	tr | Q5XI34 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Ppp2r4	tr | B2RYQ2 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Nucleoside diphosphate kinase A	sp | Q05982 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Adenylate kinase isoenzyme 1	sp | P39069 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Phosphatidylethanolamine-binding protein 1	sp | P31044 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma	sp | Q5I6B8 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	0.90	0.90	0.90
Phosphatidylinositol 5-phosphate 4-kinase type-2 beta	sp | O88377 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phosphatidylinositol transfer protein alpha isoform	sp | P16446 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.10	1.10	1.00
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1	sp | P10687 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Diphosphoinositol polyphosphate phosphohydrolase 1	sp | Q566C7 |	1	1.00	1.00	1.0	1.10	1.40	1.25	**1.25**	0.90	0.90	0.90	0.90
Inositol monophosphatase 1	tr | F1M978 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Inositol-trisphosphate 3-kinase A	sp | P17105 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	0.90	0.90	0.90
Phosphatidate cytidylyltransferase 2	sp | Q91XU8 |	1	0.90	0.95	1.0	0.90	1.10	1.00	1.05	0.80	1.00	0.90	0.95
ArfGAP with dual PH domains 1	tr | O88768 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	1.00	0.95	0.95
Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B	sp | Q01066 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	1.00	0.90	0.95	1.00
cGMP-dependent 3',5'-cyclic phosphodiesterase	tr | F8WFW5 |	1	1.30	1.15	1.0	1.10	1.20	1.15	1.00	1.20	1.10	1.15	1.00
cAMP-dependent protein kinase type II-alpha regulatory subunit	sp | P12368 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.00	1.05	1.00
cAMP-dependent protein kinase type II-beta regulatory subunit	sp | P12369 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Protein kinase, cAMP-dependent, catalytic, alpha	tr | A1L1M0 |	1	1.00	1.00	1.0	0.80	0.90	0.85	0.85	1.00	1.00	1.00	1.00
A-kinase anchor protein 5	tr | F1LPP6 |	1	1.20	1.10	1.0	1.40	1.00	1.20	1.09	1.00	1.00	1.00	0.91
Tyrosine-protein phosphatase non-receptor type 11	sp | P41499 |	1	1.00	1.00	1.0	0.80	0.90	0.85	0.85	1.10	0.90	1.00	1.00
Protein kinase C beta type (Fragment)	tr | F1LS42 |	1	0.80	0.90	1.0	0.90	1.00	0.95	1.06	1.00	1.00	1.00	1.11
Protein kinase C gamma type	sp | P63319 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Protein kinase C	tr | F1LMV8 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.00	1.10	1.05	0.95

Dual specificity mitogen-activated protein kinase kinase 1	sp | Q01986 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
MAP/microtubule affinity-regulating kinase 3	tr | F1M836 |	1	1.00	1.00	1.0	0.80	0.80	0.80	**0.80**	1.00	1.00	1.00	1.00
Mitogen-activated protein kinase 1	sp | P63086 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mitogen-activated protein kinase 9	tr | D4A5V8 |	1	1.10	1.05	1.0	0.90	0.90	0.90	0.86	1.10	1.20	1.15	1.10
Protein Map2k4 (Fragment)	tr | F1LP57 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.20	1.10	1.15	1.15
Dusp3 protein	tr | B5DFF7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Connector enhancer of kinase suppressor of ras 3	sp | Q5SGD7 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	0.90	0.95	0.95
Calcium Signalling													
Calcium/calmodulin-dependent protein kinase II, beta, isoform CRA_a	tr | G3V9G3 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Calcium/calmodulin-dependent protein kinase type II subunit alpha	sp | P11275 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	1.00	1.00	1.05
Calcium/calmodulin-dependent protein kinase type II subunit delta	tr | F1LWF6 |	1	0.90	0.95	1.0	1.10	1.10	1.10	1.16	1.00	1.10	1.05	1.11
Calcium/calmodulin-dependent protein kinase type II subunit gamma	sp | P11730 |	1	0.90	0.95	1.0	0.80	0.90	0.85	0.89	1.00	1.00	1.00	1.05
Calcium/calmodulin-dependent protein kinase type IV	sp | P13234 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	0.90	0.95	0.95
Calmodulin	sp | P62161 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.10	1.20	1.15	1.15
Neurogranin	sp | Q04940 |	1	1.10	1.05	1.0	1.20	1.10	1.15	1.10	1.00	1.10	1.05	1.00
Purkinje cell protein 4	sp | P63055 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	1.00	1.00	1.00	1.00
Neurochondrin	sp | O35095 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Calbindin	sp | P07171 |	1	1.10	1.05	1.0	1.10	0.90	1.00	0.95	1.00	1.10	1.05	1.00
Calcineurin subunit B type 1	sp | P63100 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Calretinin	sp | P47728 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.10	1.00	1.00
CaM kinase-like vesicle-associated protein	sp | Q63092 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00
Hippocalcin-like protein 1	sp | P62749 |	1	0.80	0.90	1.0	0.90	1.00	0.95	1.06	0.80	0.90	0.85	0.94
Hippocalcin-like protein 4	sp | P35332 |	1	1.20	1.10	1.0	1.10	1.00	1.05	0.95	1.30	1.20	1.25	1.14
Neurocalcin-delta	sp | Q5PQN0 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Visinin-like protein 1	sp | P62762 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.20	1.20	1.20	**1.20**
Annexin A3	sp | P14669 |	1	1.30	1.15	1.0	0.70	0.90	0.80	**0.70**	0.90	1.20	1.05	0.91
Annexin A5	sp | P14668 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Reticulocalbin-2	sp | Q62703 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	0.90	0.95	0.95

Calcium binding protein 39 (Predicted), isoform CRA_a	tr | D3ZJ77 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Neuron-specific calcium-binding protein hippocalcin	sp | P84076 |	1	0.80	0.90	1.0	1.00	0.90	0.95	1.06	0.90	0.90	0.90	1.00
Protein S100-B	sp | P04631 |	1	1.20	1.10	1.0	1.10	1.30	1.20	1.09	1.00	1.10	1.05	0.95
Neuronal membrane glycoprotein M6-a	sp | Q812E9 |	1	0.60	0.80	1.0	0.70	0.80	0.75	0.94	0.80	0.80	0.80	1.00
Other													
4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans), isoform CRA_b	tr | G3V728 |	1	0.90	0.95	1.0	1.00	1.20	1.10	1.16	1.00	0.90	0.95	1.00
14-3-3 protein beta/alpha	sp | P35213 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.20	1.15	1.10
14-3-3 protein epsilon	sp | P62260 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
14-3-3 protein eta	sp | P68511 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
14-3-3 protein gamma	sp | P61983 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
14-3-3 protein theta	sp | P68255 |	1	1.10	1.05	1.0	0.90	1.00	0.95	0.90	1.00	1.10	1.05	1.00
14-3-3 protein zeta/delta	sp | P63102 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
ERC protein 2	sp | Q8K3M6 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Homer protein homolog 1	sp | Q9Z214 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.10	0.90	1.00	1.05
Kinesin heavy chain isoform 5C (Fragment)	tr | G3V6L4 |	1	1.10	1.05	1.0	0.90	1.10	1.00	0.95	1.20	1.10	1.15	1.10
LanC-like protein 1	sp | Q9QX69 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Platelet-activating factor acetylhydrolase IB subunit alpha	sp | P63004 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Platelet-activating factor acetylhydrolase IB subunit beta	sp | O35264 |	1.1	1.00	1.05	1.0	1.20	1.20	1.20	1.14	1.00	1.00	1.00	0.95
Protein bassoon	tr | G3V984 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	0.90	0.90	0.90	0.95
Protein Dmxl2 (Fragment)	tr | F1M164 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein phosphatase 1 regulatory subunit 1B	sp | Q6J4I0 |	1	0.90	0.95	1.0	0.80	0.90	0.85	0.89	1.10	1.00	1.05	1.11
Protein phosphatase 1 regulatory subunit 7	sp | Q5HZV9 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	1.00	0.95	0.95
Protein phosphatase 1, regulatory subunit 9B	tr | B1H262 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Protein phosphatase 1E	sp | Q80Z30 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
Protein phosphatase methylesterase 1	sp | Q4FZT2 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Protein Srgap3	tr | F1M5M9 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
Stathmin	sp | P13668 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Wiskott-Aldrich syndrome protein family member 1	sp | Q5BJU7 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00

Alpha-synuclein	sp | P37377 |	1	1.20	1.10	1.0	1.20	1.10	1.15	1.05	1.20	1.30	1.25	1.14
Beta-synuclein	sp | Q63754 |	1	1.20	1.10	1.0	1.30	1.10	1.20	1.09	1.10	1.20	1.15	1.05
Glutamate decarboxylase 2	sp | Q05683 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	0.90	0.90	0.90
Glutamine synthetase	sp | P09606 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein NDRG2	sp | Q8VBU2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Receptor expression-enhancing protein 5	sp | B2RZ37 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	0.90	0.95	0.95
Tumor protein D54	sp | Q6PCT3 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Neuronal pentraxin receptor (Fragment)	tr | F1LSY2 |	1	0.90	0.95	1.0	0.80	0.80	0.80	0.84	0.70	0.80	0.75	**0.79**
Phytanoyl-CoA hydroxylase-interacting protein	sp | Q568Z9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phytanoyl-CoA hydroxylase-interacting protein-like	sp | Q6AYN4 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
Receptor-type tyrosine-protein phosphatase zeta	sp | Q62656 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.00	1.10	1.05	0.95

Redox regulation

6-phosphogluconate dehydrogenase, decarboxylating	sp | P85968 |	1	1.20	1.10	1.0	1.10	1.20	1.15	1.05	1.20	1.00	1.10	1.00
Glyceraldehyde-3-phosphate dehydrogenase	sp | P04797 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	sp | O35077 |	1	1.10	1.05	1.0	0.90	1.00	0.95	0.90	1.10	1.20	1.15	1.10
Alcohol dehydrogenase [NADP(+)]	sp | P51635 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Aldehyde dehydrogenase family 6, subfamily A1, isoform CRA_b	tr | G3V7J0 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Aldehyde dehydrogenase, mitochondrial	tr | F1LN88 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Dihydrolipoyl dehydrogenase, mitochondrial	sp | Q6P6R2 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
L-lactate dehydrogenase B chain	sp | P42123 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
L-lactate dehydrogenase	tr | B5DEN4 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Cytosolic 10-formyltetrahydrofolate dehydrogenase	sp | P28037 |	1	0.80	0.90	1.0	1.00	0.90	0.95	1.06	0.80	1.00	0.90	1.00
3-hydroxyacyl-CoA dehydrogenase type-2	tr | B0BMW2 |	1	1.20	1.10	1.0	1.10	1.00	1.05	0.95	1.10	1.20	1.15	1.05
D-3-phosphoglycerate dehydrogenase	sp | O08651 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	0.90	0.95	0.95
Succinate-semialdehyde dehydrogenase, mitochondrial	tr | D3ZWV7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Peroxiredoxin 3	tr | G3V7I0 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	0.90	0.95	0.95
Peroxiredoxin-1	sp | Q63716 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00

Peroxiredoxin-2	sp | P35704 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Peroxiredoxin-5, mitochondrial (Fragment)	tr | D3ZEN5 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.10	1.10	1.10	1.10
Peroxiredoxin-6	sp | O35244 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Thioredoxin	sp | P11232 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Thioredoxin reductase 1, cytoplasmic	tr | R9PXU4 |	1	0.80	0.90	1.0	1.00	1.00	1.00	1.11	0.80	1.00	0.90	1.00
Thioredoxin-like protein 1	sp | Q920J4 |	1	1.00	1.00	1.0	0.90	0.80	0.85	0.85	1.00	1.00	1.00	1.00
Aldose reductase	sp | P07943 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Biliverdin reductase A	sp | P46844 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.00	1.20	1.10	1.00
Biliverdin reductase B (Flavin reductase (NADPH))	tr | B5DF65 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	0.90	0.95	0.95
Carbonyl reductase [NADPH] 1	sp | P47727 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Dihydropteridine reductase	sp | P11348 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Electron transfer flavoprotein subunit alpha, mitochondrial	sp | P13803 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.10	1.05	1.05
Ketimine reductase mu-crystallin	sp | Q9QYU4 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Oxidation resistance protein 1	sp | Q4V8B0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Prenylcysteine oxidase	sp | Q99ML5 |	1	1.10	1.05	1.0	1.10	0.90	1.00	0.95	1.00	1.00	1.00	0.95
Protein Sh3bgrl3	tr | B2RZ27 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	1.00	0.90	0.95	0.95
Redox-regulatory protein FAM213A	sp | Q6AXX6 |	1	0.80	0.90	1.0	0.70	0.90	0.80	0.89	0.80	0.90	0.85	0.94

Protein synthesis/neurotrophic

Transcription

Transcription elongation factor B polypeptide 1	sp | P83941 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
Transcription elongation factor B polypeptide 2	sp | P62870 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	0.90	0.95	0.95
Transcriptional activator protein Pur-alpha	tr | F1LPS8 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Transcriptional activator protein Pur-beta	sp | Q68A21 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	1.00	0.95	0.95
Transgelin-3	sp | P37805 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Histone H1.4	sp | P15865 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Histone H2A type 1-C	sp | P0C169 |	1	1.10	1.05	1.0	1.00	0.90	0.95	0.90	1.10	1.20	1.15	1.10
Histone H2A.Z	sp | P0C0S7 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.10	1.10	1.10	1.10
Histone H2B	tr | D3ZNH4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Histone H3	tr | B0BMY8 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Histone H4	sp | P62804 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brain acid soluble protein 1	sp | Q05175 |	1	0.70	0.85	1.0	1.00	0.80	0.90	1.06	0.90	1.00	0.95	1.12
Nucleolin	sp | P13383 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Nucleosome assembly protein 1-like 1	tr | G3V6H9 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Nucleosome assembly protein 1-like 4	sp | Q5U2Z3 |	1	1.10	1.05	1.0	1.20	1.10	1.15	1.10	1.10	1.10	1.10	1.05
Heterogeneous nuclear ribonucleoprotein A1	tr | F7FEZ6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Heterogeneous nuclear ribonucleoprotein A3	sp | Q6URK4 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Heterogeneous nuclear ribonucleoprotein C (C1/C2)	tr | G3V9R8 |	1	1.10	1.05	1.0	0.90	1.00	0.95	0.90	1.10	1.20	1.15	1.10
Heterogeneous nuclear ribonucleoprotein D, isoform CRA_b	tr | G3V6A4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heterogeneous nuclear ribonucleoprotein F	sp | Q794E4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heterogeneous nuclear ribonucleoprotein H2	sp | Q6AY09 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	0.90	0.95	0.95
Heterogeneous nuclear ribonucleoprotein M	sp | Q62826 |	1	1.20	1.10	1.0	0.90	1.10	1.00	0.91	1.00	0.80	0.90	0.82
Heterogeneous nuclear ribonucleoproteins A2/B1 (Fragment)	tr | F1LNF1 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Protein Hnrnpl	tr | F1LQ48 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	0.90	0.95	0.95
Hnrpk protein	tr | Q5D059 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Small nuclear ribonucleoprotein-associated protein	tr | Q63747 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	0.80	0.90	0.90
Nucleophosmin	sp | P13084 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
LRRGT00192	tr | Q6QI16 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Leucine-rich PPR motif-containing protein, mitochondrial	tr | F1LM33 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.10	1.05	1.11
Leucine-rich repeat-containing protein 57	tr | F1LN70 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
Activated RNA polymerase II transcriptional coactivator p15	sp | Q63396 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	0.90	0.90	0.90
DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 (Fragment)	tr | B6DTP5 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	0.90	0.90	0.90
DEAH (Asp-Glu-Ala-His) box polypeptide 9 (Predicted)	tr | D4A9D6 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
ELAV (Embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	tr | B5DF91 |	1	1.10	1.05	1.0	1.20	1.10	1.15	1.10	1.00	0.90	0.95	0.90
ELAV (Embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)	tr | B0BMT8 |	1	1.20	1.10	1.0	1.00	1.20	1.10	1.00	1.10	1.00	1.05	0.95
Protein Tardbp	tr | I6L9G6 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	0.90	1.00	0.95	0.95

Spliceosome RNA helicase Ddx39b	sp | Q63413 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.20	1.15	1.10
Plasminogen activator inhibitor 1 RNA-binding protein	sp | Q6AXS5 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Matrin-3	sp | P43244 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
RNA binding motif protein, X-linked-like-1	sp | D4AE41 |	1	0.80	0.90	1.0	0.80	0.70	0.75	0.83	0.70	0.70	0.70	**0.78**
O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), isoform CRA_b	tr | G3V6F4 |	1	1.00	1.00	1.0	1.20	1.00	1.10	1.10	1.00	1.00	1.00	1.00
Scaffold attachment factor B1	sp | O88453 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	0.80	0.70	0.75	**0.75**
Non-POU domain-containing octamer-binding protein	sp | Q5FVM4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Btaf1	tr | F1LW16 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
KH domain-containing, RNA-binding, signal transduction-associated protein 1	sp | Q91V33 |	1	1.10	1.05	1.0	0.90	0.90	0.90	0.86	1.00	1.10	1.05	1.00
Protein Rnf14	tr | Q3ZAU6 |	1	1.20	1.10	1.0	0.90	1.20	1.05	0.95	1.10	1.00	1.05	0.95
Translation													
Elongation factor 1-alpha 1	sp | P62630 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Elongation factor 1-alpha 2	sp | P62632 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Elongation factor 1-delta	sp | Q68FR9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Elongation factor 1-gamma	sp | Q68FR6 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Elongation factor 2	sp | P05197 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Elongation factor Tu, mitochondrial	sp | P85834 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Eukaryotic initiation factor 4A-II	sp | Q5RKI1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Eukaryotic translation initiation factor 2 subunit 1	sp | P68101 |	1	1.20	1.10	1.0	1.20	1.10	1.15	1.05	1.20	1.30	1.25	1.14
Eukaryotic translation initiation factor 5	sp | Q07205 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.10	1.00	1.05	1.11
Eukaryotic translation initiation factor 5A-1	sp | Q3T1J1 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Eukaryotic translation elongation factor 1 beta 2	tr | B5DEN5 |	1	1.40	1.20	1.0	1.50	1.00	1.25	1.04	1.00	1.20	1.10	0.92
Poly(A) binding protein, cytoplasmic 3	tr | B5DF80 |	1	1.10	1.05	1.0	1.20	1.00	1.10	1.05	1.00	1.30	1.15	1.10
Polyadenylate-binding protein 1	sp | Q9EPH8 |	1	0.90	0.95	1.0	1.10	1.00	1.05	1.11	0.80	0.70	0.75	**0.79**
40S ribosomal protein S14	tr | Q6PDV6 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	0.90	0.90	0.90	0.90
40S ribosomal protein S17	sp | P04644 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.20	1.15	1.05

40S ribosomal protein S28	sp | P62859 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.10	1.10	1.05
40S ribosomal protein S3	sp | P62909 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	0.90	1.00	0.95	1.00
40S ribosomal protein S3a	tr | M0R6L4 |	1	1.10	1.05	1.0	1.30	1.10	1.20	1.14	1.10	1.00	1.05	1.00
40S ribosomal protein S4, X isoform	sp | P62703 |	1	1.20	1.10	1.0	1.20	0.90	1.05	0.95	1.00	1.20	1.10	1.00
40S ribosomal protein S6 (Fragment)	tr | M0RD75 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.10	1.20	1.15	1.15
40S ribosomal protein S8	tr | B2RYR8 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.90	0.90	0.90	0.95
40S ribosomal protein S9	sp | P29314 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
40S ribosomal protein SA	sp | P38983 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	1.00	0.95	0.95
60S acidic ribosomal protein P0	sp | P19945 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
60S acidic ribosomal protein P1	sp | P19944 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	0.90	0.95	0.90
60S ribosomal protein L11	sp | P62914 |	1	1.20	1.10	1.0	1.00	1.00	1.00	0.91	1.00	1.00	1.00	0.91
60S ribosomal protein L13	tr | D3ZRM9 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
60S ribosomal protein L13a	tr | Q5RK10 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
60S ribosomal protein L17	sp | P24049 |	1	0.80	0.90	1.0	0.90	0.90	0.90	1.00	0.70	0.90	0.80	0.89
60S ribosomal protein L18 (Fragment)	tr | Q0QEW8 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
60S ribosomal protein L19	sp | P84100 |	1	1.00	1.00	1.0	1.20	1.20	1.20	**1.20**	0.90	0.90	0.90	0.90
60S ribosomal protein L23	sp | P62832 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
60S ribosomal protein L24	sp | P83732 |	1	1.10	1.05	1.0	1.10	0.90	1.00	0.95	1.00	1.10	1.05	1.00
60S ribosomal protein L27	sp | P61354 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	1.00	1.00	1.00	1.00
60S ribosomal protein L27a	sp | P18445 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
60S ribosomal protein L3	sp | P21531 |	1	1.10	1.05	1.0	1.10	0.90	1.00	0.95	1.00	1.00	1.00	0.95
60S ribosomal protein L31	sp | P62902 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
60S ribosomal protein L32	sp | P62912 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	0.90	0.90	0.90
60S ribosomal protein L4	tr | Q6P3V9 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
60S ribosomal protein L6	tr | F1LQS3 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
60S ribosomal protein L7	tr | B0K031 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
RCG25732, isoform CRA_b	tr | B5DEM5 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
RCG45400	tr | G3V7C6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.10	1.10	1.10
RCG45476, isoform CRA_d	tr | B0K021 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
RCG45615, isoform CRA_a	tr | B2RYU2 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	1.00	0.95	0.95
RCG62292, isoform CRA_a	tr | B5DEL9 |	1	1.00	1.00	1.0	1.30	1.00	1.15	1.15	1.00	1.00	1.00	1.00

Ribosomal protein (Fragment)	tr | Q4KM60 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.10	1.20	1.15	1.05
Ribosomal protein L9	tr | Q6P9U5 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.10	1.10	1.10	1.16
Ribonuclease UK114	sp | P52759 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	0.90	0.95	0.90
Rps16 protein (Fragment)	tr | B0K038 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Protein LOC100909878	tr | D3ZLL8 |	1	1.10	1.05	1.0	1.00	0.90	0.95	0.90	0.90	0.90	0.90	0.86
Protein LOC100911774	tr | Q1RP74 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	0.90	1.00	0.95	0.95
Protein LOC100911918 (Fragment)	tr | M0R7M8 |	1	1.30	1.15	1.0	1.10	1.10	1.10	0.96	1.10	1.20	1.15	1.00
Protein LOC100912210 (Fragment)	tr | F1M6F4 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Protein Nars	tr | F1LPV0 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.10	1.05	1.05
Pcbp2 protein	tr | Q6AYU2 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Protein IMPACT	sp | Q5GFD9 |	1	1.10	1.05	1.0	0.90	0.90	0.90	0.86	1.00	1.00	1.00	0.95
Post-translational modification													
Peptidylprolyl cis/trans isomerase, NIMA-interacting 1	tr | B0BNL2 |	1	1.10	1.05	1.0	0.90	0.90	0.90	0.86	1.10	1.10	1.10	1.05
Peptidyl-prolyl cis-trans isomerase A	sp | P10111 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Peptidyl-prolyl cis-trans isomerase B	sp | P24368 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Peptidyl-prolyl cis-trans isomerase FKBP1A	sp | Q62658 |	1	1.00	1.00	1.0	1.20	1.20	1.20	**1.20**	1.00	1.00	1.00	1.00
Protein disulfide-isomerase A3	sp | P11598 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Protein disulfide-isomerase A6	sp | Q63081 |	1	1.00	1.00	1.0	1.00	1.20	1.10	1.10	1.00	0.90	0.95	0.95
Protein disulfide-isomerase	sp | P04785 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Endoplasmin	sp | Q66HD0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Ppidl1	tr | M0RB67 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Biosynthesis													
Acyl carrier protein	tr | D3ZF13 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00
Fatty acid synthase	sp | P12785 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Cytosolic acyl coenzyme A thioester hydrolase	sp | Q64559 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Adenosylhomocysteinase (Fragment)	tr | B5DFN2 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Adenosylhomocysteinase	sp | P10760 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Bifunctional purine biosynthesis protein PURH	sp | O35567 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
N-acetylneuraminic acid synthase	tr | B1WC26 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
NSFL1 cofactor p47	sp | O35987 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Alanine--tRNA ligase, cytoplasmic	sp | P50475 |	1	1.20	1.10	1.0	1.20	1.10	1.15	1.05	1.10	1.10	1.10	1.00

Glycine--tRNA ligase	tr | G3V7G8 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Serine--tRNA ligase, cytoplasmic OS=Rattus norvegicus GN=Sars PE=1 SV=3	sp | Q6P799 |	1	1.10	1.05	1.0	1.10	1.20	1.15	1.10	1.10	1.10	1.10	1.05
Aspartyl-tRNA synthetase	tr | A9CMB7 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95

Protein degradation

Ubiquitin carboxyl-terminal hydrolase isozyme L1	sp | Q00981 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Ubiquitin carboxyl-terminal hydrolase	tr | D3ZVQ0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Ubiquitin carboxyl-terminal hydrolase	tr | D3ZC84 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.10	1.05	1.05
Ubiquitin carboxyl-terminal hydrolase	tr | G4XKZ2 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Ubiquitin thioesterase OTUB1	sp | B2RYG6 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	0.90	0.90	0.90	0.95
Ubiquitin-conjugating enzyme E2 N	sp | Q9EQX9 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Ubiquitin-like modifier-activating enzyme 1	sp | Q5U300 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Toll-interacting protein	sp | A2RUW1 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	0.90	1.00	0.95	0.95
Protein Ubqln2	tr | D4AA63 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Protein Ube2o (Fragment)	tr | F1M403 |	1	1.00	1.00	1.0	1.10	1.20	1.15	1.15	0.90	0.90	0.90	0.90
Protein Ube2m	tr D3ZNQ6 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	1.00	1.10	1.05	1.05
Polyubiquitin-C	tr | F1LML2 |	1	1.10	1.05	1.0	1.30	1.10	1.20	1.14	1.00	1.10	1.05	1.00
Huntingtin interacting protein 2 (Predicted), isoform CRA_a	tr | D3ZXS8 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	1.00	0.95	0.95
Ubiquilin-1	sp | Q9JJP9 |	1	0.90	0.95	1.0	1.00	0.80	0.90	0.95	1.10	1.00	1.05	1.11
UV excision repair protein RAD23 homolog B	sp | Q4KMA2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Transitional endoplasmic reticulum ATPase	sp | P46462 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.00	1.05	1.00
S-phase kinase-associated protein 1	sp | Q6PEC4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
F-box/LRR-repeat protein 16	sp | Q5MJ12 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	0.90	0.90	0.90	0.95
COP9 signalosome complex subunit 2	sp | P61203 |	1	1.20	1.10	1.0	1.00	1.00	1.00	0.91	1.00	1.10	1.05	0.95
COP9 signalosome complex subunit 4	sp | Q68FS2 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
COP9 signalosome complex subunit 8	sp | Q6P4Z9 |	1	1.30	1.15	1.0	1.30	1.30	1.30	1.13	1.10	1.10	1.10	0.96
26S protease regulatory subunit 4	sp | P62193 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	0.90	1.00	0.95
26S protease regulatory subunit 6A	sp | Q63569 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	0.90	0.95	0.90
26S protease regulatory subunit 7	tr | G3V7L6 |	1	0.90	0.95	1.0	1.10	1.10	1.10	1.16	1.00	1.00	1.00	1.05

26S protease regulatory subunit 8	sp | P62198 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	1.00	1.00	1.00	1.05
Protein Psmc6	tr | G3V6W6 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	0.90	0.95	0.95
Xaa-Pro aminopeptidase 1	sp | O54975 |	1	1.00	1.00	1.0	1.20	1.00	1.10	1.10	0.90	0.90	0.90	0.90
Aspartyl aminopeptidase	tr | Q4V8H5 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	0.90	1.00	0.95	0.95
Cytosolic non-specific dipeptidase	sp | Q6Q0N1 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.10	1.00	1.05	1.05
Tripeptidyl-peptidase 2	sp | Q64560 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Dipeptidyl peptidase 3	sp | O55096 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.80	0.90	0.85	0.89
Thimet oligopeptidase	sp | P24155 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
Anionic trypsin-1	sp | P00762 |	1	1.40	1.20	1.0	1.30	1.10	1.20	1.00	1.20	1.50	1.35	1.13
Acylamino-acid-releasing enzyme	sp | P13676 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	0.90	0.80	0.85	0.85
Cathepsin D	tr | Q6P6T6 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Isoaspartyl peptidase/L-asparaginase	sp | Q8VI04 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Proprotein convertase subtilisin/kexin type 1 inhibitor	tr | G3V6X7 |	1	1.10	1.05	1.0	1.20	1.10	1.15	1.10	1.00	0.90	0.95	0.90
Protein Npepps	tr | F1M9V7 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.00	1.00	0.95
SP120	tr | Q63555 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
26S proteasome non-ATPase regulatory subunit 13	sp | B0BN93 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
26S proteasome non-ATPase regulatory subunit 2	sp | Q4FZT9 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.00	1.05	1.00
Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 6	tr | Q6PCT9 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Proteasome subunit alpha type	tr | Q6P9V6 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.20	1.15	1.10
Proteasome subunit alpha type	tr | F1LSQ6 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Proteasome subunit alpha type-1	sp | P18420 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	0.90	0.95	0.95
Proteasome subunit alpha type-3	sp | P18422 |	1	1.10	1.05	1.0	0.90	1.10	1.00	0.95	1.00	1.10	1.05	1.00
Proteasome subunit alpha type-4	sp | P21670 |	1	1.10	1.05	1.0	0.90	0.90	0.90	0.86	1.10	1.10	1.10	1.05
Proteasome subunit alpha type-6	sp | P60901 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	1.00	0.95	0.95
Proteasome subunit beta type	tr | F1LNN1 |	1	1.10	1.05	1.0	0.90	1.00	0.95	0.90	1.00	0.90	0.95	0.90
Proteasome subunit beta type	tr | G3V8U9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proteasome subunit beta type-1	sp | P18421 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.00	1.00	0.95
Protein Pithd1	tr | D4ABS5 |	1	1.10	1.05	1.0	1.00	1.20	1.10	1.05	1.10	1.00	1.05	1.00
Protein Cops7a	tr | F1MAA2 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.00	1.00	1.00	0.91
4-aminobutyrate aminotransferase,	sp | P50554 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00

mitochondrial													

Other

Hemoglobin alpha, adult chain 2	tr | B1H216 |	1	1.30	1.15	1.0	1.10	1.10	1.10	0.96	1.10	1.10	1.10	0.96
Hemoglobin subunit beta-1	sp | P02091 |	1	1.30	1.15	1.0	1.10	1.10	1.10	0.96	1.10	1.20	1.15	1.00
Hemoglobin subunit beta-2	sp | P11517 |	1	1.30	1.15	1.0	1.10	1.10	1.10	0.96	1.10	1.10	1.10	0.96
Ferritin	tr | F1M5T1 |	1	0.90	0.95	1.0	1.20	1.10	1.15	**1.21**	1.10	0.80	0.95	1.00
Heat shock 70 kDa protein 4	tr | F1LRV4 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.10	1.05	1.05
Heat shock 70 kDa protein 4L	tr | B4F772 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	1.00	0.95	1.00
Heat shock 70kDa protein 12A (Predicted), isoform CRA_a	tr | D3ZC55 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.00	1.05	1.00
Heat shock cognate 71 kDa protein	sp | P63018 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heat shock protein 105 kDa	sp | Q66HA8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heat shock protein HSP 90-alpha	sp | P82995 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heat shock protein HSP 90-beta	sp | P34058 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
28 kDa heat- and acid-stable phosphoprotein	sp | Q62785 |	1	1.20	1.10	1.0	1.40	1.20	1.30	1.18	1.00	1.10	1.05	0.95
10 kDa heat shock protein, mitochondrial	sp | P26772 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
60 kDa heat shock protein, mitochondrial	sp | P63039 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Chaperonin containing Tcp1, subunit 6A (Zeta 1)	tr | Q3MHS9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chaperonin subunit 8 (Theta) (Predicted), isoform CRA_a	tr | D4ACB8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
DnaJ (Hsp40) homolog, subfamily A, member 2	tr | Q5M9H7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
DnaJ (Hsp40) homolog, subfamily C, member 6 (Predicted)	tr | D4A0I5 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Hsp90 co-chaperone Cdc37	sp | Q63692 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.20	1.10	1.05
Hsc70-interacting protein	sp | P50503 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Stress-induced-phosphoprotein 1	sp | O35814 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.00	1.00	0.95
Stress-70 protein, mitochondrial	tr | F1M953 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
T-complex protein 1 subunit alpha	sp | P28480 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
T-complex protein 1 subunit beta	sp | Q5XIM9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T-complex protein 1 subunit delta	sp | Q7TPB1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

T-complex protein 1 subunit epsilon	sp | Q68FQ0 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
T-complex protein 1 subunit gamma	sp | Q6P502 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
6-phosphofructokinase	tr | Q52KS1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-phosphofructokinase, liver type	sp | P30835 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.10	1.05	1.05
Glucose-6-phosphate isomerase	sp | Q6P6V0 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.10	1.05	1.00
Glycogen phosphorylase, brain form (Fragment)	sp | P53534 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha glucosidase 2 alpha neutral subunit (Predicted)	tr | D3ZAN3 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.80	0.80	0.80	0.84
78 kDa glucose-regulated protein	sp | P06761 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Fructosamine-3-kinase-related protein	tr | B2RYN1 |	1	0.70	0.85	1.0	0.80	0.70	0.75	0.88	0.70	0.70	0.70	0.82
Fructose-bisphosphate aldolase A	sp | P05065 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fructose-bisphosphate aldolase C	sp | P09117 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Ab2-076	tr | Q7TP61 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
Ab2-417	tr | Q7TMC7 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	1.00	1.00	1.00
Ac1002	tr | Q7TQ90 |	1	1.20	1.10	1.0	1.00	0.90	0.95	0.86	1.00	1.10	1.05	0.95
Active BCR-related gene (Predicted)	tr | D4A6K9 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Alpha-enolase	sp | P04764 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
C-1-tetrahydrofolate synthase, cytoplasmic	tr | G3V6S5 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	0.80	0.90	0.95
C38 protein	tr | B7X6I3 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	0.90	0.95	1.00
Uncharacterized protein (Fragment)	tr | B5DEP6 |	1	1.20	1.10	1.0	1.00	1.10	1.05	0.95	1.10	1.10	1.10	1.00
Uncharacterized protein (Fragment)	tr | M0RAR9 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.10	0.80	0.95	0.95
Uncharacterized protein (Fragment)	tr | F1M8C9 |	1	1.40	1.20	1.0	1.00	1.20	1.10	0.92	1.20	1.40	1.30	1.08
Uncharacterized protein	tr | D3ZIY7 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Uncharacterized protein	tr | D4A269 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
tr|D3ZAP7|D3ZAP7_RAT-DECOY Protein Chd7	tr | D3ZAP7 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.20	1.10	1.10
tr|D3ZV60|D3ZV60_RAT-DECOY Protein Cenpe	tr | D3ZV60 |	1	0.80	0.90	1.0	1.00	1.00	1.00	1.11	0.90	0.90	0.90	1.00
tr|F1LNQ8|F1LNQ8_RAT-DECOY Uncharacterized protein (Fragment)	tr | F1LNQ8 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
tr|F1LPM1|F1LPM1_RAT-DECOY Protocadherin Fat 2	tr | F1LPM1 |	1	1.30	1.15	1.0	1.20	1.30	1.25	1.09	1.30	1.50	1.40	1.22
tr|F1LY70|F1LY70_RAT-DECOY Protein Birc6 (Fragment)	tr | F1LY70 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	1.00	0.90	0.95	1.00

Protein LOC100361103	tr | D3Z9G3 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	0.90	0.90	0.90	0.90
Protein LOC100362298 (Fragment)	tr | D3ZM33 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Protein LOC100362339	tr | D4A6G6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein LOC100362751	tr | D4A4D5 |	1	1.10	1.05	1.0	1.20	1.20	1.20	1.14	1.10	1.20	1.15	1.10
Protein LOC100365676	tr | D4ABI7 |	1	0.80	0.90	1.0	0.80	0.80	0.80	0.89	0.80	0.80	0.80	0.89
Protein LOC683961	tr | M0RCY2 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.00	1.05	1.00
Prostaglandin reductase 2	sp | Q5BK81 |	1	0.90	0.95	1.0	0.90	1.20	1.05	1.11	0.80	0.90	0.85	0.89
Nitrilase 1, isoform CRA_a	tr | F7ESM5 |	1	0.90	0.95	1.0	1.10	1.20	1.15	**1.21**	1.10	1.00	1.05	1.11
D-dopachrome decarboxylase	sp | P80254 |	1	1.20	1.10	1.0	1.20	1.30	1.25	1.14	1.10	1.20	1.15	1.05
Glutathione S-transferase Mu 1	tr | G3V983 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Glutathione S-transferase Mu 5	sp | Q9Z1B2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glutathione S-transferase omega 1	tr | B6DYQ5 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Glutathione S-transferase	tr | B6DYP8 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glutathione S-transferase pi	tr | B6DYQ7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glutathione S-transferase Yb-3	sp | P08009 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Aspartate aminotransferase, cytoplasmic	sp | P13221 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	1.00	1.00	1.00	1.00
Aspartate aminotransferase, mitochondrial	sp | P00507 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Branched-chain-amino-acid aminotransferase, cytosolic	sp | P54690 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	0.90	0.95	0.95
Hypoxanthine-guanine phosphoribosyltransferase	sp | P27605 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
CDP-diacylglycerol--inositol 3-phosphatidyltransferase	sp | P70500 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	0.90	0.90	0.90
Phosphoserine aminotransferase	tr | Q68FU2 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	sp | P22062 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	sp | B2GV06 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.00	1.00	1.00
Thiosulfate sulfurtransferase	sp | P24329 |	1	1.00	1.00	1.0	1.20	1.10	1.15	1.15	1.00	0.90	0.95	0.95
Ornithine aminotransferase, mitochondrial	sp | P04182 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Adenine phosphoribosyltransferase	sp | P36972 |	1	1.10	1.05	1.0	1.40	1.20	1.30	**1.24**	1.20	1.20	1.20	1.14
Geranylgeranyl transferase type-2 subunit alpha	sp | Q08602 |	1	0.90	0.95	1.0	1.00	0.70	0.85	0.89	0.80	0.80	0.80	0.84
Acetyl-CoA acetyltransferase, mitochondrial	sp | P17764 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Acyl-CoA synthetase isoform 6 variant2	tr | Q6IU14 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95

Pyridoxal kinase	tr | G3V647 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	0.90	0.90	0.90	0.90
UMP-CMP kinase	sp | Q4KM73 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	0.90	1.10	1.00	0.95
Phosphoglycerate kinase 1	sp | P16617 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.10	1.10	1.05
Ribose-phosphate pyrophosphokinase 1	sp | P60892 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Hexokinase-1	sp | P05708 |	1	0.90	0.95	1.0	0.90	1.00	0.95	1.00	0.90	0.90	0.90	0.95
Aminopeptidase B	sp | O09175 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Atlastin-1	sp | Q6PST4 |	1	0.90	0.95	1.0	1.10	1.00	1.05	1.11	1.00	0.90	0.95	1.00
Calreticulin	sp | P18418 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Carbonic anhydrase 1	sp | B0BNN3 |	1	1.30	1.15	1.0	0.90	1.00	0.95	0.83	1.30	1.00	1.15	1.00
Carbonic anhydrase 2	sp | P27139 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.20	1.10	1.15	1.10
Clusterin	sp | P05371 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	0.90	0.90	0.90
Contactin-1	sp | Q63198 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cullin-3	sp | B5DF89 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.20	1.15	1.05
Cullin-associated NEDD8-dissociated protein 1	sp | P97536 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Cysteine and glycine-rich protein 1	sp | P47875 |	1	1.00	1.00	1.0	1.30	1.10	1.20	**1.20**	1.00	1.20	1.10	1.10
DnaJ homolog subfamily C member 5	sp | P60905 |	1	1.00	1.00	1.0	0.90	1.00	0.95	0.95	1.10	1.00	1.05	1.05
EF-hand domain-containing protein D2	sp | Q4FZY0 |	1	1.20	1.10	1.0	1.20	1.10	1.15	1.05	1.10	1.10	1.10	1.00
Fam49b protein	tr | B2GUZ9 |	1	1.10	1.05	1.0	1.00	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Family with sequence similarity 49, member A	tr | B0BN65 |	1	1.00	1.00	1.0	1.10	1.00	1.05	1.05	0.90	1.20	1.05	1.05
Fumarylacetoacetase	tr | F1M6W1 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Fumarylacetoacetate hydrolase domain-containing protein 2	sp | B2RYW9 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
S-formylglutathione hydrolase	sp | B0BNE5 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	1.10	1.00	1.05	1.05
Galectin	tr | B4F7A3 |	1	1.00	1.00	1.0	1.00	1.10	1.05	1.05	1.00	0.90	0.95	0.95
Glia maturation factor beta	sp | Q63228 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.10	1.10	1.10	1.05
Glyoxalase domain-containing protein 4	tr | GLOD4 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Inosine triphosphate pyrophosphatase	sp | D3ZW55 |	1	0.90	0.95	1.0	0.90	0.90	0.90	0.95	1.00	1.10	1.05	1.11
Lactoylglutathione lyase	sp | Q6P7Q4 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	1.00	1.00	1.00	1.00
Low molecular weight phosphotyrosine protein phosphatase	sp | P41498 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.10	1.10	1.00
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	sp | O08557 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
Neuromodulin	sp | P07936 |	1	0.90	0.95	1.0	1.10	0.90	1.00	1.05	1.20	1.20	1.20	**1.26**

Parathymosin	tr | B3DM95 |	1	1.10	1.05	1.0	1.10	1.10	1.10	1.05	1.00	1.10	1.05	1.00
Pdxp protein	tr | B2GV79 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Pgm1 protein (Fragment)	tr | A1A5L2 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Phosphoglycerate mutase 1	sp | P25113 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.00	1.05	1.00
Phosphorylase	tr | B1WBU9 |	1	0.90	0.95	1.0	1.00	1.00	1.00	1.05	0.90	0.90	0.90	0.95
Programmed cell death 6-interacting protein	sp | Q9QZA2 |	1	0.90	0.95	1.0	1.10	1.20	1.15	**1.21**	0.90	1.00	0.95	1.00
Protein Ak5	tr | M0R7U1 |	1	1.10	1.05	1.0	0.90	1.00	0.95	0.90	1.00	1.00	1.00	0.95
Protein Cct7	tr | D4AC23 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein DJ-1	sp | O88767 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Protein Irgq	tr | M0R686 |	1	1.00	1.00	1.0	1.00	0.90	0.95	0.95	0.90	1.10	1.00	1.00
Protein Ppa1	tr | F7EPH4 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.00	1.00	1.00	0.95
Protein RGD1304884	tr | D4A3C2 |	1	1.00	1.00	1.0	1.30	1.10	1.20	1.20	1.10	1.10	1.10	1.10
Protein RGD1309586	tr | D3ZN21 |	1	1.50	1.25	1.0	1.40	1.70	1.55	**1.24**	1.90	1.70	1.80	**1.44**
Protein RGD1559864	tr | D3ZBS6 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein RGD1564698	tr | F1LT36 |	1	1.00	1.00	1.0	0.80	1.00	0.90	0.90	1.20	1.00	1.10	1.10
Protein RUFY3	sp | Q5FVJ0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein Sept6	tr | B5DFG5 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.00	1.10	1.05	1.00
Protein Ugp2	tr | Q4V8I9 |	1	1.20	1.10	1.0	0.90	1.10	1.00	0.91	0.90	0.90	0.90	0.82
Protein unc-45 homolog A	tr | M0RC57 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	0.90	0.90	0.90	0.90
Protein Nbeal2	tr | D3ZUA5 |	1	0.70	0.85	1.0	0.90	1.10	1.00	1.18	0.80	0.70	0.75	0.88
Protein Cisd2	tr | D4AAE9 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Protein Tsnaxip1	tr D3ZU07 |	1	1.00	1.00	1.0	0.90	0.90	0.90	0.90	1.00	1.00	1.00	1.00
Ptges3 protein	tr | B2GV92 |	1	1.00	1.00	1.0	1.10	0.90	1.00	1.00	1.00	1.00	1.00	1.00
Purine nucleoside phosphorylase (Fragment)	tr | D3ZXK9 |	1	1.10	1.05	1.0	1.10	1.20	1.15	1.10	1.00	1.00	1.00	0.95
Serum albumin	sp | P02770 |	1	1.10	1.00	1.0	0.90	1.00	0.95	0.95	1.00	1.10	1.05	1.05
Sulfated glycoprotein 1	tr | F7EPE0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Superoxide dismutase [Cu-Zn]	tr | Q6LDS4 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Superoxide dismutase [Mn], mitochondrial	sp | P07895 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Transaldolase	sp | Q9EQS0 |	1	1.10	1.05	1.0	1.00	0.90	0.95	0.90	1.00	1.00	1.00	0.95
Transketolase	sp | P50137 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.00	1.05	1.00
Triosephosphate isomerase	sp | P48500 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05

Tripartite motif-containing protein 2 (Fragment)	tr | D3ZM62 |	1	1.10	1.05	1.0	1.20	1.10	1.15	1.10	1.00	1.10	1.05	1.00
WD repeat-containing protein 1	sp | Q5RKI0 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
WD repeat-containing protein 7	sp | Q9ERH3 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Guanine deaminase	tr | Q9JKB7 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Casein kinase II subunit alpha	sp | P19139 |	1	1.10	1.05	1.0	1.00	1.00	1.00	0.95	1.10	1.00	1.05	1.00
NAD-dependent protein deacylase sirtuin-5, mitochondrial	sp | Q68FX9 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	0.80	0.80	0.80	0.84
NAD(P)H-hydrate epimerase	sp | B0BNM1 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Zero beta-globin (Fragment)	tr | Q63011 |	1	1.30	1.15	1.0	1.00	1.00	1.00	0.87	1.10	1.20	1.15	1.00
Monoglyceride lipase	sp | Q8R431 |	1	1.00	1.00	1.0	0.80	1.00	0.90	0.90	1.10	1.10	1.10	1.10
Cystatin-B	sp | P01041 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.20	1.15	1.05
Cystatin-C	sp | P14841 |	1	1.00	1.00	1.0	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.00
Complement component 1 Q subcomponent-binding protein, mitochondrial	sp | O35796 |	1	1.10	1.05	1.0	1.10	0.80	0.95	0.90	1.20	0.90	1.05	1.00
Translationally-controlled tumor protein	sp | P63029 |	1	1.10	1.05	1.0	1.10	1.00	1.05	1.00	1.10	1.10	1.10	1.05
Acidic leucine-rich nuclear phosphoprotein 32 family member A	sp | P49911 |	1	0.90	0.95	1.0	1.10	1.00	1.05	1.11	1.00	1.00	1.00	1.05
C-terminal-binding protein 1	sp | Q9Z2F5 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	1.00	0.90	0.95	0.95
Ganglioside-induced differentiation-associated protein 1-like 1 (Predicted)	tr | B4F774 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	1.00	1.00	1.00	1.00
LanC lantibiotic synthetase component C-like 2 (Bacterial)	tr | Q68FQ9 |	1	1.00	1.00	1.0	0.90	1.10	1.00	1.00	1.00	1.00	1.00	1.00
Latexin	sp | Q64361 |	1	0.90	0.95	1.0	1.00	0.90	0.95	1.00	1.00	1.10	1.05	1.11
Macrophage migration inhibitory factor	sp | P30904 |	1	0.90	0.95	1.0	1.10	1.00	1.05	1.11	0.90	0.90	0.90	0.95
ES1 protein homolog, mitochondrial	sp | P56571 |	1	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-phosphogluconolactonase	sp | P85971 |	1	0.90	0.95	1.0	1.00	1.10	1.05	1.11	1.10	1.20	1.15	**1.21**
Myotrophin	sp | P62775 |	1	1.20	1.10	1.0	1.10	1.10	1.10	1.00	1.10	1.20	1.15	**1.05**
Asparagine-linked glycosylation 2 homolog, isoform CRA_a	tr | G3V6U3 |	1	1.00	1.00	1.0	0.80	1.10	0.95	0.95	0.90	1.00	0.95	0.95

Table A 2: Proteomic (4-plex) profile of the PFC of Ctr and MS+RS rats. Data presented as ratio to Ctr 1. The average Ctr ratio (Avg) was calculated and normalized to 1.0 (blue). Data in red differed from the normalized Ctr/Ctr by more than 20% (1.2-fold increase or decrease).

Protein / function	Accession No	Ctr 1	Ctr 2	Avg	Ctr/Ctr	MS+RS 1	MS+RS 2	Avg	MS+RS/ Ctr
Cytoskeletal/Structural									
Actin-binding									
Actin related protein 2/3 complex, subunit 3	tr | B2GV73 |	1	1.30	1.15	1.00	1.10	1.30	1.20	1.04
Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	tr | B2RZ72 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actin, alpha cardiac muscle 1	sp | P68035 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Actin-related protein 2	sp | Q5M7U6 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Actin-related protein 2/3 complex subunit 1A	sp | Q99PD4 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actin-related protein 2/3 complex subunit 2	sp | P85970 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Actin-related protein 2/3 complex subunit 5	sp | Q4KLF8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actin-related protein 2/3 complex subunit 5-like protein	sp | A1L108 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Actin-related protein 3	sp | Q4V7C7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Actin, cytoplasmic 1	sp | P60711 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Actn1 protein	tr | Q6GMN8 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Alpha II spectrin	tr | C9EH87 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Spectrin beta 3	tr | F1MA36 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Non-erythroid spectrin beta	tr | Q6XD99 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
F-actin-capping protein subunit alpha-1	sp | B2GUZ5 |	1	1.10	1.05	1.00	0.90	1.20	1.05	1.00
F-actin-capping protein subunit alpha-2	sp | Q3T1K5 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
F-actin-capping protein subunit beta	sp | Q5XI32 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Neurabin-1	sp | O35867 |	1	0.90	0.95	1.00	1.10	1.10	1.10	1.16
ARP1 actin-related protein 1 homolog B	tr | B2RYJ7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Dynactin 1, isoform CRA_a	tr | D4A8U7 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Dynactin subunit 2	sp | Q6AYH5 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95

Protein Dctn3	tr | D4A1B8 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Alpha-actinin-4	sp | Q9QXQ0 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Tropomodulin-1	sp | P70567 |	1	0.80	0.90	1.00	1.00	1.00	1.00	1.11
Tropomodulin-2	sp | P70566 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tropomyosin 5	tr | P97726 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Tropomyosin alpha isoform	tr | Q91XN7 |	1	1.00	1.00	1.00	1.20	1.30	1.25	**1.25**
Tropomyosin alpha-4 chain	sp | P09495 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Myl6 protein	tr | B2GV99 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Myosin, heavy polypeptide 10, non-muscle, isoform CRA_b	tr | G3V9Y1 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Myosin, heavy polypeptide 9, non-muscle	tr | G3V6P7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Twf2	tr | B0BMY7 |	1	1.00	1.00	1.00	1.20	1.20	1.20	**1.20**
Plectin 6	tr | Q6S3A0 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fascin	sp | P85845 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Coactosin-like protein	sp | B0BNA5 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Destrin	sp | Q7M0E3 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Drebrin E	tr | C6L8E0 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Drebrin-like protein	sp | Q9JHL4 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Adducin 3 (Gamma), isoform CRA_b	tr | D3ZCH7 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Alpha-adducin	sp | Q63028 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Beta-adducin	tr | F8WFS9 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adenylyl cyclase-associated protein 1	sp | Q08163 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Adenylyl cyclase-associated protein 2	sp | P52481 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Band 4.1-like protein 1	tr | D3ZMI4 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Calponin-3	sp | P37397 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Cofilin-1	sp | P45592 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Coronin (Fragment)	tr | F1LMV9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Coronin	tr | G3V940 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Coronin-1A	sp | Q91ZN1 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coronin-1B	sp | O89046 |	1	1.20	1.10	1.00	1.00	0.90	0.95	0.86
Cytoplasmic FMR1 interacting protein 1 (Predicted)	tr | D4A8H8 |	1	1.10	1.05	1.00	1.20	1.10	1.15	1.10
Cytoplasmic FMR1 interacting protein 2 (Predicted)	tr | D3ZX82 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05

Epb4.9 protein	tr | B2GUY4 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Erythrocyte protein band 4.1-like 3, isoform CRA_d	tr | Q9JMB3 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Gelsolin	sp|Q68FP1 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Profilin	tr | D3ZDU5 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Profilin-1	sp | P62963 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Ank2 (Fragment)	tr | F1M9N9 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Synaptopodin	tr | B1VKB4 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Ezrin	sp | P31977 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Nck-associated protein 1	sp | P55161 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Myristoylated alanine-rich C-kinase substrate	sp | P30009 |	1	0.80	0.90	1.00	1.00	0.90	0.95	1.06
Plastin 3 (T-isoform), isoform CRA_a	tr | F1LPK7 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Protein Nrap	tr | D3Z802 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Protein Tln2	tr | D3ZA84 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sorbin and SH3 domain-containing protein 2	tr | F1LPM3 |	1	0.90	0.95	1.00	0.80	1.00	0.90	0.95
Tubulin-binding									
Tubulin beta-5 chain	sp | P69897 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tubulin alpha-1A chain	sp | P68370 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Tubulin alpha-4A chain	sp | Q5XIF6 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Tubulin beta-2A chain	sp | P85108 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Tubulin beta-3 chain	sp | Q4QRB4 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tubulin-specific chaperone A (Fragment)	tr | M0RE00 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Microtubule-actin cross-linking factor 1	tr | M9MMM9 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Microtubule-associated protein 1 A, isoform CRA_c	tr | G3V7U2 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Microtubule-associated protein 1B	tr | F1LRL9 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Microtubule-associated protein 4	sp | Q5M7W5 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Microtubule-associated protein 6	sp | Q63560 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Microtubule-associated protein	tr | F1MAQ5 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Microtubule-associated protein RP/EB family member 3	sp | Q5XIT1 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Microtubule-associated proteins 1A/1B light chain 3A	sp | Q6XVN8 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Protein Tppp	tr D3ZQL7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Tubb4a	tr | B4F7C2 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95

HMW-MAP2 (Fragment)	tr | P70652 |	1	0.90	0.95	1.00	0.80	1.00	0.90	0.95
CAP-Gly domain-containing linker protein 2	tr | G3V949 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2',3'-cyclic-nucleotide 3'-phosphodiesterase	sp | P13233 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
Neuronal migration protein doublecortin	sp | Q9ESI7 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Intermediated filament binding									
Vimentin	sp | P31000 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Lamin A, isoform CRA_b	tr | G3V8L3 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Alpha-internexin	sp | P23565 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Neurofilament heavy polypeptide	tr | F1LRZ7 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Neurofilament light polypeptide	sp | P19527 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Neurofilament medium polypeptide	sp | P12839 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glial fibrillary acidic protein	sp | P47819 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Other									
Paralemmin-1	sp | Q920Q0 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Brevican, isoform CRA_a	tr | G3V8G4 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Hyaluronan and proteoglycan link protein 1	sp | P03994 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Myelin basic protein transcript variant 1	tr | I7FKL4 |	1	0.60	0.80	1.00	1.00	0.80	0.90	1.13
Myelin proteolipid protein	sp | P60203 |	1	0.70	0.85	1.00	0.80	0.60	0.70	0.82
Dihydropyrimidinase-related protein 1	sp | Q62950 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Dihydropyrimidinase-related protein 2	sp | P47942 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Dihydropyrimidinase-related protein 3	sp | Q62952 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Dihydropyrimidinase-related protein 4 (Fragment)	sp | Q62951 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydropyrimidinase-related protein 5	sp | Q9JHU0 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95

Energy metabolism

Glycolysis									
Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	tr | F7FKI5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	sp | P49432 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pyruvate kinase PKM	sp | P11980 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00

Pyruvate carboxylase, mitochondrial	sp | P52873 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Gamma-enolase	sp | P07323 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Citric Acid Cycle									
2-oxoglutarate dehydrogenase, mitochondrial	sp | Q5XI78 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fumarate hydratase, mitochondrial	sp | P14408 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Aconitate hydratase, mitochondrial	sp | Q9ER34 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cytoplasmic aconitate hydratase	sp | Q63270 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
ATP-citrate synthase	sp | P16638 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Citrate synthase, mitochondrial	sp | Q8VHF5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex), isoform CRA_a	tr | G3V6P2 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	sp | P08461 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glutamate dehydrogenase 1, mitochondrial	sp | P10860 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Malate dehydrogenase, cytoplasmic	sp | O88989 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Malate dehydrogenase, mitochondrial	sp | P04636 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Malic enzyme (Fragment)	tr | F1LQQ1 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Malic enzyme	tr | F1M5N4 |	1	0.90	0.95	1.00	0.90	1.00	0.95	1.00
Succinyl-CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	sp | P13086 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Trifunctional enzyme subunit alpha, mitochondrial	sp | Q64428 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Sucla2 protein (Fragment)	tr | B2RZ24 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oxidative phosphorylation									
Cytochrome b-c1 complex subunit 1, mitochondrial	sp | Q68FY0 |	1	0.80	0.90	1.00	0.80	0.60	0.70	**0.78**
Cytochrome b-c1 complex subunit 2, mitochondrial	sp | P32551 |	1	0.80	0.90	1.00	0.90	0.70	0.80	0.89
Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	sp | P10888 |	1	0.80	0.90	1.00	0.90	0.70	0.80	0.89
Cytochrome c oxidase subunit 5B, mitochondrial	sp | P12075 |	1	0.70	0.85	1.00	0.90	0.60	0.75	0.88
Cytochrome c oxidase subunit 6B1	tr | D3ZD09 |	1	0.70	0.85	1.00	0.70	0.60	0.65	**0.76**
Cytochrome c, somatic	sp | P62898 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	tr | F1LNF7 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Isocitrate dehydrogenase [NAD] subunit beta,	sp | Q68FX0 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00

mitochondrial									
Isocitrate dehydrogenase [NADP] cytoplasmic	sp | P41562 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Isocitrate dehydrogenase [NADP], mitochondrial	sp | P56574 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Isocitrate dehydrogenase 3 (NAD), gamma	tr | Q5XIJ3 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NADH dehydrogenase (Ubiquinone) Fe-S protein 7	tr | Q5RJN0 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
NADH dehydrogenase (Ubiquinone) flavoprotein 1	tr | Q5XIH3 |	1	0.80	0.90	1.00	0.80	0.80	0.80	0.89
Succinate dehydrogenase complex subunit A (Fragment)	tr | Q0QF18 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
ATP synthase F(0) complex subunit B1, mitochondrial	sp | P19511 |	1	0.70	0.85	1.00	0.80	0.50	0.65	**0.76**
ATP synthase gamma chain	tr | Q6QI09 |	1	0.80	0.90	1.00	0.80	0.60	0.70	**0.78**
ATP synthase subunit alpha, mitochondrial	sp | P15999 |	1	0.80	0.90	1.00	0.90	0.70	0.80	0.89
ATP synthase subunit beta, mitochondrial	sp | P10719 |	1	0.70	0.85	1.00	0.90	0.70	0.80	0.94
ATP synthase subunit O, mitochondrial	sp | Q06647 |	1	0.80	0.90	1.00	1.00	0.80	0.90	1.00
ADP/ATP translocase 1	sp | Q05962 |	1	0.70	0.85	1.00	0.80	0.60	0.70	0.82
ADP/ATP translocase 2	sp | Q09073 |	1	0.80	0.90	1.00	0.80	0.70	0.75	0.83
Mitochondrial 2-oxoglutarate/malate carrier protein	tr | G3V6H5 |	1	0.90	0.95	1.00	0.80	0.70	0.75	**0.79**
Phosphate carrier protein, mitochondrial	tr | G3V741 |	1	0.70	0.85	1.00	0.90	0.60	0.75	0.88
Phosphagen System									
Creatine kinase B-type	sp | P07335 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Creatine kinase M-type	sp | P00564 |	1	1.50	1.25	1.00	1.00	1.90	1.45	1.16
Creatine kinase, mitochondrial 1, ubiquitous	tr | Q5BJT9 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Other									
3'(2'),5'-bisphosphate nucleotidase 1	sp | Q9Z1N4 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95

Neurotransmission/signalling

Cell Adhesion									
Cell adhesion molecule 2	sp | Q1WIM2 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Limbic system-associated membrane protein	sp | Q62813 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Neural cell adhesion molecule 1 (Fragment)	tr | F1LNY3 |	1	0.90	0.95	1.00	1.00	0.80	0.90	0.95
Neurocan core protein	tr | G3V8R2 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00

Neurofascin	sp | P97685 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Neuronal growth regulator 1	sp | Q9Z0J8 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Neurotrimin	tr | G3V964 |	1	1.00	1.00	1.00	1.10	0.80	0.95	0.95
Obg-like ATPase 1	sp | A0JPJ7 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Opioid-binding protein/cell adhesion molecule	sp | P32736 |	1	1.00	1.00	1.00	0.80	0.70	0.75	**0.75**
Peripheral plasma membrane protein CASK	sp | Q62915 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Protein Cdh13 (Fragment)	tr | F1M7X3 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Protein Cttn	tr | D3ZGE6 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Synaptosomal-associated protein 25	sp | P60881 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Tenascin-R	sp | Q05546 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Versican core protein (Fragments)	sp | Q9ERB4 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Thy-1 membrane glycoprotein	sp | P01830 |	1	0.90	0.95	1.00	0.80	0.90	0.85	0.89
Neuroplastin	tr | D3ZDF0 |	1	0.80	0.90	1.00	1.00	0.80	0.90	1.00
Protein Omg	tr | F7EYB9 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Liprin-alpha-3	tr | F1LSE6 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Neurexin-3	sp | Q07310 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Neuronal cell adhesion molecule	sp | P97686 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Protein Ncam2 (Fragment)	tr | F1M8G9 |	1	0.80	0.90	1.00	0.80	0.80	0.80	0.89
Vesicle Regulation									
Vesicle associated membrane protein 2B	tr | Q9WUW2 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Vesicle-associated membrane protein-associated protein A	sp | Q9Z270 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Vesicle-fusing ATPase (Fragment)	tr | F1LQ81 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Vesicle-trafficking protein SEC22b	sp | Q4KM74 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Vesicular glutamate transporter 1	sp | Q62634 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Vesicular inhibitory amino acid transporter	sp | O35458 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
AP-1 complex subunit beta-1	tr | G3V9N8 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
AP-1 complex subunit mu-1	sp | Q32Q06 |	1	1.00	1.00	1.00	0.80	1.00	0.90	0.90
AP-2 complex subunit alpha-2	sp | P18484 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
AP-2 complex subunit beta	sp | P62944 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AP-2 complex subunit mu	sp | P84092 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
AP-2 complex subunit sigma	sp | P62744 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10

AP2-associated protein kinase 1	sp | P0C1X8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
AP-3 complex subunit mu-2	sp | P53678 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Adaptin ear-binding coat-associated protein 1	sp | P69682 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Adaptor protein complex AP-2, alpha 1 subunit (Predicted)	tr | D3ZUY8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Adaptor-related protein complex 1, gamma 1 subunit, isoform CRA_b	tr | B2RYN6 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Adaptor-related protein complex 3, delta 1 subunit, isoform CRA_b	tr | B5DFK6 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha-soluble NSF attachment protein	sp | P54921 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Beta-soluble NSF attachment protein	tr | F8WFM2 |	1	0.90	0.95	1.00	1.10	1.00	1.05	1.11
Calnexin	sp | P35565 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Clathrin coat assembly protein AP180	tr | F1LRK0 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Clathrin heavy chain 1	sp | P11442 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Clathrin light chain A	sp | P08081 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Clathrin light chain B	sp | P08082 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coatomer protein complex, subunit zeta 1 (Predicted)	tr | D4A8T3 |	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Complexin-2	sp | P84087 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
EH domain-containing protein 3	sp | Q8R491 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Endophilin-A1	tr | F1LQ05 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Endophilin-A2	sp | O35964 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Endophilin-B2	tr | D4A7V1 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Epsin-1	sp | O88339 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
General vesicular transport factor p115	sp|P41542 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Myc box-dependent-interacting protein 1	sp | O08839 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Protein Psd3	tr | D3ZUW0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Protein Snx2	tr | B2RYP4 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Reticulon	tr | F1LQN3 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Reticulon-1	sp | Q64548 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Reticulon-3	sp | Q6RJR6 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Secernin-1	sp | Q6AY84 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Secretory carrier-associated membrane protein 1	sp | P56603 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Secretory carrier-associated membrane protein 5	tr | F1M882 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00

(Fragment)									
SH3-containing GRB2-like protein 3-interacting protein 1	sp | P0DJJ3 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
SRC kinase signaling inhibitor 1	sp | Q9QXY2 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Synapsin-1	sp | P09951 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Synapsin-2	sp | Q63537 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Synaptic vesicle glycoprotein 2A	sp | Q02563 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Synaptic vesicle glycoprotein 2B	sp | Q63564 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Synaptobrevin homolog YKT6	sp | Q5EGY4 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Synaptogyrin-1	sp | Q62876 |	1	0.80	0.90	1.00	0.90	0.90	0.90	1.00
Synaptojanin-1	sp | Q62910 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Synaptophysin	sp | P07825 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Synaptotagmin-1	sp | P21707 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Syntaxin 1A	tr | Q9QXG3 |	1	0.80	0.90	1.00	1.10	0.90	1.00	1.11
Syntaxin-1B	sp | P61265 |	1	0.80	0.90	1.00	1.00	0.90	0.95	1.06
Syntaxin-binding protein 1	sp | P61765 |	1	0.90	0.95	1.00	1.10	1.00	1.05	1.11
Unconventional myosin-Va	sp | Q9QYF3 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Amphiphysin	sp | O08838 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein kinase C and casein kinase substrate in neurons protein 1	tr | F1LPP3 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein Eea1 (Fragment)	tr | F1LUA1 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein piccolo	sp | Q9JKS6 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Dynamin-1	sp | P21575 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Dynamin-1-like protein	sp | O35303 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dynamin-like 120 kDa protein, mitochondrial	tr | D4A8U5 |	1	0.70	0.85	1.00	1.10	0.90	1.00	1.18
Calcium-dependent secretion activator 1	sp | Q62717 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Transport									
Excitatory amino acid transporter 1	sp | P24942 |	1	0.70	0.85	1.00	1.00	0.90	0.95	1.12
Excitatory amino acid transporter 2	sp | P31596 |	1	0.70	0.85	1.00	1.00	0.70	0.85	1.00
Glutamate receptor 2	tr|F1LNE4 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Gamma-aminobutyric acid receptor subunit alpha-1	tr | U6DDM1 |	1	0.80	0.90	1.00	0.90	0.60	0.75	0.83
Gamma-aminobutyric acid receptor-associated protein-like 2	sp | P60522 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95

Acyl-CoA-binding protein	sp | P11030 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Disks large homolog 1	sp | Q62696 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Disks large homolog 3	sp | Q62936 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1	tr | Q5M7T6 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
ATPase, H+ transporting, V1 subunit D, isoform CRA_c	tr | Q6P503 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
ATPase, H+ transporting, V1 subunit E isoform 1, isoform CRA_a	tr | G3V7L8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ATPase, H+ transporting, V1 subunit G isoform 2	tr | Q8R2H0 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
V-H+ATPase subunit a1-III	tr | Q2I6B2 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
V-type proton ATPase subunit B, brain isoform	sp | P62815 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
V-type proton ATPase subunit C 1	sp | Q5FVI6 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
V-type proton ATPase subunit F	sp | P50408 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Protein Atp8a1 (Fragment)	tr | F1M585 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Protein Atp6v1a	tr | D4A133 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Atp6v1h	tr | E9PTI1 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Voltage-dependent anion-selective channel protein 1	sp | Q9Z2L0 |	1	0.70	0.85	1.00	0.80	0.50	0.65	**0.76**
Voltage-dependent anion-selective channel protein 3	sp | Q9R1Z0 |	1	0.80	0.90	1.00	0.90	0.70	0.80	0.89
Voltage-dependent calcium channel subunit alpha-2/delta-1	sp | P54290 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84
Voltage-gated potassium channel subunit beta-2	sp | P62483 |	1	1.10	1.05	1.00	0.90	0.80	0.85	0.81
Sodium/calcium exchanger 2	tr | F1M9A2 |	1	0.80	0.90	1.00	0.90	0.90	0.90	1.00
Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	sp | P11507 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Plasma membrane calcium-transporting ATPase 1	sp | P11505 |	1	0.90	0.95	1.00	1.00	0.80	0.90	0.95
Plasma membrane calcium-transporting ATPase 2	tr | D3ZCE9 |	1	0.90	0.95	1.00	1.10	0.90	1.00	1.05
Plasma membrane calcium-transporting ATPase 4	tr | D3ZH00 |	1	0.70	0.85	1.00	1.00	0.70	0.85	1.00
Sodium/potassium-transporting ATPase subunit alpha-1	sp | P06685 |	1	0.80	0.90	1.00	1.00	0.80	0.90	1.00
Sodium/potassium-transporting ATPase subunit alpha-2	sp | P06686 |	1	0.70	0.85	1.00	0.90	0.70	0.80	0.94
Sodium/potassium-transporting ATPase subunit alpha-3	sp | P06687 |	1	0.70	0.85	1.00	1.00	0.70	0.85	1.00
Sodium/potassium-transporting ATPase subunit beta-1	sp | P07340 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94

Sodium-dependent neutral amino acid transporter SLC6A17	sp | P31662 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Solute carrier family 12 member 5	sp | Q63633 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
ATPase Asna1	tr | G3V9T7 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Astrocytic phosphoprotein PEA-15	sp | Q5U318 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Cytoplasmic dynein 1 heavy chain 1	tr | M0R9X8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cytoplasmic dynein 1 light intermediate chain 1	sp | Q9QXU8 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
sp|Q9JJ79|DYHC2_RAT-DECOY Cytoplasmic dynein 2 heavy chain 1	sp | Q9JJ79 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Dynein light chain 1, cytoplasmic	sp | P63170 |	1	1.10	1.05	1.00	1.20	1.30	1.25	1.19
Dynein light chain 2, cytoplasmic	sp | Q78P75 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**
Exportin-1	sp | Q80U96 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Protein Tnpo2	tr | D3ZER6 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Fatty acid-binding protein, brain	sp | P55051 |	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Fatty acid-binding protein, epidermal	sp | P55053 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Importin 7 (Predicted), isoform CRA_c	tr | D4AE96 |	1	0.90	0.95	1.00	1.00	1.10	1.05	1.11
Importin subunit beta-1	tr | F2Z3Q8 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
LIM and SH3 domain protein 1	sp | Q99MZ8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Mitochondrial import inner membrane translocase subunit Tim13	sp | P62076 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Mitochondrial import receptor subunit TOM70	tr | R9PXR4 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Phosphofurin acidic cluster sorting protein 1	tr | F1LPG3 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein Napg	tr | D4A0E2 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Ranbp1	tr | D4A2G9 |	1	1.00	1.00	1.00	1.30	1.20	1.25	**1.25**
Protein Tom1l2	tr D4A6C9 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein Vps26b	tr | B1WBS4 |	1	1.20	1.10	1.00	1.00	1.30	1.15	1.05
Rabphilin-3A	tr | F1LPB9 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
Vacuolar protein sorting-associated protein 29	sp | B2RZ78 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Vacuolar protein sorting-associated protein 35	tr | G3V8A5 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Hepatocyte growth factor-regulated tyrosine kinase substrate	sp | Q9JJ50 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Oxysterol-binding protein	tr | M0R916 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Ap3b2	tr | D4AE00 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Protein Chmp4bl1	tr | D4A9Z8 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85

Protein Kif21a (Fragment)	tr | D3ZCG2 |	1	0.90	0.95	1.00	0.80	0.90	0.85	0.89
Protein lin-7 homolog A	tr | M0R7K1 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Protein transport protein Sec31A	tr | G3V699 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
SEC14-like 2 (S. cerevisiae)	tr | Q5EBD0 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Transmembrane emp24 domain-containing protein 10	sp | Q63584 |	1	0.80	0.90	1.00	0.90	0.70	0.80	0.89
GTPase Signalling									
CB1 cannabinoid receptor-interacting protein 1	sp | Q5M7A7 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
GTP-binding nuclear protein Ran	sp | P62828 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Sar1a	tr | Q6AY18 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Sept9 protein (Fragment)	tr | B2GVB4 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Septin 5, isoform CRA_d	tr | D3ZDH8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Septin 7	tr | A2VCW8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Septin 8 (Predicted)	tr | G3V9Z6 |	1	0.90	0.95	1.00	0.90	1.00	0.95	1.00
Septin-11	sp | B3GNI6 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Septin-2	sp | Q91Y81 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Neuronal-specific septin-3	tr | D3ZPP8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Diras2	tr | D3ZHX3 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Arf-GAP with SH3 domain, ANK repeat and PH domain-containing protein 1	sp | Q1AAU6 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
ADP-ribosylation factor 1	sp | P84079 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
ADP-ribosylation factor 5	sp | P84083 |	1	1.20	1.10	1.00	1.20	1.20	1.20	1.09
ADP-ribosylation factor-like protein 3	sp | P37996 |	1	1.10	1.05	1.00	1.00	1.20	1.10	1.05
Centaurin alpha	tr | Q63629 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Cell division control protein 42 homolog	sp | Q8CFN2 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Guanine nucleotide binding protein, alpha q polypeptide, isoform CRA_a	tr | D4AE68 |	1	0.80	0.90	1.00	0.80	0.70	0.75	0.83
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	sp | P54311 |	1	0.70	0.85	1.00	1.00	0.90	0.95	1.12
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	sp | P54313 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Guanine nucleotide-binding protein G(o) subunit alpha	sp | P59215 |	1	0.70	0.85	1.00	1.00	0.80	0.90	1.06
Guanine nucleotide-binding protein subunit beta-2-like 1	sp | P63245 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90

Guanine nucleotide-binding protein subunit beta-5	sp | P62882 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Protein Gng13	tr | G3V8Y0 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Ras-related C3 botulinum toxin substrate 1	sp | Q6RUV5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ras-related protein Rab-10	sp | P35281 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Ras-related protein Rab-11A	sp | P62494 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ras-related protein Rab-18	sp | Q5EB77 |	1	0.80	0.90	1.00	0.90	0.90	0.90	1.00
Ras-related protein Rab-1A	tr | E9PU16 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Ras-related protein Rab-2A	sp | P05712 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Ras-related protein Rab-3A	sp | P63012 |	1	0.90	0.95	1.00	1.10	1.10	1.10	1.16
Ras-related protein Rab-3C	sp | P62824 |	1	0.90	0.95	1.00	1.10	1.00	1.05	1.11
Ras-related protein Rab-4B	sp | P51146 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Ras-related protein Rab-6A	sp | Q9WVB1 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Ras-related protein Rab-7a	sp | P09527 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Ras-related protein Ral-A	sp | P63322 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Ras-related protein Rap-1b	sp | Q62636 |	1	0.80	0.90	1.00	0.90	0.90	0.90	1.00
Protein Rab5b	tr | A1L1J8 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Protein Rab5c	tr | B0BNK1 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
GTP-binding protein Rheb	tr | M0R3K1 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Rho GDP-dissociation inhibitor 1	sp | Q5XI73 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Rho guanine nucleotide exchange factor 2	sp | Q5FVC2 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Rho-associated protein kinase 2	sp | Q62868 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Transforming protein RhoA	sp | P61589 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00
Protein Rap1gds1	tr | F1M7Y3 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Rab GDP dissociation inhibitor alpha	sp | P50398 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Rab GDP dissociation inhibitor beta	sp | P50399 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
RAB14, member RAS oncogene family	tr | B0BMW0 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Sbf1	tr | D3ZNN0 |	1	0.90	0.95	1.00	0.90	1.00	0.95	1.00
MAP kinase-activating death domain protein	sp | O08873 |	1	1.10	1.05	1.00	0.90	1.10	1.00	0.95
Regulator of G-protein-signaling 6	tr | F1LS67 |	1	0.90	0.95	1.00	1.20	1.20	1.20	**1.26**
Serine/threonine-protein kinase DCLK1	sp | O08875 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Serine/threonine-protein kinase MARK1 (Fragment)	tr | F1LNE7 |	1	1.00	1.00	1.00	1.10	0.90	1.00	1.00

Serine/threonine-protein kinase PAK 1	sp | P35465 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	sp | P36876 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	sp | P63331 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	sp | P63329 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine/threonine-protein phosphatase 2B catalytic subunit beta isoform	sp | P20651 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Serine/threonine-protein phosphatase 5	sp | P53042 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Serine/threonine-protein phosphatase PP1-beta catalytic subunit	sp | P62142 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein Ppp2r1a	tr | Q5XI34 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Ppp2r4	tr | B2RYQ2 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein Ppp2r5e	tr | D3ZHI9 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nucleoside diphosphate kinase A	sp | Q05982 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Nucleoside diphosphate kinase B	sp | P19804 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Phosphatidylethanolamine-binding protein 1	sp | P31044 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma	tr | F1M8H6 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha	sp | Q9R0I8 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Phosphatidylinositol 5-phosphate 4-kinase type-2 beta	sp | O88377 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Phosphatidylinositol transfer protein alpha isoform	sp | P16446 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phosphatidylinositol-binding clathrin assembly protein	tr | E9PTD2 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1	sp | P10687 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Type I inositol 3,4-bisphosphate 4-phosphatase	tr | D3ZAN1 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84
Inositol monophosphatase 1	tr | F1M978 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Inositol-trisphosphate 3-kinase A	sp | P17105 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Membrane-associated phosphatidylinositol transfer protein 1	sp | Q5U2N3 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B	sp | Q01066 |	1	0.90	0.95	1.00	0.70	0.80	0.75	**0.79**
cGMP-dependent 3',5'-cyclic phosphodiesterase	tr | F8WFW5 |	1	0.80	0.90	1.00	1.00	0.90	0.95	1.06
cAMP-dependent protein kinase catalytic subunit	sp | P68182 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95

beta									
cAMP-dependent protein kinase type II-beta regulatory subunit	sp | P12369 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
cAMP-regulated phosphoprotein 19	sp | Q712U5 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein kinase, cAMP-dependent, regulatory, type 2, alpha, isoform CRA_a	tr | G3V8Q6 |	1	0.90	0.95	1.00	1.10	0.90	1.00	1.05
Protein Pde1a	tr | F1LMC0 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
A-kinase anchor protein 5	tr | F1LPP6 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Tyrosine-protein phosphatase non-receptor type 11	sp | P41499 |	1	1.10	1.05	1.00	0.90	1.10	1.00	0.95
Protein kinase C beta type	tr | F1LS42 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein kinase C gamma type	sp | P63319 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Protein kinase C	tr | F1LMV8 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Dual specificity mitogen-activated protein kinase kinase 1	sp | Q01986 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Mitogen-activated protein kinase 1	sp | P63086 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Mitogen-activated protein kinase 3	sp | P21708 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
Protein Map2k4 (Fragment)	tr | F1LP57 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
JNK3 protein	tr | B0VXR6 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Calcium Signalling									
Calcium/calmodulin-dependent protein kinase II, beta, isoform CRA_a	tr | G3V9G3 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Calcium/calmodulin-dependent protein kinase type II subunit alpha	sp | P11275 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Calcium/calmodulin-dependent protein kinase type II subunit delta	tr | F1LWF6 |	1	1.00	1.00	1.00	1.00	1.20	1.10	1.10
Calcium/calmodulin-dependent protein kinase type II subunit gamma (Fragment)	tr | F1LMC3 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Calcium/calmodulin-dependent protein kinase type IV	sp | P13234 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Calmodulin	sp | P62161 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Neurogranin	sp | Q04940 |	1	1.20	1.10	1.00	1.00	1.10	1.05	0.95
Purkinje cell protein 4	sp | P63055 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Neurochondrin	sp | O35095 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calbindin	sp | P07171 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Calcineurin subunit B type 1	sp | P63100 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Calpain-2 catalytic subunit	sp | Q07009 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86

Calretinin	sp | P47728 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
CaM kinase-like vesicle-associated protein	sp | Q63092 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Hippocalcin-like protein 4	sp | P35332 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Neurocalcin-delta	sp | Q5PQN0 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Neuronal membrane glycoprotein M6-a	sp | Q812E9 |	1	0.70	0.85	1.00	0.90	0.80	0.85	1.00
Neuron-specific calcium-binding protein hippocalcin	sp | P84076 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein S100-B	sp | P04631 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Visinin-like protein 1	sp | P62762 |	1	0.90	0.95	1.00	1.10	1.10	1.10	1.16
Annexin A3	sp | P14669 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Annexin A5	sp | P14668 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Other									
Brain-specific angiogenesis inhibitor 1-associated protein 2	sp | Q6GMN2 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Amyloid beta A4 protein	sp | P08592 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans), isoform CRA_b	tr | G3V728 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
14-3-3 protein epsilon	sp | P62260 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
14-3-3 protein eta	sp | P68511 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
14-3-3 protein theta	sp | P68255 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
14-3-3 protein zeta/delta	sp | P63102 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
CDGSH iron-sulfur domain-containing protein 1	sp | B0K020 |	1	0.80	0.90	1.00	0.80	0.80	0.80	0.89
ERC protein 2	sp | Q8K3M6 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Gephyrin isoform	tr|B3GS92 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Growth factor receptor-bound protein 2	sp|P62994 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Homer protein homolog 1	sp | Q9Z214 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Kinesin heavy chain isoform 5C (Fragment)	tr | G3V6L4 |	1	1.20	1.10	1.00	1.10	1.20	1.15	1.05
Kinesin-like protein KIF2A	tr | F1M745 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
LanC-like protein 1	sp | Q9QX69 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Platelet-activating factor acetylhydrolase IB subunit alpha	sp | P63004 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein bassoon	tr | G3V984 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Dmxl2 (Fragment)	tr | F1M164 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00

Protein phosphatase 1 regulatory subunit 7	sp | Q5HZV9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein phosphatase 1, regulatory subunit 9B	tr | B1H262 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein phosphatase 1A	sp | P20650 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Protein phosphatase 1E	sp | Q80Z30 |	1	1.10	1.05	1.00	1.00	1.20	1.10	1.05
Protein phosphatase methylesterase 1	sp | Q4FZT2 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Stathmin	sp | P13668 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Wiskott-Aldrich syndrome protein family member 1	sp | Q5BJU7 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Alpha-synuclein	sp | P37377 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Beta-synuclein	sp | Q63754 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Glutamate decarboxylase 2	sp | Q05683 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Glutamine synthetase	sp | P09606 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein NDRG2	sp | Q8VBU2 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein NDRG3	sp | Q6AYR2 |	1	1.10	1.05	1.00	0.80	0.90	0.85	0.81
Protein NDRG4	tr | D3Z831 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Receptor expression-enhancing protein 5	sp | B2RZ37 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Membrane protein, palmitoylated 2 (MAGUK p55 subfamily member 2), isoform CRA_a	tr | D3ZAA9 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phytanoyl-CoA hydroxylase-interacting protein	sp | Q568Z9 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Phytanoyl-CoA hydroxylase-interacting protein-like	sp | Q6AYN4 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Receptor-type tyrosine-protein phosphatase zeta	sp | Q62656 |	1	1.20	1.10	1.00	1.00	1.10	1.05	0.95

Redox regulation

6-phosphogluconate dehydrogenase, decarboxylating	sp | P85968 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Glyceraldehyde-3-phosphate dehydrogenase	sp | P04797 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	sp | O35077 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Alcohol dehydrogenase [NADP(+)]	sp | P51635 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Aldehyde dehydrogenase family 5, subfamily A1	tr | G3V945 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Aldehyde dehydrogenase family 6, subfamily A1, isoform CRA_b	tr | G3V7J0 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Aldehyde dehydrogenase, mitochondrial	tr | F1LN88 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydrolipoyl dehydrogenase, mitochondrial	sp | Q6P6R2 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95

Isovaleryl-CoA dehydrogenase, mitochondrial	sp | P12007 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
L-lactate dehydrogenase B chain	sp | P42123 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
L-lactate dehydrogenase	tr | B5DEN4 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
D-3-phosphoglycerate dehydrogenase	sp | O08651 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Protein Gpd1l	tr | D3ZAP9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Peroxiredoxin 3	tr | G3V7I0 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Peroxiredoxin-1	sp | Q63716 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Peroxiredoxin-2	sp | P35704 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Peroxiredoxin-5, mitochondrial (Fragment)	tr | D3ZEN5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Peroxiredoxin-6	sp | O35244 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Acad9 protein	tr | B1WC61 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Thioredoxin	sp | P11232 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Thioredoxin reductase 1, cytoplasmic	tr | R9PXU4 |	1	1.20	1.10	1.00	1.00	1.20	1.10	1.00
Thioredoxin-like protein 1	sp | Q920J4 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Aldose reductase	sp | P07943 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Biliverdin reductase A	sp | P46844 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Biliverdin reductase B (Flavin reductase (NADPH))	tr | B5DF65 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Carbonyl reductase [NADPH] 1	sp | P47727 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dihydropteridine reductase	sp | P11348 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Electron transfer flavoprotein subunit alpha, mitochondrial	sp | P13803 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Electron transfer flavoprotein subunit beta	sp | Q68FU3 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Ketimine reductase mu-crystallin	sp | Q9QYU4 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Oxidation resistance protein 1	sp | Q4V8B0 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Prenylcysteine oxidase	sp | Q99ML5 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
Protein Sh3bgrl3	tr | B2RZ27 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Redox-regulatory protein FAM213A	sp | Q6AXX6 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Protein Vat1l	tr | D3ZE32 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00

Protein synthesis/neurotrophic

Transcription

Transcription elongation factor B polypeptide 2	sp | P62870 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Tceal5	tr | M0RDJ7 |	1	0.90	0.95	1.00	0.80	1.00	0.90	0.95
Transcription factor Pur-beta	tr | F1LSL1 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Transcription intermediary factor 1-beta	sp | O08629 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Transcriptional activator protein Pur-alpha	tr | F1LPS8 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Transgelin-3	sp | P37805 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Btf3l4	tr | D4A3I4 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Core histone macro-H2A.1	sp | Q02874 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Histone H1.0	sp | P43278 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Histone H1.4	sp | P15865 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Histone H1.5	sp | D3ZBN0 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Histone H2A	tr | D3ZVK7 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Histone H2B	tr | D3ZNH4 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Histone H3	tr | B0BMY8 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Histone H4	sp | P62804 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heterochromatin protein 1-binding protein 3	sp | Q6P747 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Brain acid soluble protein 1	sp | Q05175 |	1	0.60	0.80	1.00	1.00	0.80	0.90	1.13
Nucleolin	sp | P13383 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
Nucleosome assembly protein 1-like 1	tr | G3V6H9 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Heterogeneous nuclear ribonucleoprotein A1	tr | F7FEZ6 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Heterogeneous nuclear ribonucleoprotein A3	sp | Q6URK4 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Heterogeneous nuclear ribonucleoprotein C (C1/C2)	tr | G3V9R8 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Heterogeneous nuclear ribonucleoprotein D, isoform CRA_b	tr | G3V6A4 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Heterogeneous nuclear ribonucleoprotein H	tr | D3ZYW2 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heterogeneous nuclear ribonucleoprotein K	sp | P61980 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Heterogeneous nuclear ribonucleoprotein M	tr | F1LV13 |	1	0.90	0.95	1.00	1.10	0.90	1.00	1.05
Heterogeneous nuclear ribonucleoprotein Q	sp | Q7TP47 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Heterogeneous nuclear ribonucleoproteins A2/B1	tr | F1LM82 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Hnrnpl protein (Fragment)	tr | B5DFG2 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Hnrnpul2	tr | D4ABT8 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Far upstream element-binding protein 2	sp | Q99PF5 |	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Matrin-3	sp | P43244 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Nucleophosmin	sp | P13084 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
LRRGT00192	tr | Q6QI16 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Leucine-rich PPR motif-containing protein, mitochondrial	tr | F1LM33 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
ATP-dependent RNA helicase DDX1	sp | Q641Y8 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Activated RNA polymerase II transcriptional coactivator p15	sp | Q63396 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CUGBP Elav-like family member 2	sp | Q792H5 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
AU RNA binding protein/enoyl-coenzyme A hydratase (Predicted), isoform CRA_a	tr | F1LU71 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 (Fragment)	tr | B6DTP5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ELAV (Embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	tr | B5DF91 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Protein Tardbp	tr | I6L9G6 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Spliceosome RNA helicase Ddx39b	sp | Q63413 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase), isoform CRA_b	tr | G3V6F4 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine-threonine kinase receptor-associated protein	sp | Q5XIG8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Splicing factor proline/glutamine rich (Polypyrimidine tract binding protein associated)	tr | Q4KM71 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Histidine triad nucleotide-binding protein 1	sp | P62959 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Ilf2 protein	tr | B2RZC6 |	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Paraspeckle component 1	sp | Q4KLH4 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Proliferation-associated 2G4	tr | Q6AYD3 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00
Protein Alyref	tr | D3ZXH7 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Translation									
Elongation factor 1-alpha 1	sp | P62630 |	1	1.00	1.00	1.00	1.00	1.20	1.10	1.10
Elongation factor 1-alpha 2	sp | P62632 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Elongation factor 1-gamma	sp | Q68FR6 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95

Elongation factor 2	sp | P05197 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Elongation factor Tu, mitochondrial	sp | P85834 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Eukaryotic initiation factor 4A-II	sp | Q5RKI1 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Eukaryotic translation elongation factor 1 beta 2	tr | B5DEN5 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Eukaryotic translation initiation factor 2 subunit 1	sp | P68101 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Eukaryotic translation initiation factor 4H	sp | Q5XI72 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Eukaryotic translation initiation factor 5	sp | Q07205 |	1	1.20	1.10	1.00	1.00	1.10	1.05	0.95
Eukaryotic translation initiation factor 5A-1	sp | Q3T1J1 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
40S ribosomal protein S10	sp | P63326 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
40S ribosomal protein S12	tr | M0R9I8 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**
40S ribosomal protein S13	sp | P62278 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
40S ribosomal protein S14	tr | Q6PDV6 |	1	1.10	1.05	1.00	0.90	1.10	1.00	0.95
40S ribosomal protein S18	sp | P62271 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
40S ribosomal protein S23	sp | P62268 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
40S ribosomal protein S24	tr | D4ACJ1 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
40S ribosomal protein S3	sp | P62909 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
40S ribosomal protein S3a	sp | P49242 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
40S ribosomal protein S4, X isoform	sp | P62703 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
40S ribosomal protein S6 (Fragment)	tr | M0RD75 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
40S ribosomal protein S8	tr | B2RYR8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
40S ribosomal protein S9	sp | P29314 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
40S ribosomal protein SA	sp | P38983 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
60S acidic ribosomal protein P0	sp | P19945 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
60S ribosomal protein L11	sp | P62914 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
60S ribosomal protein L13	tr | D3ZRM9 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
60S ribosomal protein L13a	tr | Q5RK10 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
60S ribosomal protein L18	sp | P12001 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
60S ribosomal protein L18a	sp | P62718 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
60S ribosomal protein L23	sp | P62832 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
60S ribosomal protein L24	sp | P83732 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
60S ribosomal protein L3	sp | P21531 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90

60S ribosomal protein L30	sp | P62890 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
60S ribosomal protein L34	tr | B2RZD4 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
60S ribosomal protein L36	tr | D3ZZ95 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84
60S ribosomal protein L4	tr | Q6P3V9 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
60S ribosomal protein L5	sp | P09895 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
60S ribosomal protein L6	tr | F1LQS3 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
60S ribosomal protein L7	tr | B0K031 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
60S ribosomal protein L8	sp | P62919 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
RCG25732, isoform CRA_b	tr | B5DEM5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
RCG45400	tr | G3V7C6 |	1	1.10	1.05	1.00	1.20	1.20	1.20	1.14
RCG45476, isoform CRA_d	tr | B0K021 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86
RCG45615, isoform CRA_a	tr | B2RYU2 |	1	0.90	0.95	1.00	0.70	0.80	0.75	**0.79**
RCG55853, isoform CRA_b	tr | F1LVX3 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00
RCG62292, isoform CRA_a	tr | B5DEL9 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Ribosomal protein (Fragment)	tr | Q4KM60 |	1	1.00	1.00	1.00	1.00	0.80	0.90	0.90
Ribosomal protein S11	tr | Q6PDV9 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Ribonuclease UK114	sp | P52759 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Rps16 protein (Fragment)	tr | B0K038 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein LOC100359563	tr | D3ZZK1 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Protein LOC100360057 (Fragment)	tr | F7FLF2 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein LOC100360604	tr | D3ZPN7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein LOC100360791	tr | M0R8Q2 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein LOC100362339	tr | D4A6G6 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Protein LOC100909464	tr | F1MAA3 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Protein LOC100909878	tr | D3ZLL8 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein LOC100910017	tr | D3ZX87 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Protein LOC100911422 (Fragment)	tr | M0R9U5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein LOC100911472	tr | M0RBD4 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein LOC100911774	tr | Q1RP74 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Protein LOC100912618	tr | D3ZFY8 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Protein LOC689899	tr | D3ZTH8 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90

Protein Nars	tr | F1LPV0 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Pcbp2 protein	tr | Q6AYU2 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Protein IMPACT	sp | Q5GFD9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Posttranslational modification									
Peptidylprolyl cis/trans isomerase, NIMA-interacting 1	tr | B0BNL2 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Peptidyl-prolyl cis-trans isomerase A	sp | P10111 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Peptidyl-prolyl cis-trans isomerase B	sp | P24368 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Peptidyl-prolyl cis-trans isomerase D	sp | Q6DGG0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Peptidyl-prolyl cis-trans isomerase FKBP1A	sp | Q62658 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Peptidyl-prolyl cis-trans isomerase FKBP4	sp | Q9QVC8 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein disulfide-isomerase A3	sp | P11598 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Protein disulfide-isomerase A6	sp | Q63081 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Protein disulfide-isomerase	sp | P04785 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Endoplasmic reticulum resident protein 29	sp | P52555 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Endoplasmin	sp | Q66HD0 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Biosynthesis									
Acyl carrier protein	tr | D3ZF13 |	1	0.90	0.95	1.00	0.90	0.80	0.85	0.89
Fatty acid synthase	sp | P12785 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Cytosolic acyl coenzyme A thioester hydrolase	sp | Q64559 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Adenosylhomocysteinase (Fragment)	tr | B5DFN2 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Adenosylhomocysteinase	sp | P10760 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Bifunctional purine biosynthesis protein PURH	sp | O35567 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
N-acetylneuraminic acid synthase	tr | B1WC26 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Alanine--tRNA ligase, cytoplasmic	sp | P50475 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Glycine--tRNA ligase	tr | G3V7G8 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Serine--tRNA ligase, cytoplasmic	sp | Q6P799 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Tryptophan--tRNA ligase, cytoplasmic	tr | F8WFH8 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Tyrosine--tRNA ligase, cytoplasmic	sp | Q4KM49 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Aspartyl-tRNA synthetase	tr | A9CMB7 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Methionine adenosyltransferase 2 subunit beta	sp | Q5U2R0 |	1	1.20	1.10	1.00	1.00	0.90	0.95	0.86
Phosphoribosyl pyrophosphate synthase-associated protein 2	sp | O08618 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95

Phosphoribosyl pyrophosphate synthetase 1-like 1 (Predicted)	tr | M0RBK1 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
S-adenosylmethionine synthase	tr | F1LRB8 |	1	1.10	1.05	1.00	0.80	0.90	0.85	0.81

Protein degradation

Ubiquitin carboxyl-terminal hydrolase isozyme L1	sp | Q00981 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Ubiquitin carboxyl-terminal hydrolase	tr | D3ZVQ0 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Ubiquitin carboxyl-terminal hydrolase	tr | Q5U2N2 |	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Ubiquitin carboxyl-terminal hydrolase	tr | D3ZC84 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ubiquitin thioesterase OTUB1	sp | B2RYG6 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Ubiquitin-conjugating enzyme E2 D2 (Fragment)	tr | F1M5C9 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Ubiquitin-conjugating enzyme E2 N	sp | Q9EQX9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Ubiquitin-like modifier-activating enzyme 1	sp | Q5U300 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Protein Ube2l3	tr | B2RZA9 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Protein Ube2m	tr D3ZNQ6 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Ube2o (Fragment)	tr | F1M403 |	1	1.20	1.10	1.00	1.10	1.20	1.15	1.05
Protein Ubqln2	tr | D4AA63 |	1	0.90	0.95	1.00	1.00	1.00	1.00	1.05
Polyubiquitin-C	tr | F1LML2 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
SUMO-activating enzyme subunit 1	sp | Q6AXQ0 |	1	1.20	1.10	1.00	0.80	1.00	0.90	0.82
UV excision repair protein RAD23 homolog B	sp | Q4KMA2 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Transitional endoplasmic reticulum ATPase	sp | P46462 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
S-phase kinase-associated protein 1	sp | Q6PEC4 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
DNA damage-binding protein 1	sp | Q9ESW0 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
F-box only protein 2	tr | G3V774 |	1	0.90	0.95	1.00	1.40	1.10	1.25	**1.32**
COP9 (Constitutive photomorphogenic) homolog, subunit 5 (Arabidopsis thaliana)	tr | Q4KM69 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
COP9 (Constitutive photomorphogenic) homolog, subunit 7a (Arabidopsis thaliana) (Predicted)	tr | G3V8Z9 |	1	0.90	0.95	1.00	1.10	1.20	1.15	**1.21**
COP9 signalosome complex subunit 2	sp | P61203 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
COP9 signalosome complex subunit 3	sp | Q68FW9 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
COP9 signalosome complex subunit 4	sp | Q68FS2 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
26S protease regulatory subunit 4	sp | P62193 |	1	1.10	1.05	1.00	0.90	0.90	0.90	0.86

26S protease regulatory subunit 6A	sp | Q63569 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
26S protease regulatory subunit 6B	sp | Q63570 |	1	1.10	1.05	1.00	0.80	0.90	0.85	0.81
26S protease regulatory subunit 7	sp | Q63570 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
26S protease regulatory subunit 8	sp | P62198 |	1	1.10	1.05	1.00	1.00	1.20	1.10	1.05
Lon protease homolog, mitochondrial	sp | Q924S5 |	1	0.90	0.95	1.00	0.90	1.00	0.95	1.00
Protein Psmc6	tr | G3V6W6 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Xaa-Pro aminopeptidase 1	sp | O54975 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Xaa-Pro dipeptidase	sp | Q5I0D7 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Carboxypeptidase E	sp | P15087 |	1	1.10	1.05	1.00	0.80	0.80	0.80	**0.76**
Cytosolic non-specific dipeptidase	sp | Q6Q0N1 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Tripeptidyl-peptidase 2	sp | Q64560 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Dipeptidyl peptidase 3	sp | O55096 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Prolyl endopeptidase-like	tr | D3ZZ32 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Cathepsin D	tr | Q6P6T6 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Isoaspartyl peptidase/L-asparaginase	sp | Q8VI04 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Proprotein convertase subtilisin/kexin type 1 inhibitor	tr | G3V6X7 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Protein Npepps	tr | F1M9V7 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
SP120	tr | Q63555 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
26S proteasome non-ATPase regulatory subunit 1	tr | G3V8B6 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
26S proteasome non-ATPase regulatory subunit 13	sp | B0BN93 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
26S proteasome non-ATPase regulatory subunit 9	tr | G3V9P0 |	1	0.90	0.95	1.00	0.80	0.90	0.85	0.89
Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 3	tr | Q5U2S7 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 6	tr | Q6PCT9 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Proteasome subunit alpha type	tr | F1LSQ6 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Proteasome subunit alpha type-1	sp | P18420 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Proteasome subunit alpha type-2	sp | P17220 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Proteasome subunit alpha type-3	sp | P18422 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proteasome subunit alpha type-4	sp | P21670 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Proteasome subunit alpha type-5	sp | P34064 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Proteasome subunit alpha type-6	sp | P60901 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90

Proteasome subunit beta type	tr | Q6PDW4 |	1	1.10	1.05	1.00	0.80	1.10	0.95	0.90
Proteasome subunit beta type	tr | F1LNN1 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00
Proteasome subunit beta type	tr | G3V7Q6 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Proteasome subunit beta type-2	sp | P40307 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Proteasome subunit beta type-6	sp | P28073 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Alpha-1-inhibitor 3	sp | P14046 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Alpha-1-antiproteinase	sp | P17475 |	1	1.20	1.10	1.00	0.90	0.90	0.90	0.82
4-aminobutyrate aminotransferase, mitochondrial	sp | P50554 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90

Other

Hemoglobin alpha, adult chain 2	tr | B1H216 |	1	1.30	1.15	1.00	1.00	1.10	1.05	0.91
Hemoglobin subunit beta-1	sp | P02091 |	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Hemoglobin subunit beta-2	sp | P11517 |	1	1.40	1.20	1.00	1.00	1.00	1.00	0.83
Hemopexin	sp | P20059 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
0 beta-2 globin	tr | Q62670 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Heat shock 70 kDa protein 4	tr | F1LRV4 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Heat shock 70 kDa protein 4L	tr | B4F772 |	1	0.90	0.95	1.00	1.10	1.10	1.10	1.16
Heat shock 70kDa protein 12A (Predicted), isoform CRA_a	tr | D3ZC55 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Hypoxia up-regulated protein 1	tr | F1LN18 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heat shock cognate 71 kDa protein	sp | P63018 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Heat shock factor-binding protein 1	sp | Q8K3X8 |	1	1.10	1.05	1.00	0.90	1.10	1.00	0.95
Heat shock protein 105 kDa	sp | Q66HA8 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Heat shock protein HSP 90-alpha	sp | P82995 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Heat shock protein HSP 90-beta	sp | P34058 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
10 kDa heat shock protein, mitochondrial	sp | P26772 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
60 kDa heat shock protein, mitochondrial	sp | P63039 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Chaperonin containing Tcp1, subunit 6A (Zeta 1)	tr | Q3MHS9 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chaperonin subunit 8 (Theta) (Predicted), isoform CRA_a	tr | D4ACB8 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Hsc70-interacting protein	sp | P50503 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95

Small glutamine-rich tetratricopeptide repeat-containing protein alpha	sp | O70593 |	1	1.10	1.05	1.00	1.10	1.30	1.20	1.14
DnaJ (Hsp40) homolog, subfamily C, member 6 (Predicted)	tr | D4A0I5 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DnaJ homolog subfamily C member 5	sp | P60905 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
Hsp90 co-chaperone Cdc37	sp | Q63692 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stress-70 protein, mitochondrial	tr | F1M953 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Stress-induced-phosphoprotein 1	sp | O35814 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T-complex protein 1 subunit alpha	sp | P28480 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
T-complex protein 1 subunit beta	sp | Q5XIM9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
T-complex protein 1 subunit delta	sp | Q7TPB1 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
T-complex protein 1 subunit epsilon	sp | Q68FQ0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
T-complex protein 1 subunit gamma	sp | Q6P502 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
6-phosphofructokinase	tr | Q52KS1 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6-phosphofructokinase, liver type	sp | P30835 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00
Fructose-bisphosphate aldolase A	sp | P05065 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Fructose-bisphosphate aldolase C	sp | P09117 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glucose-6-phosphate isomerase	sp | Q6P6V0 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
78 kDa glucose-regulated protein	sp | P06761 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Ab2-076	tr | Q7TP61 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Ab2-417	tr | Q7TMC7 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Abi1 protein	tr | A2VD09 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Ac1002	tr | Q7TQ90 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Active BCR-related gene (Predicted)	tr | D4A6K9 |	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Acyl-CoA synthetase family member 2, mitochondrial	sp | Q499N5 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Acyl-CoA synthetase isoform 6 variant2	tr | Q6IU14 |	1	0.80	0.90	1.00	0.90	0.80	0.85	0.94
Acyl-CoA thioesterase 9	tr | Q5U2X8 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Acetyl-CoA acetyltransferase, mitochondrial	sp | P17764 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Alpha-enolase	sp | P04764 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
C-1-tetrahydrofolate synthase, cytoplasmic	tr | G3V6S5 |	1	0.80	0.90	1.00	0.90	1.10	1.00	1.11
C38 protein	tr | B7X6I3 |	1	0.80	0.90	1.00	1.00	0.80	0.90	1.00
Uncharacterized protein (Fragment)	tr | B5DEP6 |	1	1.00	1.00	1.00	0.90	1.10	1.00	1.00

Uncharacterized protein (Fragment)	tr | M0R5N3 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Uncharacterized protein	tr | M0R9D5 |	1	1.00	1.00	1.00	0.70	0.80	0.75	**0.75**
Uncharacterized protein	tr | D3ZFD0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Uncharacterized protein	tr | E9PT76 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LOC681996 protein	tr | B0BN63 |	1	0.90	0.95	1.00	1.00	1.10	1.05	1.11
LOC684097 protein	tr | B0BMY2 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein LOC679748	tr | D3ZE63 |	1	1.10	1.05	1.00	0.80	0.70	0.75	**0.71**
Glutathione S-transferase Mu 1	tr | G3V983 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Glutathione S-transferase Mu 5	sp | Q9Z1B2 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Glutathione S-transferase omega 1	tr | B6DYQ5 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Glutathione S-transferase	tr | B6DYP8 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Glutathione S-transferase pi	tr | B6DYQ7 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glutathione S-transferase Yb-3	sp | P08009 |	1	1.20	1.10	1.00	1.00	1.10	1.05	0.95
Aspartate aminotransferase, cytoplasmic	sp | P13221 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Aspartate aminotransferase, mitochondrial	sp | P00507 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Glycylpeptide N-tetradecanoyltransferase 1	sp|Q8K1Q0 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84
Branched-chain-amino-acid aminotransferase, cytosolic	sp | P54690 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Hypoxanthine-guanine phosphoribosyltransferase	sp | P27605 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
CDP-diacylglycerol--inositol 3-phosphatidyltransferase	sp | P70500 |	1	0.90	0.95	1.00	0.80	0.90	0.85	0.89
Phosphoserine aminotransferase	tr | Q68FU2 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	sp | P22062 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
Succinyl-CoA:3-ketoacid coenzyme A transferase 1, mitochondrial	sp | B2GV06 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Thiosulfate sulfurtransferase	sp | P24329 |	1	1.00	1.00	1.00	1.10	1.60	1.35	**1.35**
Protein arginine N-methyltransferase 1	sp | Q63009 |	1	1.10	1.05	1.00	1.00	0.90	0.95	0.90
GTP:AMP phosphotransferase AK3, mitochondrial	sp|P29411 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Hydroxymethylglutaryl-CoA synthase, cytoplasmic	sp | P17425 |	1	1.00	1.00	1.00	0.90	0.80	0.85	0.85
Maleylacetoacetate isomerase	sp | P57113 |	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Pdhx protein (Fragment)	tr | Q5BJX2 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Protein Pgm2l1	tr | D3Z955 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Adenosine kinase	sp | Q64640 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Adenylate kinase isoenzyme 1	sp | P39069 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Pyridoxal kinase	tr | G3V647 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
UMP-CMP kinase	sp | Q4KM73 |	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
3-hydroxyacyl-CoA dehydrogenase type-2	tr | B0BMW2 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
4-trimethylaminobutyraldehyde dehydrogenase	sp | Q9JLJ3 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Aminopeptidase B	sp | O09175 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Apolipoprotein E	sp | P02650 |	1	0.90	0.95	1.00	1.10	1.00	1.05	1.11
Atlastin-1	sp | Q6PST4 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Calreticulin	sp | P18418 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Carbonic anhydrase 2	sp | P27139 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Clusterin	sp | P05371 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Contactin-1	sp | Q63198 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
C-terminal-binding protein 1	sp | Q9Z2F5 |	1	1.00	1.00	1.00	0.80	0.80	0.80	**0.80**
Cullin-3	sp | B5DF89 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Cullin-associated NEDD8-dissociated protein 1	sp | P97536 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Cystathionine beta-synthase	sp | P32232 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Cystatin-C	sp | P14841 |	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
Cysteine and glycine-rich protein 1	sp | P47875 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cysteine-rich protein 2	sp | P36201 |	1	1.20	1.10	1.00	1.10	1.00	1.05	0.95
D-tyrosyl-tRNA(Tyr) deacylase	tr | B0K014 |	1	1.20	1.10	1.00	0.90	0.90	0.90	0.82
EF-hand domain-containing protein D2	sp | Q4FZY0 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Enoyl-CoA hydratase, mitochondrial	sp | P14604 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
ES1 protein homolog, mitochondrial	sp | P56571 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fam49b protein	tr | B2GUZ9 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Fibroblast growth factor	tr | Q794I6 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Four and a half LIM domains protein 1	sp | Q9WUH4 |	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Fumarylacetoacetate hydrolase domain-containing protein 2	sp | B2RYW9 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Galectin	tr | B4F7A3 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95
Glia maturation factor beta	sp | Q63228 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Glutamic acid decarboxylase 1, isoform CRA_a	tr | C9E895 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glyoxalase domain-containing protein 4	tr | GLOD4 |	1	1.00	1.00	1.00	1.00	0.90	0.95	0.95

Growth arrest-specific protein 7	sp|O55148|	1	1.20	1.10	1.00	1.00	1.30	1.15	1.05
Hexokinase-1	sp|P05708|	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Inosine triphosphate pyrophosphatase	sp|D3ZW55|	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Lactoylglutathione lyase	sp|Q6P7Q4|	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Low molecular weight phosphotyrosine protein phosphatase	sp|P41498|	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	sp|O08557|	1	1.00	1.00	1.00	1.10	1.00	1.05	1.05
Neuromodulin	sp|P07936|	1	0.80	0.90	1.00	1.00	0.80	0.90	1.00
Parathymosin	tr|B3DM95|	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Pdxp protein	tr|B2GV79|	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Pgm1 protein (Fragment)	tr|A1A5L2|	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Phosphoglycerate kinase 1	sp|P16617|	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Phosphoglycerate mutase 1	sp|P25113|	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Phosphorylase	tr|B1WBU9|	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Programmed cell death 6-interacting protein	sp|Q9QZA2|	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Ak5	tr|M0R7U1|	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
Protein Cct7	tr|D4AC23|	1	1.10	1.05	1.00	0.90	1.00	0.95	0.90
Protein DJ-1	sp|O88767|	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Irgq	tr|M0R686|	1	1.10	1.05	1.00	1.10	1.30	1.20	1.14
Protein Ppa1	tr|F7EPH4|	1	1.20	1.10	1.00	1.00	1.00	1.00	0.91
Protein RGD1304884	tr|D4A3C2|	1	1.00	1.00	1.00	1.10	1.20	1.15	1.15
Protein RGD1309586	tr|D3ZN21|	1	0.90	0.95	1.00	1.00	0.90	0.95	1.00
Protein RGD1559864	tr|D3ZBS6|	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Protein RUFY3	sp|Q5FVJ0|	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Protein Sept6	tr|B5DFG5|	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Ugp2	tr|Q4V8I9|	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Protein unc-45 homolog A	tr|M0RC57|	1	1.10	1.05	1.00	1.10	1.00	1.05	1.00
Purine nucleoside phosphorylase (Fragment)	tr|D3ZXK9|	1	1.00	1.00	1.00	1.20	1.10	1.15	1.15
Serum albumin	sp|P02770|	1	1.20	1.10	1.00	1.10	1.10	1.10	1.00
Sulfated glycoprotein 1	tr|F7EPE0|	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Superoxide dismutase [Cu-Zn]	tr|Q6LDS4|	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Superoxide dismutase [Mn], mitochondrial	sp|P07895|	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Transaldolase	sp | Q9EQS0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Transketolase	sp | P50137 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Triosephosphate isomerase	sp | P48500 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Tripartite motif-containing protein 2 (Fragment)	tr | D3ZM62 |	1	1.00	1.00	1.00	1.00	1.10	1.05	1.05
WD repeat-containing protein 1	sp | Q5RKI0 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
WD repeat-containing protein 7	sp | Q9ERH3 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Guanine deaminase	tr | Q9JKB7 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Alpha-endosulfine	sp | P60841 |	1	0.90	0.95	1.00	1.00	0.70	0.85	0.89
NSFL1 cofactor p47	sp | O35987 |	1	1.10	1.05	1.00	1.10	1.10	1.10	1.05
Haloacid dehalogenase-like hydrolase domain-containing protein 2	tr | Q6QI86 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Hepatoma-derived growth factor	tr | F1LPC7 |	1	1.10	1.05	1.00	0.90	1.10	1.00	0.95
Ig kappa chain C region, A allele	sp | P01836 |	1	1.00	1.00	1.00	1.10	1.30	1.20	**1.20**
Long-chain-fatty-acid--CoA ligase ACSBG1	sp | Q924N5 |	1	1.00	1.00	1.00	1.10	1.10	1.10	1.10
Non-specific lipid-transfer protein	tr | F1LQ55 |	1	0.90	0.95	1.00	0.90	0.90	0.90	0.95
Omega-amidase NIT2	sp | Q497B0 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Protein Aimp1	tr | Q4G079 |	1	1.10	1.05	1.00	1.10	1.20	1.15	1.10
Protein Appl1 (Fragment)	tr | D3ZWA8 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Nova2 (Fragment)	tr | F1M4H5 |	1	0.90	0.95	1.00	0.80	0.80	0.80	0.84
Protein Wdr37	tr | D3ZQ02 |	1	1.00	1.00	1.00	0.90	1.00	0.95	0.95
Protein Wdr47	tr | G3V9M3 |	1	1.10	1.05	1.00	1.00	1.10	1.05	1.00
Set protein	tr | B0BMV1 |	1	1.10	1.05	1.00	1.20	1.10	1.15	1.10
Myotrophin	sp | P62775 |	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
tRNA-splicing ligase RtcB homolog	sp | Q6AYT3 |	1	1.10	1.05	1.00	1.00	1.00	1.00	0.95
Casein kinase II subunit alpha	sp | P19139 |	1	1.00	1.00	1.00	0.80	0.90	0.85	0.85
Casein kinase II subunit beta	sp | P67874 |	1	1.00	1.00	1.00	0.90	0.90	0.90	0.90