A ROLE FOR THE ORGANIC CATION TRANSPORTER-3 IN STRESS-INDUCED PSYCHOPATHOLOGY

By

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To my father, for always believing in me

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LIST OF ABBREVIATIONS

5H1AA	5-hydroxy-indole acetic acid
5HT	5-hydroxytryptamine or serotonin
5HT1A	5-hydroxytryptamine 1A
5HTT	5-hydroxytryptamine transporterRemember to use a tab between the abbreviations and the definitions
5HTTLPR	5-hydroxytryptamine transporter-linked promoter region
ACC	anterior cingulate cortex
ACTH	adrenocorticotropic hormone
BDNF	brain-derived neurotrophic factor
BNST	bed nucleus of stria terminalis
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
CVS	chronic variable stress
D22	decynium 22
DMH	dorsomedial nucleus of the hypothalamus
DA	dorsal area of the hypothalamus
EMT	extraneuronal monoamine transporter
GR	glucocorticoid receptor
GRE	glucocorticoid response element
HPA	hypothalamic-pituitary-adrenocortical
HPLC	high performance liquid chromatography
LE	Long Evans
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mPFC	medial prefrontal cortex

OCD	Obsessive compulsive disorder
OCT3	Organic cation transporter-3
PET	Positron emission tomography
PTSD	Post Traumatic Stress Disorder
PVN	Parvoventricular nucleus of the hypothalamus
SSRI	Selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
WKY	Wistar-Kyoto

Abstract of Dissertation Presented to the Graduate School
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The molecular genetic basis of individual susceptibility to stress-related psychiatric disorders is a subject of intense interest in the neuroscience community. For example, studies have shown that genetic polymorphisms in the 5-hydroxytryptamine transporter (5HTT) gene are associated with an increased likelihood of developing Major Depression, and it is likely that multiple other important signaling mechanisms may be disrupted in these knockouts. Genetic expression of organic cation transporter-3 (OCT3), a corticosterone-sensitive monoamine transporter, is upregulated in the hippocampus of 5HTT-deficient mice, suggesting that the OCT3 may play an important role in the regulation of cellular function relevant to major depression. In the present study, we characterized basal and stress-induced expression of the (OCT3) in the hippocampus, medial prefrontal cortex (mPFC), and striatum of Wistar-Kyoto (WKY) and Long-Evans (LE) rats by real-time PCR and Western blot. The WKY strain exhibits depressive-like behavior, heightened sensitivity to stress and a muted response to conventional antidepressants of the SSRI class, whereas the LE strain has been described as stress-resilient, exhibiting more active coping responses to stress. In our study, 5HTT gene expression was significantly downregulated in the hippocampus of

WKY relative to LE rats with a corresponding basal elevation of the OCT3 in both the hippocampus and mPFC. These results indicate that the OCT3, which serves as a “back-up” transporter when the 5HTT is saturated or in low abundance, may be a critical modulator of 5HT clearance in the WKY rat.

Rats were randomly assigned to the following groups: 1) control 2) acute restraint stress and 3) repeated social defeat + acute restraint stress. OCT3 gene expression was upregulated in the WKY rats exposed to acute restraint stress after a history of social defeat, but not in WKY rats exposed to acute restraint stress alone. By contrast, acute stress induced a robust increase in OCT3 gene expression in the hippocampus of LE rats, and this effect was not seen in the LE rats that had a history of social defeat. These results also suggest that the WKY rats exhibit a delayed genomic adaptation to stress.

The pattern of stress-induced hippocampal gene expression of the glucocorticoid receptor (GR) was similar to the pattern of expression of the OCT3 gene within both strains, but the within-groups differences did not reach statistical significance. However, there was a significant correlation between OCT3 and GR gene expression in the hippocampus within each strain, suggesting that glucocorticoid signaling may regulate both GR and OCT3 gene expression. OCT3 gene expression was not significantly altered by stress in the mPFC or the striatum of WKY rats or LE rats, which is consistent with previous studies in 5HTT-knockout mice in which upregulation of the OCT3 was restricted to the hippocampus.

Tissues for western blot analysis of OCT3 protein expression were collected 3 hours after the onset of the acute stressor (or equivalent time in the controls).

Consistent with our gene expression data, basal expression of OCT3 protein in the hippocampus was significantly elevated overall in WKY compared to LE rats. The WKY rats exhibited an interesting pattern of elevated cytosolic OCT3 but suppressed membrane OCT3 in the acutely-stressed rats with a history of social defeat. In the LE rats, there was a trend toward a decrease in plasma membrane OCT3 expression in the hippocampus after repeated stress that did not reach statistical significance. However, there was a significant increase in OCT3 protein expression in the cytosol after repeated stress in LE rats. Taken together, these data suggest that the OCT3 may be internalized when bound by corticosterone, which would explain why the OCT3 is localized to the cytosol 3 hours after stress.

Basal elevation of OCT3 mRNA and protein levels in the hippocampus and mPFC of WKY rats compared to LE rats may indicate that the OCT3 has been recruited to maintain 5HT clearance due to the relatively low abundance of 5HTT. These same high levels of OCT3 expression in the hippocampus may allow WKY rats to maintain high rates of serotonin clearance even when corticosterone is present, which may increase their vulnerability to stress-induced psychopathology. In order to test this hypothesis, we administered decynium 22 (0, 1 and 10 ug/kg), a specific inhibitor of the OCT3, to WKY rats shortly before behavioral testing on the forced swim test. It was found that both 1 ug/kg and 10 ug/kg decynium 22 produced a profound antidepressant effect, suggesting that the OCT3 may be a useful target for antidepressant pharmacotherapy in patients who, like the WKY rat, are unresponsive to selective serotonin reuptake inhibitors (SSRIs).

CHAPTER 1
GENERAL INTRODUCTION

Clinical Significance

Major depression is one of the leading causes of disability, lost productivity and diminished quality of life across the world. An estimated 121 million people suffer the devastating physical, emotional, and social consequences of depression, which constitutes the fourth leading contributor to the global burden of disease (World Health Organization, n.d.). In fact, it is estimated that by 2020, depression will be the second leading cause of death behind heart disease, making this one of the most pressing public health concerns of our time.

Although there are a variety of commercially available drugs that are commonly used to treat depression, it is estimated that between 10-30% of depressed patients do not achieve remission after an initial trial with a selective serotonin reuptake inhibitor (SSRI), the most widely prescribed antidepressant drugs. Furthermore, the success rate drops significantly for subsequent trials with antidepressant drugs after the first trial, such that a large proportion of initially unresponsive patients will eventually be classified as “refractory” to antidepressant treatment. Even for those that do respond, however, the time-to-onset of antidepressant action can be rather prolonged (5-7 weeks) which may not meet the needs of severely depressed patients who cannot otherwise function normally in their daily lives.

Yet despite its demographic prevalence and drastic socioeconomic and interpersonal impact, the etiology of depression remains elusive. From a biomedical standpoint, progress in this field has been limited by the paucity of animal models that recapitulate the neurochemical and physiological sequelae of major depression (O’Neil

and Moore, 2003). In our studies we have attempted to address this by performing depressogenic (i.e. stress) manipulations in the Wistar-Kyoto (WKY) rat, a strain that exhibits innate depressive-like characteristics. We used this model to characterize neurobiological correlates of depression that may lead to the discovery of new potential targets for antidepressant pharmacotherapy. Our focus is on the organic cation transporter-3 (OCT3), a polyspecific monoamine transporter that has been linked with a variety of psychiatric conditions in humans including obsessive-compulsive disorder (OCD) and methamphetamine use disorder (Aoyama et al., 2006;Fujimoto et al., 2007;Nagayama et al., 2007;Lazar et al, 2008). Here we present a series of studies that help to elucidate the role of the OCT3 stress-induced psychopathology, particularly depression. We propose that the results of these studies may pave the way toward clinical studies in humans.

Pathophysiology of Depression

Depression can best be described as a physiological and behavioral syndrome characterized by flattened affect, and loss of motivation, interest and pleasure in daily activities, as well as physical and mental fatigue that is both pervasive and enduring (>2 weeks) (American Psychiatric Association [DSM-IV-TR], 2000). There is no singular cause of depression but it is instead thought to arise from a complex interplay of genetic, experiential and developmental factors.

Genetic Factors

Both genetic linkage studies and the candidate gene approach have led to useful insights into the genetic basis of depression. Although there is no one gene that causes depression, researchers have identified a number of susceptibility genes that, when exposed to the toxic milieu of childhood trauma, parental abuse or neglect, or other

forms of developmental and social stress, can substantially increase the likelihood of developing depression. Modern estimates of the heritability of depression from genetic linkages studies vary from 30% to 70% depending on the stringency of the criteria used to define depression in clinical studies, suggesting the need for further investigations into the genetic origins of this elusive disease (McGuffin et al., 2007). One recently identified region of interest located on chromosome 15q has been the subject of at 3 independent large-scale studies, including the Genetics of Recurrent Early-Onset Major Depression (GenRED) study, the European-U.S. Depression Network (DeNt) study, and a Utah-based study (McGuffin et al., 2007).

The serotonin transporter

Studies have identified a link between specific genetic loci, such as 5' regulatory region of the serotonin transporter (5HTT) gene, and an enhanced susceptibility to depression and post-traumatic stress disorder (PTSD) (Lin et al., 2004;Lee et al., 2005;Pezewas et al., 2005). This 5HTT-linked promoter region (5HTTLPR), located on 17q11.2, controls transcription of the 5HTT and thus has important implications for 5HT transmission in the central nervous system. The 5HTT is expressed on the postsynaptic membrane of serotonergic neurons and is primarily involved in reuptake of extracellular 5HT from the synapse. This not only terminates 5HT neurotransmission but also facilitates the repackaging of 5HT into synaptic vesicles. One particular variant of the 5HTTLPR polymorphism, the *s* allele, results in a reduced transcription rate of 5HTT gene and lower 5HTT mRNA expression (Bradley et al., 2005). In contrast, the long (*l*) allele confers high transcriptional efficacy resulting in greater 5HTT expression. In agreement with these findings, *in vitro* studies in lymphoblastoid cell lines derived from

individuals who are homogyous for the *s* allele have verified that 5HTT mRNA content is significantly lower and that 5HT reuptake is reduced by half relative to carriers of the *ℓ* allele (Lesch et al., 1996). Carriers of the *s* allele also exhibit anatomical differences in brain regions that mediate mood, attention and sensitivity to threatening or emotionally-evocative stimuli. Regions that play a pivotal role in the modulation of emotional responses, such as the anterior cingulate cortex (ACC) and the amygdala, are reduced in volume, consistent with findings from other studies in depressed patients. Interestingly, the thalamus is enlarged by a similar margin (11-12%) in both depressed subjects and those with the *ss* genotype (Young et al., 2008). This increased capacity of the thalamus may lead to heightened sensitivity to environmental stimuli, which in turn could overload the limbic system (e.g. the ACC and amygdala) and cause a compensatory downsizing of these brain regions. Further evidence for a direct relationship between thalamic volume and emotional behavior comes from the fact that treatment with antidepressant drugs reverses the trend; that is, people taking antidepressants show a reduction in thalamic volume relative to controls (Young et al., 2008).

There is evidence of a strong gene-environment interaction between the *ss* genotype and stress, which may be mediated by differential activation of the thalamus and other limbic regions that are modified by 5HTT gene expression. Studies in mice reveal that behavioral differences between 5HTT knockout and wild-type mice are only apparent under stress but not baseline conditions (Murphy et al., 2001). Similarly in macaques, carriers of the *s* allele only exhibit decreased serotonergic function when reared under elevated stress conditions (Bennett et al., 2002). Thus the effects of *ss*

genotype on emotional behavior appear to be kindled by stress, an observation that has been confirmed in humans. For instance, functional MRI studies have shown that an emotional task which involves identifying fearful facial expressions elicits a greater amygdala response in human carriers of the *s* allele compared to non-carriers, which again points toward a role of the 5HTT in modulating limbic response to stress and anxiety-provoking events (Hariri et al., 2002). This overactivation of the amygdala in response to stress may also produce a corresponding loss of amygdalar volume that has been previously observed in individuals with the *ss* genotype.

The moderating influence of the *ss* genotype on individual response to stressful life events is aptly demonstrated using epidemiological data from the Dunedin Multidisciplinary Health and Development study (Caspi et al., 2003). The probability of a major depressive episode in adulthood does not vary significantly with genotype in individuals with a relatively normal, stress-free upbringing. However, depression rates climb significantly among *s* carriers as a function of childhood trauma. Even more striking is the fact that individuals of the *ll* genotype appear to be remarkably resilient to the effects of childhood trauma, with depression rates remaining at a relatively low 30% even among survivors of severe trauma. The same holds true even when the stress occurs later on in adulthood, that is, the incidence of depression among *s* carriers, but not *ll* individuals, increases with cumulative life stress between the ages of 18-26. Thus the effects of stress on emotional behavior in genetically vulnerable individuals are not limited to the childhood years.

These data suggest that genes themselves may not necessarily cause depression but instead interact with complex array of developmental, experiential, and social factors

that predispose an individual to depression. Severe or prolonged stress, in conjunction with certain “risk” genes such as the 5HTT, seems to be a precipitating factor that sets in motion the neurobiological processes that actually cause depression. However, we cannot attribute a multifactorial disease such as depression to a single gene, as Risch et al. remind us in their 2009 meta-analysis of 14 published studies which revealed no significant interaction between stress, depression, and the 5HTT-s allele (Risch et al., 2009). Yet the failure to replicate the gene x environment effect for a single gene, the 5HTT gene, does not exclude the possibility of other genetic, epigenetic, or environmental factors that may alter expression or penetrance of a gene of interest and thus neutralize (or augment) its effect on phenotype (e.g. gene x gene x environment interactions).

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) has a wide distribution in parts of the brain known to mediate emotional responses, and studies have found that hippocampal BDNF is not only depleted in depressed subjects (Chen et al., 2001), but plays a key role in the dendritic remodeling observed in the hippocampus following antidepressant treatment. It has even been suggested that failure to upregulate BDNF early on may predict failure to respond to antidepressants (Tadic et al., 2010). Furthermore, there is some evidence of cross-talk between BDNF and 5HT pathways which increases the likelihood of a gene x gene interaction effect on depression outcome. The BDNF val(66)met polymorphism has been the subject of new investigations into the genetic basis of depression and has yielded some novel insights into the gene-environment debate. The BDNF val(66)met polymorphism in the 5’ pro-region of the BDNF protein results in lower depolarization-induced BDNF secretion from neurons, which negatively

impacts hippocampal excitability and episodic memory (Egan et al., 2003). Studies have shown a direct association between the BDNF val(66)met polymorphism and characteristics that are prevalent in a variety of psychiatric disorders, such as anxiety (Chen et al., 2006) and suicidality (Pregelj et al., 2010), while others have shown that the interaction of BDNF val(66) met polymorphism with childhood adversity (gene x environment) elevates the risk of depression in adulthood (Aguilera et al., 2009). Moreover, there appears to be a higher-order interaction between the BDNF val(66)met polymorphism, the 5HTTLPR polymorphism, and childhood events (gene x gene x environment) that exerts a strong influence on the developmental course of depression (Kaufman et al., 2006).

Antidepressant response and the 5HT1A receptor

So far we have presented evidence that inheritance of multiple susceptibility genes confers sensitivity to the depressogenic effects of chronic life stress. Further evidence suggests that response time to SSRI antidepressants, such as sertraline, is also affected by variation in the 5HTT and other genes. Individuals with one or more copies of the *s* allele of the 5HTT tend to have a slower response time to sertraline (Durham et al, 2004), whereas *ll* individuals exhibit the most rapid response. This differential response may be explained in terms of the mechanism of SSRI action. SSRIs directly curtail high 5HTT expression generated by the variant of the 5HTTLPR polymorphism, resulting in a better prognosis for pharmacotherapy. On the other hand, the primary target of SSRIs is in lower abundance in carriers of the *s* allele, which may render individual carriers of the *s* allele less sensitive to the antidepressant effects of SSRIs. Once again, we find that gene x gene interactions affect the outcome of antidepressant

therapy, much as they do the etiology of depression itself. Combination of the ss genotype of the 5HTT with the 5HT1A receptor -1018C/G polymorphism results in a phenotype that is refractory to the antidepressant citalopram (Arias et al., 2005). The 5HT1A receptor plays an important role in the antidepressant response, since ultimately the goal of therapy is to restore 5HT signaling which is mediated through postsynaptic 5HT1A receptors. However, the inhibitory effects of somatodendritic 5HT1A autoreceptors are thought to predominate in the initial phase of treatment when there is an immediate spike in 5HT in the synapse (Artigas et al., 1996). This excess 5HT binds to the autoreceptors, inhibiting further 5HT release which may account for the initial lag phase of 3-4 weeks in antidepressant treatment (Blier et al., 1987). Continued treatment with SSRIs is thought to desensitize 5HT1A autoreceptors, restoring 5HT release to pretreatment levels so that postsynaptic 5HT1A receptors are activated (Blier and de Montigny, 1998). The contribution of 5HT1A autoreceptors to antidepressant action may help to explain why the 5HT1A -1018 C/G polymorphism, which occurs in a region that controls transcription of the 5HT1A gene, has such a profound effect on antidepressant response time (Arias et al., 2005). The 5HT1A -1018 C/G polymorphism in particular has been associated with higher rates of transcription of the 5HT1A gene, which may translate to more autoreceptors that inhibit firing of 5HT neurons.

The glucocorticoid receptor

The glucocorticoid receptor (GR) is a key regulator of stress-responsive neurohormonal signaling, in which one or more polymorphisms may contribute to vulnerability for depression. In particular, the 22/23EK polymorphism in the GR gene, much like the 5HTTLPR and BDNF val(66)met polymorphism, contributes to depressive psychopathology only in the presence of childhood trauma (Bet et al., 2008). This

particular allele affects responsiveness of the GR, rendering it less sensitive to cortisol. The GR is widely distributed in parts of the brain that send inhibitory inputs to the parvaventricular nucleus (PVN) of the hypothalamus and essentially switch off the stress response, so this desensitized version of the GR would conceivably render the individual more vulnerable to the deleterious effects of stress.

Role of Stress

Depression has been characterized as a disorder of stress regulation that typically manifests under conditions of prolonged, and/or severe stress against a background of genetic vulnerability. Under normal circumstances, stress is a highly adaptive series of physiological and behavioral responses to perturbations in the environment that have been co-opted to restore and maintain homeostasis. This definition of *stress* as a response of the organism to some somatic or external event is not to be confused with the stimulus that evokes it, which will be referred to as a *stressor*. Activation of brainstem catecholaminergic neurons, the amygdala, and the bed nucleus of stria terminalis (BNST) send converging, excitatory inputs to the paraventricular nucleus (PVN), which serves as the linchpin for activation of the central stress axis. Stimulation of the PVN initiates a neuroendocrine signaling cascade known as the hypothalamic-pituitary-adrenocortical (HPA) axis that begins with secretion of corticotropin-releasing factor (CRF) and arginine-vasopressin into the pituitary portal circulation. This in turn stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary, which is released into systemic circulation. The binding of ACTH in the adrenal cortex releases glucocorticoids such as cortisol (corticosterone in rats) into the circulation (for a review see Herman et al., 2005). It is important to note that any gross interruption of this vital pathway (e.g. adrenalectomy in rats) is fatal without corticosterone

replacement, emphasizing that basal function of the HPA axis is essential for survival. When we speak of stress in the negative, we are referring to severe, prolonged or chronic stress.

Glucocorticoids exert their effects on the sympathetic nervous system by increasing heart rate, constricting blood vessels, and reducing digestive and other vegetative functions in preparation for the “fight or flight” response. In the brain they also bind to glucocorticoid receptors (GR), which are enriched in parts of the limbic system that mediate feedback to the PVN during stress. (Sapolsky et al., 1984;Herman et al., 1989;Herman and Cullinan, 1997;Herman et al., 2005). The key players in this feedback pathway will be discussed in the next sections.

Prolonged or severe stressors such as childhood abuse, neglect, social isolation, or maternal separation may induce neurobiological adaptations in the stress axis that are conducive to those extreme environments but become maladaptive under normal circumstances. Associations between childhood trauma and perturbations in the HPA response are well-documented in the literature, although there are some discrepancies between reports about the directionality of these changes that may be attributed to confounding factors such as genetic variation, the type and timing of abuse, gender, age and comorbid psychiatric or substance abuse disorders. One study reporting elevated ACTH and cortisol in response to the dexamethasone/CRF suppression test in victims of childhood abuse suggest an exaggerated pituitary response to CRF or insensitivity to the inhibitory effect of glucocorticoids (Heim et al., 2001), which is characteristic of certain types of depression. In fact, childhood abuse sufferers with comorbid depression had the most robust response, which supports the notion that

altered stress responsiveness, mediated by childhood experience and genetic vulnerability, can predispose individuals to psychopathology. On the other hand, depressed individuals without childhood adversity exhibited a slightly lower HPA response relative to controls, highlighting that depression is a disease of diverse etiology. Individuals with and without a history of childhood trauma may become depressed, but the developmental trajectories leading to this common endpoint may differ by a considerable margin.

Early life stress has also been linked with an increased risk of depression, which is independently associated with a dysregulated HPA response that is marked by an elevated, yet flattened sinusoidal rhythm of cortisol secretion (Stokes et al., 1995). Animal studies concur with these findings, and reports of CRF involvement in depression abound (Binder and Nemeroff, 2010). For instance, those with a genetic predisposition to depression, such as carriers of the ss genotype of the 5HTT gene, are shown to have elevated waking cortisol and ACTH levels compared to other genotypes (Chen et al., 2009;Wüst et al., 2009).

Hippocampus

The hippocampus is a limbic structure located inside the medial temporal lobe which derives its name from the Greek *hippos* meaning “horse” and *kampos* meaning “seamonster”. Although well-known for its role in learning and memory, the hippocampus is also a key modulator of the stress axis as evidenced by the presence of GRs on hippocampal neurons, which provide an important source of negative feedback to the PVN.

The hippocampus is also exquisitely sensitive to the deleterious effects of stress on neurogenesis and synaptic integrity. Sustained elevations in glucocorticoid levels

negatively impact cell proliferation and BDNF expression in the hippocampus, leading to eventual neuronal loss and hippocampal atrophy (Stokes et al., 1995;Gould et al., 1997;Schmidt and Duman, 2007). Thus prolonged periods of stress interrupts negative feedback to the PVN, allowing excessive glucocorticoid release to exert further hippocampal damage.

Depression has also been associated with hippocampal volume loss (Chen et al., 2001;Nichols et al., 2001;Marin et al., 2007;Lanfumey et al., 2008) and concomitant learning and memory impairments that tend to worsen with age (Kim and Diamond, 2002;Schmidt and Duman, 2007). In fact, a lifetime history of depression has been identified as a potential risk factor in the cognitive impairment of elderly subjects (Sierksma et al., 2010). Similarly, depressogenic manipulations in animals result in neuronal degeneration in the CA3 region of the hippocampus and profound deficits in neurogenesis. Reversal of these effects is achieved with antidepressant treatment, and in fact may be essential to achieve a therapeutic response to these drugs (Santarelli et al., 2003).

Prefrontal cortex

The medial prefrontal cortex (mPFC) plays a somewhat more complex role in negative feedback regulation of the HPA axis, although its overall actions are inhibitory. Stress evokes c-fos expression in the anterior cingulate and prelimbic subdivisions of the mPFC, which have been shown to modulate ACTH and corticosterone secretion and cfos induction in the PVN, possibly via projections to the ventrolateral preoptic area, dorsomedial hypothalamus, and peri-PVN region (Hurley et al., 1991;Sesack et al., 1989). The infralimbic division of the mPFC, on the other hand, opposes the actions of the other two and sends afferent projections to the bed nucleus of stria terminalis

(BNST), medial and central amygdala and the nucleus of the solitary tract, all of which activate the HPA axis. However, the inhibitory effects of the mPFC on the HPA axis predominate, and structural abnormalities in the mPFC have been implicated in the pathophysiology of a variety of psychiatric disorders. Hypofrontality, or reduced activation of the frontal cortex, contributes to the dysregulation of central stress pathways in the brain and inhibition of emotional and behavioral responses to stress, and these effects tend to be lateralized to the left hemisphere. Depression is associated with gray matter loss in the ACC and orbital and ventrolateral PFC based on MRI and postmortem histological analysis with a corresponding decrease in total metabolic activity as reported by positron emission tomography (PET) studies (Drevets et al., 2008). We also know from animal studies that chronic stress, which has depressogenic effects, induces dendritic spine loss (Radley et al., 2006), apical dendritic retraction (Radley et al., 2004) and volumetric reductions in the mPFC (Kitayama et al., 2006), paralleling morphological changes seen in the hippocampus under similar experimental conditions.

Role of Monoamines

The monoaminergic hypothesis of depression emerges from the fact that depression is closely linked with the dysregulation of serotonergic and other monoamine systems in the brain. In the 1950s it was observed that many patients taking the antihypertensive drug reserpine, which depletes monoamines, developed symptoms of depression (Musselman et al., 1998), giving rise to animal models of depression that are based on monoamine depletion. Perhaps the most compelling evidence stems from yet another fortuitous discovery that iproniazid, an experimental drug used to combat tuberculosis infection, has antidepressant effects (Smith et al., 1953;Loomer et al.,

1957; Kline et al., 1958;Salzer and Lurie, 1953). Furthermore, iproniazid pretreatment appeared to reverse the depressogenic effects of reserpine (Saunders et al., 1959; Kline, 1959), suggesting a possible mode of action that involved potentiation of monoaminergic activity. Reserpine depletes monoamines by interfering with uptake of monoamines into synaptic vesicles through the vesicular monoamine transporter (VMAT), leaving them vulnerable to enzymatic digestion by monoamine oxidases (MAOs). Iproniazid, a monoamine oxidase inhibitor (MAOI), prevents this degradation, and as a result monoamine concentrations at nerve terminals are vastly increased (Assael et al., 1960). And while this drug and other MAOIs were abandoned in favor of tricyclic antidepressants (TCA) shortly thereafter, they played an indispensible role in the formulation of the so-called “catecholamine hypothesis” of depression (Schildkraut, 1965). It was originally thought that catecholamines, namely norepinephrine and dopamine, were responsible for the antidepressant action of MAOIs and TCAs. This was supported by the fact that imipramine, a TCA, was shown to block reuptake of norepinephrine into presynaptic terminals (Axelrod et al., 1961). A growing body of evidence, however, pointed to serotonin as the culprit. Tryptophan, the precursor to serotonin synthesis, augmented the antidepressant effects of the MAOI tranylcypromine, while tryptophan depletion had the opposite effect on mood. Furthermore, it was found that imipramine blocked serotonin reuptake in platelets. Depressed patients exhibit reduced concentrations of the serotonin metabolite 5-hydroxy-indole acetic acid (5-HIAA) in the cerebrospinal fluid (CSF) and post-mortem brain tissue (Owens et al., 1994). Suicide victims, which represent the most severe case of depression, manifest other signs of serotonin deficiency such as increased $5HT_2$

receptor density, decreased 5HTT density and decreased $5HT_{1A}$ autoreceptor binding (Owens et al., 1994;Drevets, 1998). These findings led to the eventual design of selective serotonin reuptake inhibitors (SSRIs) which averted many of the side effects incurred with previous antidepressants due to their actions on cholinergic systems. These drugs represent the gold standard of antidepressant therapy today, which again lends credence to the monoamine hypothesis of depression, now modified to include serotonin.

Nodes of Interaction between Stress and Monoaminergic systems

Dysregulation of the stress response, which can lead to certain types of psychopathology including depression, is thought to stem from low serotonergic drive (Stokes, 1995). As noted earlier, depression is associated with impaired negative feedback to the HPA axis which may be mediated by extended glucocorticoid action at the hippocampus and other limbic sites. Serotonin is a key modulator of two important players in the stress network, hippocampal BDNF and GR, which may be protective against hippocampal damage and diminished feedback drive. Thus, individual differences in serotonergic tone, whether genetically determined or environmentally induced, may confer vulnerability for affective disorders.

The hippocampus is an important site of interaction between glucocorticoid and serotonin systems in the brain, given its role in learning and memory processes, stress regulation, and mood disorders. Corticosterone elicits a rapid response from 5HT systems by increasing the activity of tryptophan hydroxylase, the rate limiting enzyme in 5HT synthesis, and increasing the firing rates of 5HT neurons that project to the hippocampus. A variety of acute stressors have also been shown to increase 5HT release in the hippocampus (Keeney et al., 2006;Linthorst and Reul, 2008). This

increase in 5HT release in the hippocampus is important for habituation to chronic stress (Keeney et al., 2006), whereas serotonin depletion (Zhou et al., 2008) or lesions in serotonergic neurons projecting to the hippocampus (Robertson et al., 2005) resulted in loss of glucocorticoid receptors, anxiety-like behavior and anhedonia. Studies in rodents have also found that $5HT_{1A}$ autoreceptors, which inhibit 5HT release from presynaptic terminals, are desensitized in the hippocampus following chronic stress (Buwalda et al., 2005;Lanfumey et al., 2008). These facilitatory actions of glucocorticoids on 5HT neurotransmission are thought to promote behavioral adaptation through selective modifications in corticolimbic circuitry.

Excessive stimulation by glucocorticoids exerts an inhibitory effect on serotonin function through mechanisms which are not yet clearly understood. As mentioned earlier, acute stress stimulates 5HT release in the hippocampus, but this response is inhibited under conditions of prolonged, unmitigated stress. For instance, mice reared in isolation have a blunted 5HT response to novelty stress compared to group-reared mice (Bickerdike et al., 1993; Muchimapura et al., 2002). Furthermore, chronic social defeat stress decreases the number of 5HTT binding sites in the CA3 region of the hippocampus, similar to what has been observed in suicide victims. We propose a role for OCT3 as a molecular interface between glucocorticoid actions at limbic regulatory sites and serotonin signaling in the brain that mediates neurobiological adaptations to stress.

The Organic Cation Transporter-3

Neuroanatomical Distribution

The OCT3 belongs to a family of polyspecific solute carriers that have been shown to play a significant role in the elimination of cationic species in tissues such as

the liver and kidney (Prichard et al., 1993;Ullrich, 1994). The OCT1 was the first to be cloned from rat kidney, and both OCT1 and OCT2 subtypes are predominantly expressed in the kidney, liver and to a lesser extent the intestine. More recently OCT3 mRNA was discovered to be abundantly expressed in the placenta, but is also detectable by Northern blot analysis in certain parts of the brain (Kekuda et al, 1998). Further characterization of OCT3 distribution in the brain by *in situ* hybridization reveals robust expression within the cerebellum, hippocampus, pontine nucleus and cortex (Wu et al., 1998). High levels of expression are also detected along the brain-CSF border and choroid plexus (CP), emphasizing the putative role of the OCT3 in regulating the bioavailability of cationic neurotoxins in the brain. However, the OCT3 also has a hand in what is referred to as uptake$_2$, a low-affinity, steroid-sensitive, Na^+- and Cl^--independent and phylogenetically older system for removing extracellular monoamines. Given its role in uptake$_2$, OCT3 expression is found to cluster along monoaminergic pathways innervating the dorsal raphe, striatum, cortex, PVN of the hypothalamus, thalamus, hippocampus and nucleus accumbens (Amphoux et al., 2006;Gasser et al., 2009). The OCT3 also colocalizes with dopamine D1 receptors in parts of the amygdala, but not with tyrosine hydroxylase or dopamine β-hydroxylase, suggesting that the OCT3 is situated on postsynaptic membranes (Hill et al., 2009).

The cellular distribution of the OCT3 is not uniform across the entire brain, although it was originally named the “extraneuronal monoamine transporter” (EMT) due to its association with glia in some parts of the brain. For instance in the nigrostriatal pathway, the OCT3 is predominantly expressed in astrocytes. In the hippocampus, however, the OCT3 is primarily localized to neurons where it likely participates in postsynaptic

clearance of extracellular monoamines (Cui et al., 2009). In our work we have focused primarily on the GR-rich limbic regions of the hippocampus and medial prefrontal cortex where monoaminergic systems and stress pathways converge in the brain.

Pharmacology

An alternative catecholamine transport system, dubbed uptake$_2$, was proposed by Iversen in 1965 to account for the accumulation of norepinephrine in cardiac muscle after perfusion at very high concentrations (>1 µg/ml). (Iversen, 1965). This mode of transport, unlike high-affinity presynpatic reuptake, is characterized as a low-affinity, high-capacity, Na^+- and Cl^- -independent system that is driven by membrane potential and is pharmacological sensitive to steroids, cyanine dye derivatives and O-methylated catecholamines such as normetanephrine (Gründemann et al., 1997;Wu et al., 1998;Amphoux et al., 2006). The OCT3 has been shown to exhibit all of these properties and was subsequently established as the molecular identity of uptake$_2$ (Wu et al., 1998).

Role in monoamine transport

A variety of monoamines, including norepinephrine, dopamine, serotonin and histamine, can serve as substrates for the OCT3, albeit at concentrations in the micromolar range. (Gründemann et al., 1998;Gründemann et al., 1999;Schomig et al., 2006). This low-affinity, high capacity transport system is especially salient when the high-affinity transporters are saturated or in low abundance. This was aptly demonstrated in mice with a homozygous deletion of the 5HTT, in which 5HT levels were kept at relatively normal concentrations due to upregulation of the OCT3 in the hippocampus (Schmitt et al., 2003;Baganz et al., 2008). As previously noted, stress

provokes the rapid release of monoamines which may provide the necessary conditions for the OCT3 to exert a discernible effect on monoaminergic neurotransmission.

Regulation by corticosterone

Corticosteroids have been shown to elicit rapid increases in extracellular monoamine concentrations in the dorsomedial hypothalamus (DMH) (Lowry et al., 2001), possibly through their acute inhibitory actions on the OCT3 (Gasser et al., 2006). Previous studies have demonstrated elevated dopamine levels in the DMH of salamanders within 20 minutes of receiving an intracerbroventricular (ICV) injection of CRF. Similar effects were observed following CORT injection except that both dopamine and 5HT concentrations were elevated (Lowry et al., 2001). Acute restraint stress induced simultaneous increases in 5HT, DA and NE concentrations in the dorsal hypothalamic area (DHA) of Lewis rats (Lowry et al., 2003), which is suggestive of extracellular accumulation mediated by blockade of the OCT3. Conversely in Fischer rats, only NE decreases in the DHA after restraint stress, revealing possible strain-dependent differences in stress responsiveness. This blunted monoaminergic response to acute stress in Fischer rats may partially explain their hyperreactivity to stress as indicated by their plasma corticosterone levels. There was also a negative correlation between 5HT and plasma corticosterone concentrations in unstressed rats, suggesting that 5HT activity in the DHA may suppress excitability of the HPA axis.

Other studies have demonstrated that intra-DMH injection of the cyanine dye derivative decynium 22, which specifically inhibits the OCT3, elicits robust increases in extracellular 5HT as quantified by *in vivo* microdiaysis (Feng et al., 2005). Contrary to the findings of Lowry and colleagues, this group did not observe a significant increase in 5HT following corticosterone infusion in the DMH. However, it should be noted that

Feng et al. used in vivo microdialysis combined with high performance liquid chromatography (HPLC) on live animals to obtain a measure of extracellular monoamine concentrations. Lowry, on the other hand, performed HPLC analysis on postmortem tissues, which reflects both intracellular and extracellular levels of the analyte. However, Feng et al. did observe that corticosterone potentiated the effects of fenfluramine on extracellular 5HT in the DMH. Fenfluramine increases serotonin release by disrupting vesicular storage, causing a flood of 5HT in the synapse that overwhelms high-affinity transport (Feng et al., 2009). Taken together, these findings illustrate that the contributions of the OCT3 to 5HT neurotransmission are “unmasked” in the presence of unusually high 5HT levels. The same is true when restraint stress is used in place of corticosterone. While acute restraint stress alone did not elevate extracellular corticosterone or 5HT levels in Sprague-Dawley rats as measured by in vivo microdialysis, it did augment the effects of decynium 22 (Feng et al., 2010). It should be noted that the effects of OCT3 on 5HT neurotransmission are not limited to the DMH and DHA, as other studies have noted that decynium 22 reduces 5HT clearance in the CA3 region of the hippocampus using in vivo chronoamperometry. However, these effects were only apparent in mice lacking at least one copy of the 5HTT gene (Baganz et al., 2008), illustrating once again that uptake$_2$ via the OCT3 is important in the homeostatic regulation of 5HT when high-affinity transport is absent or disabled.

Interactions with the 5HTT and antidepressant response

Recent studies have shown that the OCT3 is upregulated in the hippocampus of mice lacking 5HTT, which may serve to buffer against elevations in synaptic 5HT concentrations (Schmitt et al., 2003;Baganz et al., 2008). This implies that processes that disrupt normal 5HTT function lead to the recruitment of OCT3 for the removal of

extracellular 5HT, at least in the hippocampus. It stands to reason that the OCT3 may negate the therapeutic benefit derived from antidepressant drugs, which typically exert their effects by increasing synaptic levels of 5HT. Human carriers of the *s* allele may be especially vulnerable to the buffering effects of OCT3 on extracellular 5HT, since they presumably have lower 5HTT expression which has been associated with OCT3 upregulation in mice. It would be interesting and informative to see if OCT3 is also upregulated in depressed patients with the *s* variant of the 5HTTLPR polymorphism, which may both explain their unique depressive phenotype and their insensitivity to conventional antidepressants. Furthermore, targeting the OCT3 may offer new therapeutic strategies for treating such patients, as indicated by preliminary studies in which the OCT3 inhibitor decynium 22 effectively reduced depressive-like behavior in 5HTT knockout mice, but not in wild-type controls (Baganz et al., 2008).

Role in Psychopathology

The OCT3 has been associated with a variety of psychiatric disorders including obsessive-compulsive disorder (OCD) and methamphetamine use disorder in humans(Lazar et al., 2008;Aoyama et al., 2006;Fujimoto et al., 2007;Nagayama et al., 2007) and depressive- and anxiety-like behavior in rodents (Wieland et al., 2000;Kitaichi et al., 2005;Baganz et al., 2008;Vialou et al., 2008;Wultsch et al., 2009). These results are not surprising given the role of the OCT3 in 5HT transport and clearance, which is demonstrated in 5HTT-knockout mice (Baganz et al., 2008). It has also been shown that OCT3 antisense and OCT3 inhibitors such as decynium 22 and normetanephrine (Kitaichi et al., 2005;Baganz et al., 2008;Schildkraut et al., 2004;Mooney et al., 2008) can exert an antidepressant effect in the Porsolt swim test. Furthermore, genetic deletion of the OCT3 was found to reduce anxiety-like behavior on the elevated plus

maze and open field tests (Wultsch et al., 2009). Still others have argued that the OCT3 deletion may disrupt neuronal function (Vialou et al., 2008). This ambiguity with regard to the role of OCT3 in psychiatric morbidity is partially resolved during the course of our study of the comparative expression of OCT3 in the WKY rat, which represents an animal model of depression with both face and construct validity. We provide the first evidence that OCT3 gene expression is differentially regulated in the Wistar-Kyoto rat, which may give rise to an aberrant stress response and vulnerability to psychopathology.

Project Rationale

The goal of these studies is to characterize the role of the OCT3 in the etiology of stress-related psychopathology. Chronic stress is associated with the onset and exacerbation of depression, a limbic-mediated mood disorder that is marked by anhedonia, despair, and psychomotor retardation. Stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis and triggers the release of corticosterone (CORT) into circulation, which can directly modulate 5HT signaling through their actions on OCT3. As such, individual differences in susceptibility to Major Depression may be associated with genetic variations in the expression or functionality of the OCT3.

In our studies we made use of the Wistar-Kyoto (WKY) rat as an animal model of depression. These animals exhibit depressive-like behavior in the Porsolt forced swim test (Paré, 1994;Armario et al., 1995;Lopez-Rubalcava and Lucki, 2000), which is the current standard in screening new compounds for antidepressant efficacy (Porsolt et al., 1978). Acute stress in these animals also elicits an exaggerated and protracted neuroendocrine response that fails to habituate with repeated stress exposure (Pare and Redei, 1993;Rittenhouse et al., 2002;De la Garza and Mahoney, 2004). Stress

provokes increased rates of 5HT turnover, increased $5HT_{1A}$ receptor sites and a decrease in 5HTT sites (De La Garza and Mahoney, 2004), which may be associated with altered expression of the OCT3 in this strain of rats. This idea is supported by the fact that 7-day treatment with corticosterone alters 5HT uptake in the hippocampus of WKY rats in a 5HTT-independent fashion (Fernandez et al., 2001). In the experiments reported in Chapter 2 we assessed gene and protein expression of the OCT3 in the WKY rat. We postulated OCT3 expression would be differentially regulated in the WKY rat relative to the more stress-resilient Long-Evans (LE) strain, which may account for strain-specific deficits in serotonergic tone and subsequent expression of depressive-like behavior.

In the experiments of Chapter 3 we assessed the effects of an OCT3 antagonist on depressive-like behavior in the forced swim test. Decynium 22, a specific inhibitor of the OCT3, has been shown to induce a rapid increase in extracellular 5HT content in the dorsomedial hypothalamus of rats. Elevations in 5HT due to inhibition of OCT3 are associated with a reduction in depressive-like behavior in the Porsolt forced swim test, We hypothesized that decynium 22 would have an antidepressant effect on the forced swim test in WKY rat.

As described earlier, the OCT3 is a high-capacity, low affinity transporter of 5HT that participates in 5HT clearance and regulates serotonin neurotransmission when the high-affinity 5HTT is saturated or in low abundance. Such is the case in human carriers of the s allele of the 5HTT who have both reduced expression of the 5HTT and a higher propensity toward depressive behavior. Individuals with the ss genotype are also refractory to conventional antidepressants of the selective serotonin reuptake inhibitor

(SSRI) class. Due to the low abundance of the 5HTT, there is reason to believe that the OCT3 may be upregulated in these individuals and may also serve as a novel target for antidepressant intervention. The WKY rat shares many characteristics in common with these genetically predisposed individuals including low 5HTT expression and a poor response to SSRI drugs on the forced swim test (Lahmame and Armario, 1996;Griebel et al., 1999;Lopez-Rubalcava and Lucki, 2000;Tejani-Butt et al., 2003), indicating that the WKY rat may also serve as a model of antidepressant resistance that can be used to test the effectiveness of new pharmacological approaches to treatment, such as OCT3 inhibition.

CHAPTER 2
EFFECTS OF ACUTE AND REPEATED STRESS ON EXPRESSION OF THE OCT3 IN AN ANIMAL MODEL OF DEPRESSION

Introduction

Several lines of evidence indicate that chronic exposure to stress can invoke depressive symptoms in a subset of individuals with certain genetic, developmental and psychosocial profiles. We have attempted to identify some of these factors that can predispose an individual to stress-induced psychopathology using a model that recapitulates some of the core features of depressive disorders. Although depression is a multi-factorial disease with phenotypic heterogeneity that is difficult to reproduce in an animal model, there are at least two symptoms that manifest in almost all cases of depression: anhedonia, or the loss of pleasure in almost all activities and helplessness, and the inability to act in one's own interest to avoid harmful or unpleasant circumstances (Zacharko and Anisman, 1991;Snaith, 1993;O'Neil and Moore, 2003; Bürgy, 2008). The WKY rat embodies these core elements of depressive illness, exhibiting excessive immobility on the forced swim test (which is used as a proxy for helplessness behavior) and prolonged elevation in plasma CORT levels (which suggests hyperactivity of the HPA axis). In these rats, stress is also accompanied by reduced 5HT levels (De La Garza and Mahoney, 2004), increased $5HT_{1A}$ receptor binding in the hippocampus and hypothalamus, and a decrease in 5HTT sites in the cortex and hippocampus (Páre and Tejani-Butt, 1996), whereas in other strains the directionality of these parameters is reversed. This leads us to postulate that stress may differentially affect the 5HT systems of WKY rats compared to other strains, giving rise to neurobiological adaptations in corticolimbic circuitry that manifest as depression.

The OCT3 is a stress-responsive, monoamine transporter in the brain that has profound influence on 5HT neurotransmission in the limbic system. The OCT3 is acutely inhibited by corticosterone, which produces local increases in 5HT in limbic regions of the brain that may facilitate stress recovery. Previous studies have shown that the OCT3 is upregulated in the hippocampus of mice lacking the 5HTT. Upregulation of the OCT3 can increase the rate of 5HT clearance in the hippocampus which in turn may inhibit behavioral adaptation to stress. In these studies, we examined strain-specific differences in OCT3 expression between WKY and Long-Evans (LE) rats with a view toward elucidating the molecular mechanisms that underlie stress-induced psychopathology. We hypothesized that differential expression of OCT3 in the hippocampus of WKY rats may contribute to their stress-vulnerable phenotype.

Induction of a depressive-like state in animals can be accomplished by exposure to a variety of stressful stimuli that promote physiological and behavioral changes. In the resident-intruder paradigm or “social defeat” model, a large male “resident” rat comes into brief contact with the test animal, a smaller “intruder” rat. This interaction, which almost always results in the defeat of the test animal, aims to recapitulate the dominance-subordination hierarchies that impact mood regulation and affect. The social defeat model of depression possesses high face validity, since the most common source of stress in humans is of a psychosocial nature (Bjorkqvist, 2001).

Social defeat is positively associated with elevated glucocorticoid and ACTH activity and behavioral sensitization to mild stress (Buwalda et al., 2005); both core symptoms of human depression (Stokes, 1995). Social defeat also invokes dendritic remodeling and inhibition of neurogenesis in tree shrews (Magarinos et al., 1996; Gould

et al., 1997), which correlates with the hippocampal atrophy that occurs in human depression (Sala et al., 2004). On a behavioral level, chronic social defeat also induces anhedonia on the sucrose preference test and helplessness behavior on the forced swim test which is attended by histone modifications in the hippocampus (Rygula et al., 2005;Rygula et al., 2006;Hollis et al., 2010). Furthermore, antidepressants have been shown to reverse these behavioral adaptations induced by chronic social defeat (Rygula et al., 2006).

In this study, we applied the social defeat model to the genetically vulnerable WKY rat to simulate the genes-environment interactions that give rise to major depression. Our goal was to determine if the OCT3 gene is differentially regulated by stress in WKY rats compared to LE rats. Given that LE rats readily adapt to stress and exhibit none of the behavioral characteristics associated with depression, this strain was used as a control throughout all of our experiments. We also analyzed GR gene expression and BDNF protein expression in the hippocampus as biomarkers of stress-induced neuroplasticity. In order to investigate the putative correlation between 5HTT and OCT3, we analyzed 5HTT gene expression in our samples as well.

Methods

Experimental Design

Separate cohorts of WKY and LE rats were used to obtain tissues for real-time PCR and Western blot analysis of the OCT3. In each cohort, WKY and LE rats were randomly assigned to control, acute, and repeated stress groups. All rats in the repeated stress group were exposed to daily social defeat for 7 days. On day 8, rats in the acute and repeated stress groups were exposed to 30 minutes of restraint stress, then returned to their home cages for the remainder of 2 hours (real-time PCR) or 3

hours (Western blot) since the beginning of the restraint session. Control rats were handled daily but were otherwise undisturbed (Figure 2-1).

General Methods

Experimental animals

Twenty-four adult male WKY and and twenty-four adult male LE rats weighing 250-300 g at the beginning of the experiment were pair-housed in standard polycarbonate cages (43 x 21.5 x 25.5 cm) in a climate controlled vivarium with a 12-hour light/dark schedule (lights on at 7 am). The rats were allowed *ad libitum* access to standard laboratory chow (Lab Diet 5001) and tap water. The rats were allowed 7 days to adapt to the housing facility before the experiments began. At the end of the experiment, the animals were terminated by rapid decapitation.

Post-mortem tissue processing

Rat brains were dissected at 4°C to remove the mPFC, hippocampus and striatum according to a modified version of the technique first published by Iversen and Glowinski (Iversen and Glowinski, 1966). Dissected tissues were frozen in isopentane maintained at -40°C and subsequently stored at -80°C until further processing.

Stress Procedures

Social defeat

Male retired breeder rats of the Long-Evans strain weighing at least 600 g at the start of experiments were isolation-housed in standard polycarbonate cages in a climate-controlled vivarium with a reverse 12-hour light/dark schedule (lights on at 7 pm). Rats were allowed 7 days to acclimate to the housing facility before screening for dominance behavior. During the screening test, an intruder rat was placed inside the resident's home cage. If the intruder exhibited submissive behavior (supine posture 3

times for a minimum of 2 sec each time), the resident was classified as dominant. Once dominance was established, the test session was terminated. This procedure was repeated daily (up to 30 days) until there are 3 consecutive days in which the intruder was defeated 3 times within the 5 min session.

Once the residents met the minimum criteria for dominance behavior, they were used for subsequent social defeat experiments. The social defeat procedure consisted of two stages. In the first stage, the intruder rat was placed into a 10 x 10 x 15 cm (inner dimensions) double-walled wire mesh cage protective cage. The rat was then placed, inside the protective cage, into the home cage of the resident rat for 45 minutes. The cage served to separate the rats, avoiding physical contact but maintaining stressful sensory stimuli. During the second stage, the intruder rat was removed from the wire mesh cage and placed directly inside the resident's home cage for 5 minutes. Dominance/submission was scored as above. This direct interaction between resident and intruder rats continued for 5 min before the test session was terminated. After exposure to the resident rat, the intruder rat was removed and returned to its home cage (Figure 2-2).

Restraint stress

Restraint stress was performed according to methods we previously described by our laboratory (Devine et al., 2003;Simpkiss and Devine, 2003;King et al., 2007). Briefly, each rat was individually removed from its home cage and placed into a restraining tube for 30 minutes. The restraint tube was composed of a soft flexible sheet of plastic (11" X 7 ¾") mounted to a rigid Plexiglas cradle (8 ½" X 3' X 3") by means of two small bolts with convex heads. The securing nuts were on the bottom of the cradle to prevent any

discomfort to the animal. The cradle was open on the two long sides to allow the rat to be placed on the plastic sheet while flat. There were ventilation holes at one end to allow unrestricted breathing, and the other end had a vertical slot to allow comfortable placement of the tail during the restraint process. The plastic sheet was then gently rolled around the animal and held securely in place with two 12” X 1” Velcro strips. Following the 30 min exposure to restraint stress, the rat was returned to its home cage.

Biochemical and Molecular Assays

Real-time PCR

RNA isolation was carried out according to the protocol by Molecular Research Center (MRC), Cincinnati OH. Briefly, tissues were homogenized in 1 ml TRI Reagent RT (MRC, Cincinatti, OH) with a sonifier and mixed with 50 ul 4-bromoanisole (BAN), then incubated on ice for 5 minutes. Samples were then centrifuged at 12,000 x g for 15 minutes at 4°C and 0.5 ml of the aqueous phase was transferred to a new tube for subsequent wash and precipitation steps. The resulting RNA pellet was reconstituted in nuclease-free water (Applied Biosystems/Ambion, Austin, TX) and the final concentration was quantified using a NanoDrop spectrophotometer. cDNA was generated from total RNA using the High Capacity RNA-to-cDNA kit according to the manufacturer’s protocol. (Applied Biosystems, Foster City, CA). Real-time PCR was carried out using Taqman Gene Expression reagents with primer/probe sets from Applied Biosystems that are specific for the OCT3 (Assay ID-Rn00570264_m1), 5HTT (Assay ID-Rn00564737_m1), GR (Assay ID-Rn00561369_m1) or eukaryotic 18S (Assay ID-Hs99999901_s1) (Table 2-1). All samples were analyzed in duplicate on the Applied Biosystems 7500 Fast Real-time PCR system.

Cycle thresholds (Ct) values were used to compute the relative quantification (RQ) for each gene of interest. RQ values were calculated according to the formula below:

ΔCt = Ct (gene of interest) – Ct(18S)

$\Delta\Delta Ct$ = mean ΔCt (sample) – mean ΔCt (reference)

RQ = $2^{-\Delta\Delta Ct}$ relative quantification

The mean ΔCt (reference) used to compute the $\Delta\Delta Ct$ was the mean ΔCt of the LE control group in cases where both strains were analyzed together or the LE strain was analyzed separately. In cases where the WKY group was analyzed separately, the mean ΔCt (reference) is the mean ΔCt of the WKY control group.

Western blot

Tissues were processed for Western blot analysis using the Plasma Membrane Protein Extraction Kit (Biovision, Mountain View, CA) to obtain separate fractions of cell surface, organelle, and cytosolic proteins. OCT3 and BDNF protein expression was quantified by Western blot using antibodies directed against the rat OCT3 (Abcam, Cambridge, MA). N-Cadherin (Abcam, Cambridge, MA) or β-actin (Cell Signaling Technology, Danvers, MA) were used as endogenous controls in plasma membrane and cytosol extracts, respectively.

Protein electrophoresis was performed on a Nu-PAGE 4-12% Tris-glycine gel (Invitrogen, Carlsbad, CA). Each sample was diluted in loading buffer (50mM Tris-HCl (pH 6.8), 2% SDS (sodium dodecyl sulfate), 0.1% bromophenol blue, 10% glycerol, 50 mM DTT). Ten µg of protein was loaded per lane, with molecular weight markers run in parallel. Electrophoresis was performed at 195 volts for approximately 60 min. The gels were then equilibrated in protein transfer buffer containing 25 mM Tris, 192 mM glycine, and 10% (v/v) methanol. The proteins were electrophoretically transferred onto a

polyvinylidene difluoride (PVDF) membrane using a semi-dry transfer apparatus (Biorad, Hercules, CA). After the transfer, the membrane was rinsed with Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBS-T) with pH of 7.4 and blocked for 1 hour with 5% non-fat dry milk in TBS-T. The membrane was then incubated with rabbit anti-rat OCT3 or BDNF IgG (Abcam, Cambridge, MA) (Table 2-2) diluted 1:1000 in 5% non-fat dry milk in TBS-T overnight at 4°C. After the overnight incubation they were washed three times in TBS-T and incubated for 1 hr at room temperature in horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:2000 goat anti-rabbit peroxidase conjugate (Cell Signaling Technology, Beverly, MA) in 5% non-fat dry milk in TBS-T. Membranes were washed 3 × 10 min in TBS-T at room temperature with rocking, then developed using Phototope®-HRP Western Blot Detection System (Cell Signaling Technology) and exposed to Kodak Biomax Light chemiluminescence film. Chemiluminescent bands were quantified by optical densitometry using Image J software.

The PVDF membranes were stripped by immersion in SDS stripping buffer (containing 62.5 mM Tris, 2% SDS and 150 mM 2-mercaptoethanol, pH 6.8) for 15 min. at 37°C. Membranes were then washed 3 x 10 min in TBS-T, reblocked in 5% non-fat dry milk in TBS-T, and re-probed with an antibody for N-cadherin (plasma membrane marker) or β-actin (cytosolic marker) to verify equivalent expression of this stable marker is present in blots from all the rats in each treatment group. Chemiluminescent bands were quantified by optical densitometry using the MCID camera system and Image J software. The integrated optical density (OD) of bands corresponding to the

OCT3, BDNF, N-cad, and β-actin were measured using this program and used to compute the relative OD for each gene of interest.

Adjusted OD = OD (gene of interest)/OD (endogenous control)

Relative OD= Adjusted OD (sample)/mean adjusted OD (reference)

In cases where both strains were analyzed together and the LE strain was analyzed separately, the reference is the LE control group. In cases where the WKY strain was analyzed separately, the reference is the WKY control group.

Statistical Analyses

Gene and protein expression data for the OCT3, GR, and 5HTT were analyzed by two-way analyses of variance (ANOVAs) using Graphpad Prism software to determine significant main effects of strain and stress and significant strain x stress interactions. Data from all groups are expressed relative to the LE control group. Individual Student t-tests were used to determine significant differences between WKY and LE rats in each stress category. The results of these analyses are presented in the first graph (A) of each figure.

In cases where the two-way ANOVAs revealed a significant main effect of stress, we analyzed both strains separately using a one-way ANOVA so that pairwise comparisons could be made between different stress categories with Newman-Keuls post-tests. In this case, all data were expressed relative to the LE control group for the comparisons of stress effects across the LE groups and to the WKY control group for comparisons of stress effects across WKY groups. The results of these analysis are depicted in (B) and (C) of each figure.

We performed correlation analysis between OCT3 gene expression and 1) GR and 2) 5HTT gene expression within each strain using the Pearson's correlation in Graphpad Prism.

Results

Real-time PCR Data

OCT3 gene expression

Hippocampus. There were significant main effects of strain ($F(1,41)=1630$, $p<0.0001$) and stress ($F(2,41)=266$, $p<0.0001$) on hippocampal gene expression of the OCT3, and a significant interaction between strain and stress ($F(2,41)=310.3$, $p<0.0001$) (Figure 2-3A). Student t-tests revealed OCT3 gene expression was significantly higher in WKY compared to LE rats in all stress groups.

The effects of stress on OCT3 gene expression were also analyzed separately for each strain. A one-way analysis of variance (ANOVA) revealed a significant effect of stress on OCT3 gene expression in the hippocampus of LE rats ($F(2,21)=9.804$, $p=0.001$). Post-hoc analysis showed that acute, but not repeated stress induces a robust increase in hippocampal OCT3 gene expression compared to all other groups in LE rats (Figure 2-3B). An identical analysis in WKY rats also revealed a significant effect of stress ($F(2,20)=23.15$, $p<0.0001$) on hippocampal gene expression of the OCT3 (Figure 2-3C) . In this case, however, it was repeated, but not acute stress that invoked upregulation of the OCT3 compared to all other groups.

Medial prefrontal cortex. A main effect of strain on OCT3 gene expression in the mPFC was apparent ($F(1,41)=169.6$, $p<0.0001$), but there was no main effect of stress ($F(2,41)=1.357$), $p=0.2688$). There was, however a significant stress x strain interaction

(F(2,41)=4.863, p=0.0127) (Figure 2-4A). Student t-tests revealed a significant elevation in OCT3 in WKY relative to LE rats in all stress categories.

Striatum. There were no main effects of strain (F(1,41)=0.03982, p=0.8428) or stress (F(2,41)=0.4063, p=0.6687) on striatal OCT3 gene expression There was no significant interaction between stress and strain (F(2,41)=0.5070, p=0.6061) and (Figure 2-5A).

GR gene expression

There was no main effects of strain (F(1,41)=0.3087, p=0.5815) on hippocampal GR expression. Although the effect of stress on GR gene expression did not quite reach significance (F(2,41)=2.941, p=0.0641), there was a trend toward an increase after acute stress. There was also no significant interaction stress x strain interaction (F(2,39)=2.236, p=0.1197) (Figure 2-6A). There was, however, a significant positive correlation between OCT3 and GR expression in both strains (r(20)=0.5056, p=0.0164 for WKY group and r(21)=0.4165, p=0.048 for LE group) (Figure 2-8A, B).

5HTT gene expression

There was a significant main effect of strain (F(1,41)=11.65, p=0.0015) but not of stress (F(2,38)=0.8443, p=0.4372) on 5HTT expression in the hippocampus. There was also no significant stress x strain interaction (F(2,38)=1.196, p=0.3127) (Figure 2-7A). Student t-tests revealed a significant decrease in 5HTT expression in WKY rats in the acute and repeated stress groups, but not in the control group.

As with the GR, there was a positive correlation between OCT3 and 5HTT expression in the WKY group (r(20)=0.4892), p=0.0209), but not the LE group (r(21)=0.2750,p=0.2154) (Figure 2-8C, D).

Western Blot Results

OCT3 protein expression

Plasma membrane. OCT3 protein expression was quantified in the plasma membrane fractions of the hippocampus. Significant main effects of both strain ($F(1,42)=11.03$, $p=0.0019$) and stress ($F(2,42)=9.417$, $p=0.0004$) were observed (Figure 2-9A). However, there was no significant interaction between stress and strain on OCT3 protein expression in the hippocampus ($F(2,42)=0.3682$, $p=0.6942$). Student t-tests indicated a significant elevation in OCT3 in WKY relative to LE rats in the control and repeated stress groups.

In LE rats there was a trend toward a decrease in hippocampal OCT3 expression at the plasma membrane with stress that did not reach significance ($F(2,21)=2.141$, $p=0.1425$) (Figure 2-9B). In WKY rats there was a significant effect of stress in hippocampal OCT3 protein expression at the plasma membrane ($F(2,21)=11.77$), $p=0.0004$), with post-tests indicating a significant downregulation of the OCT3 after repeated stress compared to the control and acute stress groups (Figure 2-9C).

Cytosol. Plasma membrane protein expression of the OCT3 did not correspond to our gene expression data, so we decided to perform these assays in cytosolic fractions to account for any OCT3 protein that may be associated with vesicular membranes. There was a significant main effect of stress ($F(2,42)=22.8$, $p<0.0001$) but not of strain ($F(1,42)=1.876$, $p=0.1780$) on OCT3 protein expression in the cytosol and a significant interaction between stress and strain ($F(2,42)=8.516$, $p=0.0008$) (2-10A). However, strain effects on OCT3 expression may have been missed due to the fact that lower OCT3 expression in WKY rats in the control and acute stress conditions was counterbalanced by higher expression in the repeated stress condition. According to

Student t-tests, cytosolic OCT3 was lower in WKY relative to LE rats in the control and acute stress conditions and elevated by a similar margin in the repeated stress condition.

Stress effects within each strain were analyzed separately by one-way ANOVA. In LE rats there was an effect of stress ($F(2,21)=4.426$, $p=0.0249$) and post-tests indicated a significant increase in cytosolic OCT3 in the repeated stress group relative to controls (Figure 2-10B). In WKY rats there was a significant effect of stress on OCT3 protein expression in the cytosol ($F(2,21)=18.78$, $p<0.0001$) and post-hoc analysis revealed that the OCT3 was upregulated after repeated stress relative to all other groups (Figure 2-10C).

BDNF protein expression

BDNF protein expression was quantified in cytosolic fractions of the hippocampus. There was a significant main effect of both strain ($F(1,42)=4.349$, $p=0.0431$) and stress ($F(2,42)=9.001$, $p=0.0006$), but no significant stress x strain interaction on BDNF protein expression in the hippocampus ($F(2,42)=1.106$, $p=0.3403$) (Figure 2-11A). Student t-tests did not indicate an effect of strain within individual stress groups.

A one-way ANOVA detected a significant effect of stress on BDNF protein expression in LE ($F(2,21)=5.026$, $p=0.0165$) rats (Figure 2-11B) and WKY ($F(2,21)=5.06$, $p=0.0161$) (Figure 2-11C) rats. Post-hoc analysis indicated that in both strains, BDNF expression was upregulated after repeated stress compared to all other groups.

Discussion

OCT3 Gene Expression

The results we obtained indicate a profound elevation in basal OCT3 gene and protein expression in the hippocampus and mPFC of WKY relative to LE rats which may translate to increased 5HT clearance in these brain regions. Corticosterone, which inhibits the OCT3, elevates extracellular 5HT which in turn facilitates normalization of the HPA axis after stress (Robertson et al., 2005;Keeney et al., 2006;Zhou et al., 2008). This heightened expression of the OCT3 in the WKY rat (roughly 5X that of LE rats) may simply overwhelm the inhibitory actions of corticosterone, such that the change in extracellular OCT3 between stress and basal conditions is low. The passive behavioral response (e.g. floating) of WKY rats in the forced swim test compared to most other strains supports this view. Acute injections of drugs that increase extracellular serotonin, including corticosterone, have been shown to increase active swimming responses on the forced swim test. The relative passivity of the WKY rat suggests that corticosterone, which is released during swim, fails to produce a sufficient increase in extracellular 5HT.

It is notable that OCT3 gene expression was upregulated after acute stress in LE rats, then returned to basal levels after repeated stress exposure. A single exposure to mild stress has a number of beneficial effects on cognition, mood and memory which may be mediated by local increases in monoamine levels, and stress-induced 5HT signaling plays a key role in physiological and behavioral adaptation to stress. It has been suggested that inhibition of the OCT3 by corticosterone may underlie this stress-induced increase in extracellular 5HT in the dorsomedial hypothalamus and possibly other brain regions (Lowry et al., 2001; Lowry et al., 2003; Gasser et al., 2006). Thus, the transient upregulation after a single stress exposure may provide an outlet to boost

5HT levels in times of stress. The absence of any effect of acute stress on OCT3 gene expression in WKY rats supports this hypothesis, since WKY rats are known to habituate much more slowly to stress. Elevated basal expression of the OCT3 in WKY rats may also inhibit any further upregulation by stress until the cumulative pressure of multiple stress exposures eventually overcomes this initial hurdle.

The fact that rats with divergent behavioral and neurochemical profiles could be differentiated based upon their OCT3 gene expression pattern suggests a close association between the OCT3 and emotional responses to stress. WKY rats are slow to adapt to the effects of repeated stress and manifest a helpless, depressive phenotype under stress conditions whereas LE rats adapt more readily to stress and respond in a more aggressive, proactive manner. These behavioral characteristics correlate nicely with gene expression of the OCT3 under acute and repeated stress conditions.

Interestingly, the effects of stress observed in the hippocampus were not replicated in the mPFC or the striatum, indicating that the hippocampus may be the locus of interaction between stress and the OCT3. It has been reported in the literature that the OCT3 is mainly localized to astrocytes in the striatum, which is how it acquired its original name of the extraneuronal monoamine transporter (EMT), but in the hippocampus the OCT3 colocalizes with postsynaptic neurons where it may play a more direct role in monoaminergic signaling and stress adaptation. The apparent dissociation between hippocampal and striatal gene regulation of the OCT3 may also be attributed to their disparate roles in affect regulation. For instance, 5HT signaling has been shown to modulate dopamine release in the striatal reward circuitry, but in the hippocampus

5HT release not only promotes adaptive and behaviorally appropriate responses to stress but also stimulates brain-derived neurotrophic factor (BDNF) signaling and neurogenesis.

In the mPFC we did observe a basal elevation of the OCT3 in WKY rats, but the effects of stress were not apparent as they were in the hippocampus. This was surprising, given the high density of GRs in the mPFC and its similar role in feedback inhibition of the PVN (Diorio et al., 1993). Although we cannot rule out the possibility that the OCT3 is upregulated in other brain regions that were not included in our analysis, the data we have collected so far suggests that the effects of stress on OCT3 expression are restricted to the hippocampus. This expression pattern is also found in 5HTT knockout mice, suggesting that OCT3 gene expression under stress conditions may be controlled by a similar mechanism, namely excess extracellular 5HT. However, stress also increases extracellular 5HT in the mPFC (Mokler et al., 2007), so we cannot fully explain the differential response of the hippocampus and mPFC at this time.

Clearly, stress exerts robust effects on OCT3 gene expression in both strains, at suggesting that the OCT3 promoter may be responsive to glucocorticoids themselves or some downstream signaling pathway. Any discussion of the precise mechanism of OCT3 gene regulation by stress at this point would be premature since stress invokes a myriad of biochemical pathways in the brain, any one of which may be responsible for the observed effects. However, there are two logical candidates for this role which warrant further exploration due to their known interactions with the OCT3; corticosterone and 5HT. Corticosterone interfaces directly with the OCT3 to inhibit monoamine transport, resulting in a transient increase in 5HT levels in limbic regions of

the brain. Given the significance of stress-induced 5HT signaling in modulating stress responsiveness, it seems reasonable to postulate that the corticosterone-OCT3 pathway would be tightly controlled. Corticosterone has been shown to regulate transcription of GR receptors, which mediate negative feedback to the HPA axis, and may exert similar effects on expression of the OCT3 gene. In support of this hypothesis, we found that the expression pattern of the OCT3 is highly correlated with that of the GR. Stress has been shown to upregulate GR gene expression via glucocorticoids and may also upregulate OCT3 gene expression by a similar mechanism. However, our data show that the effects of stress on OCT3 gene expression are much more robust, suggesting that the OCT3may not be acting alone to regulate OCT3 gene expression. This idea is further supported by the fact that WKY rats expressed elevated OCT3 mRNA in the hippocampus and mPFC relative to LE rats under basal conditions.

Stress is also known to activate 5HT neurotransmission in the hippocampus, and previous studies have provided some evidence that upregulation of the OCT3 may be a homeostatic mechanism for regulating extracellular 5HT concentrations. The compensatory upregulation of the OCT3 in the absence of the 5HTT, which creates conditions of excess extracellular 5HT, lends support to idea that genetic expression of the OCT3 is regulated, directly or indirectly, by 5HT. In fact, since stress-induced 5HT release in the hippocampus has been shown to promote habituation to repeated stress by presumably lowering the HPA responsiveness, this mechanism provides built-in a feedback loop that would also turn down OCT3 gene expression after repeated expression, which would explain our findings in LE rats. The absence of any change in OCT3 expression after acute stress in WKY rats may reflect weak 5HT signaling which

in turn may underlie poor stress coping and a propensity toward depressive-like behavior as previously demonstrated in the forced swim test.

OCT3 Protein Expression

We obtained cytosolic, plasma membrane, and organelle membrane fractions from hippocampal lysates and initially used the plasma membrane fractions to quantify OCT3 protein expression. We observe a significant decrease in OCT3 plasma membrane expression in the hippocampus after repeated stress in WKY rats, and although a similar trend was observed in LE rats, this effect did not reach significance. These results stand in contrast to our real-time PCR results, which revealed a robust increase in OCT3 expression in both strains of rats after stress. To reconcile this, we also quantified OCT3 expression in cytosolic fractions which may contain internalized vesicles with OCT3 embedded in the vesicular membrane. Hippocampal OCT3 protein expression was increased in cytosolic fractions after repeated stress in both WKY and LE rats, which suggests that the OCT3 is being sequestered in vesicles. One possibility is that vesicular OCT3 is internalized shortly after the binding of CORT and relocated to cytosolic vesicles.

GR Gene Expression

In our study we did not observe any significant effects of stress on GR expression, contrary to previous reports that single exposures to stress upregulate the GR (Marini et al., 2006). There was, however, a trend toward an increase in GR gene expression after acute stress in LE rats and repeated stress in WKY rats. Furthermore, we found a significant correlation between OCT3 and GR expression in both strains, which may indicate that these two genes are regulated by a similar mechanism

5HTT Gene Expression

Contrary to our hypothesis, basal 5HTT gene expression in the hippocampus did not significantly differ between LE and WKY rats. The basal elevation of OCT3 mRNA in the hippocampus of WKY rats lead us to hypothesize that the OCT3 may have been recruited to compensate for low 5HTT expression, however this turns out not to be the case.

Several lines of evidence led us to believe that the 5HTT gene may be downregulated in WKY rats by stress in our model. One study in WKY rats demonstrated a robust decrease in 5HTT binding after a 21-day chronic variable stress (CVS) regimen that included foot shock (Páre and Tejani-Butt, 1996), which suggested we may observe a similar downregulation in the WKY rat after repeated social defeat. Furthermore, we observed a robust upregulation of the OCT3 in the hippocampus of WKY rats after chronic social defeat. This same effect was reported in 5HTT-knockout mice while 5HT levels remained relatively constant compared to wild-type mice, suggesting that the OCT3 is recruited when 5HTT is scarce to keep 5HT levels in check. We considered the possibility that upregulation of the OCT3 may be secondary to stress-induced downregulation of the 5HTT

5HTT expression was significantly lower in the hippocampus of WKY relative to LE rats in the acute and repeated stress condition, but overall there was no significant change in 5HTT expression within either strain after stress. These data do not support the hypothesis that upregulation of the OCT3 after stress is driven by the downregulation of 5HTT.

BDNF Protein Expression in the Hippocampus

Previous studies have shown that chronic social defeat stress reduces neurogenesis in the dentate gyrus of the hippocampus (Magarinos et al., 1996; Gould et al., 1997), which may be associated with reduced BDNF production after stress as reported by Smith and colleagues (1995). However, Bergström and colleagues report an increase in BDNF expression following a 5-week chronic mild stress regimen (Bergström et al., 2008). At this time, it is unclear whether this discrepancy is caused by differences in the length or severity of the stress regimen, but in our study we observed an increase in BDNF in both strains of rats consistent with the latter report. This result is also consistent with increased 5HT signaling in the hippocampus subsequent to stress, since BDNF expression is positively regulated by 5HT and vice versa.

In summary, we have demonstrated that stress differentially regulates expression of the OCT3 in rats displaying a depressive phenotype and poor stress coping, indicating that the OCT3 may play a significant role in stress adaptation. Further investigations into the mechanism of this regulation and the time course of OCT3 trafficking to and from the plasma membrane are warranted.

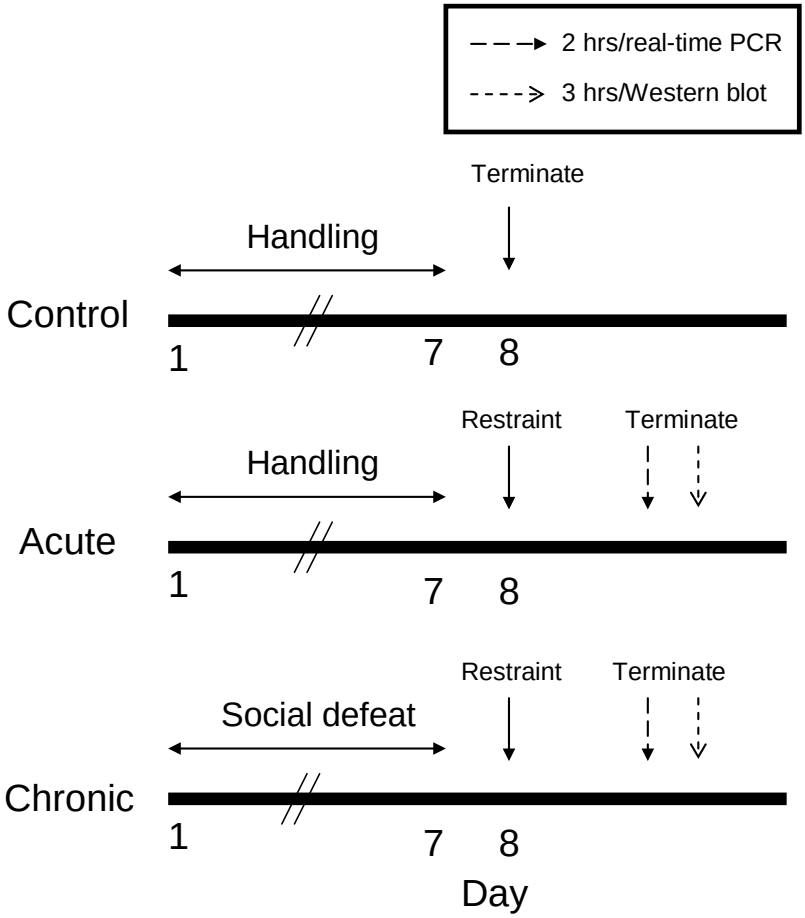

Figure 2-1. Schematic of experimental design.

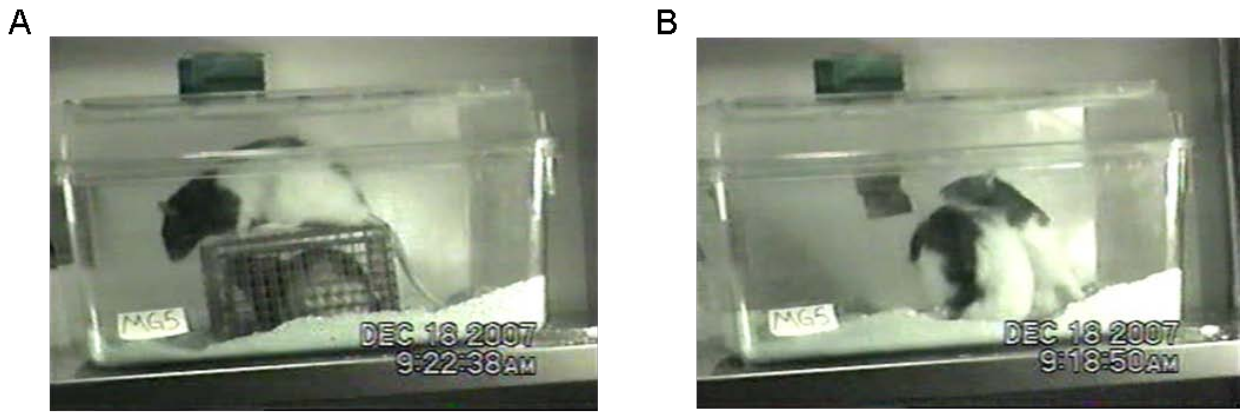

Figure 2-2. The social defeat procedure in action. Social defeat consists of a 45 minute indirect interaction phase (A) followed by a 5 minute direct interaction phase (B) in which the intruder is usually defeated by the resident.

Table 2-1. Real-time PCR Primers

Primer	Assay ID
OCT3	Rn00570264_m1
SERT	Rn00564737_m1
GR	Rn00561369_m1
18S	Hs99999901_s1

Table 2-2. Antibodies for Western Blot

Label	Company	Host	Dilution	Exposure time
OCT3	Abcam	Rabbit	1:1000	5 min
BDNF	Abcam	Rabbit	1:1000	30 sec
N-Cadherin	Abcam	Rabbit	1:2000	10 sec
β-actin	Cell Signaling	Rabbit	1:1000	10 sec

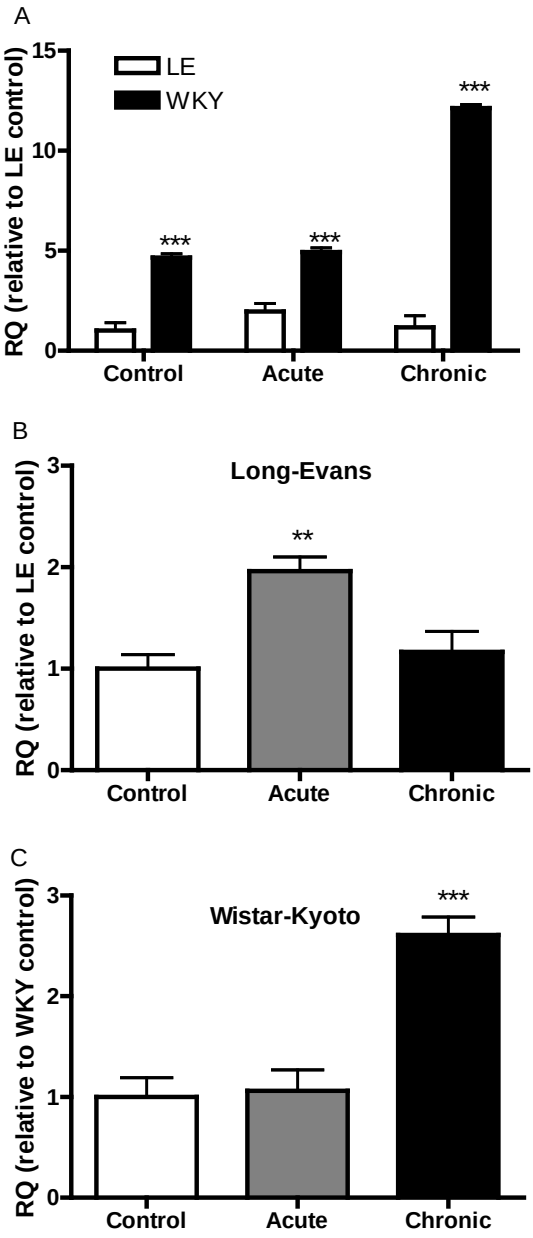

Figure 2-3. Effects of stress on OCT3 gene expression in the hippocampus. Data are expressed as relative change or relative quantification (RQ) of gene expression levels and values are mean ± SEM. (A) * ($p<0.05$) and *** ($p<0.001$) denote statistical significance relative to the LE group in each stress category. (B) *** ($p<0.001$) denotes statistical significance compared to the LE group. (C-D) * ($p<0.05$) and ** ($p<0.01$) denote statistical significance relative to all other groups.

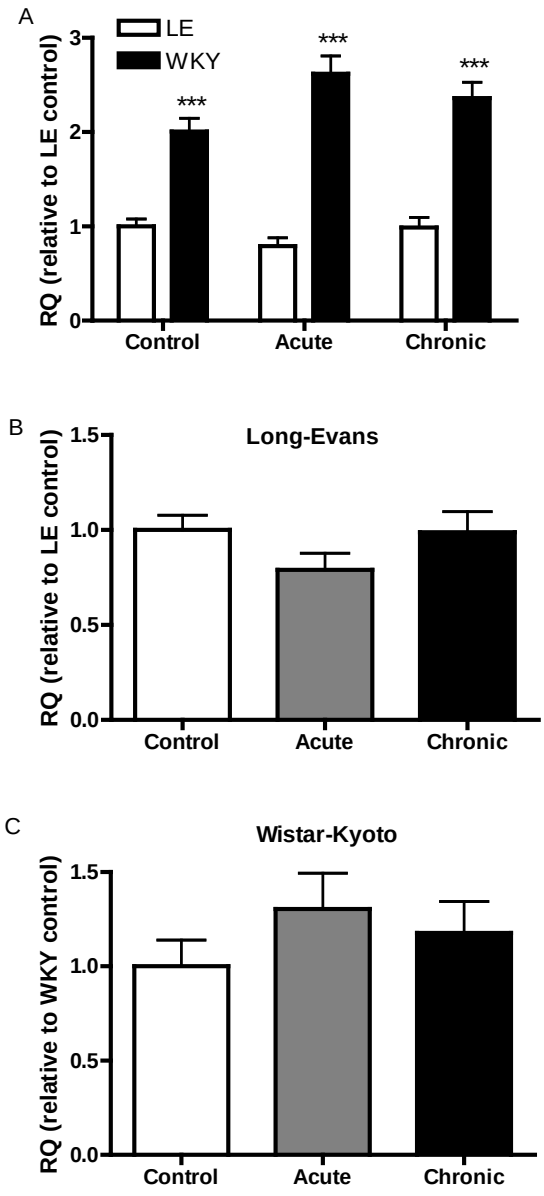

Figure 2-4. Effects of stress on OCT3 gene expression in the mPFC. Data are expressed as relative change or relative quantification (RQ) of gene expression levels and values are mean ± SEM. (A) * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$) denote statistical significance relative to the LE group in each stress category. (B) *** ($p<0.001$) denotes statistical significance compared to the LE group.

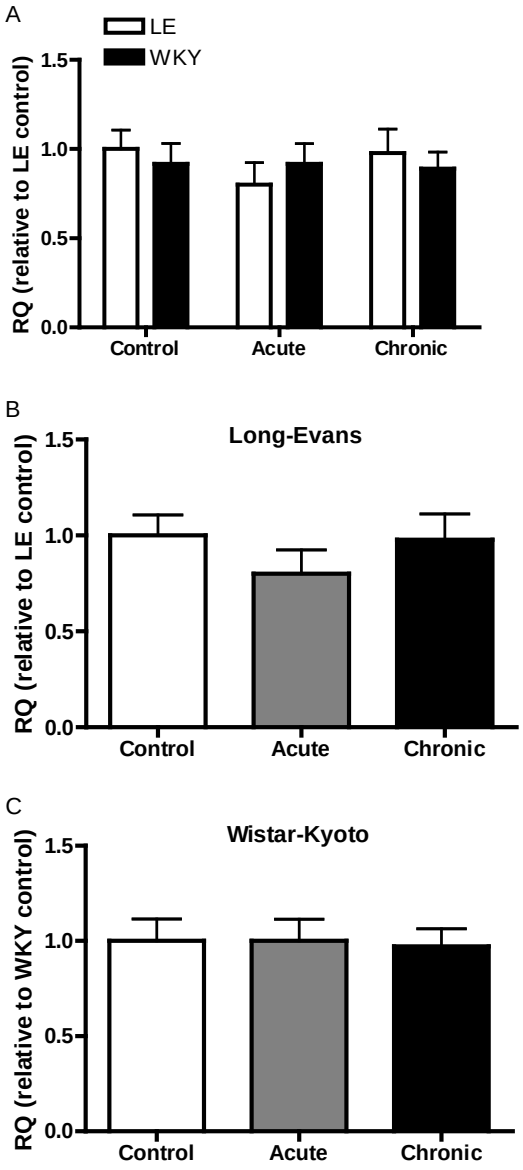

Figure 2-5. Effects of stress on OCT3 gene expression in the striatum. Data are expressed as relative change or relative quantification (RQ) of gene expression levels and values are mean ± SEM.

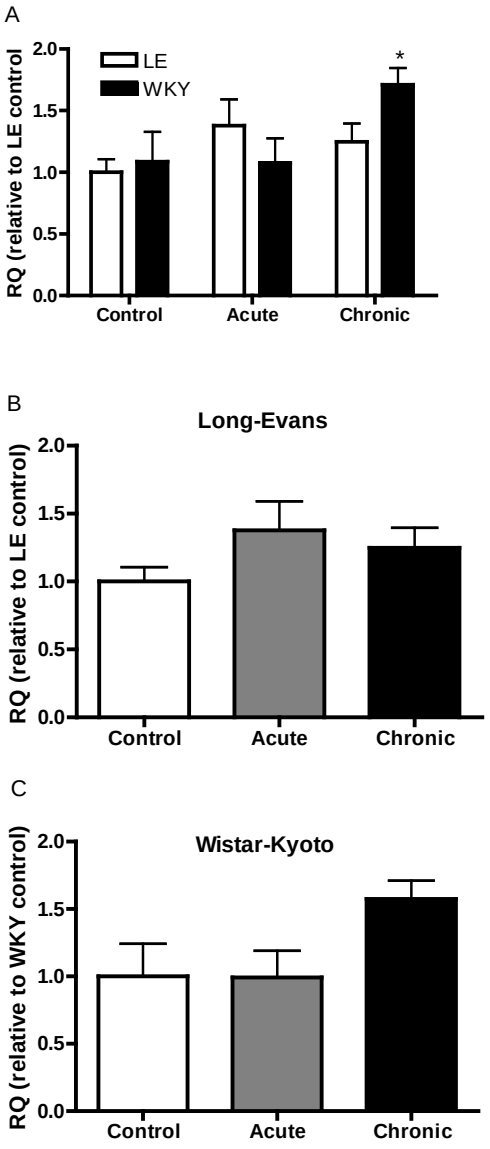

Figure 2-6. Effects of stress on GR gene expression in the hippocampus. Data are expressed as relative change or relative quantification (RQ) of gene expression levels and values are mean ± SEM. * ($p<0.05$) denotes statistical significance relative to the LE group in the repeated stress group.

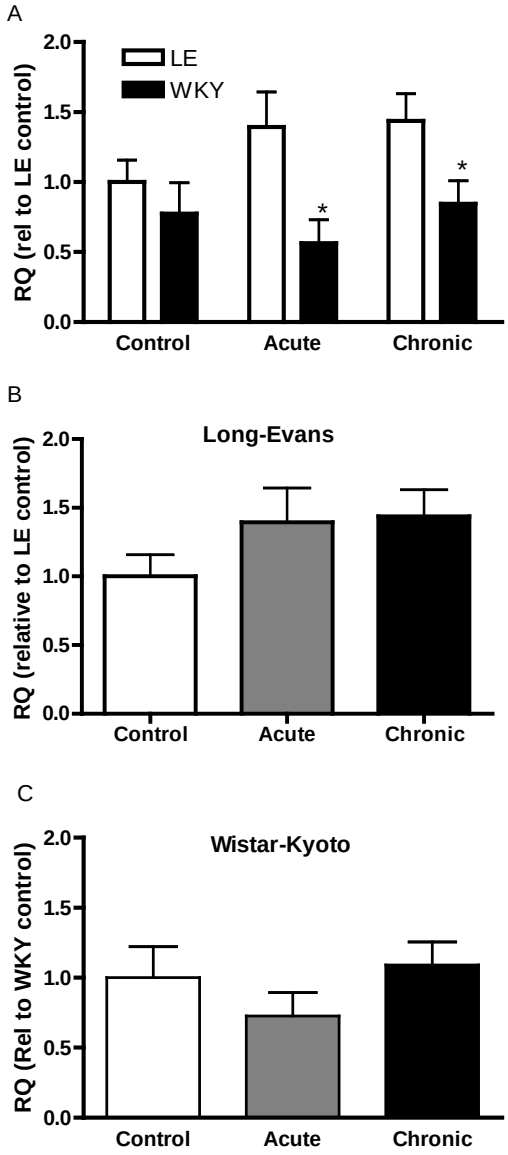

Figure 2-7. Effects of stress on 5HTT gene expression in the hippocampus. Data are expressed as relative change or relative quantification (RQ) of gene expression levels and values are mean ± SEM. (A) * ($p<0.05$) denotes statistical significance relative to the LE group in each stress category. (B) ** ($p<0.01$) denotes statistical significance compared to the LE group.

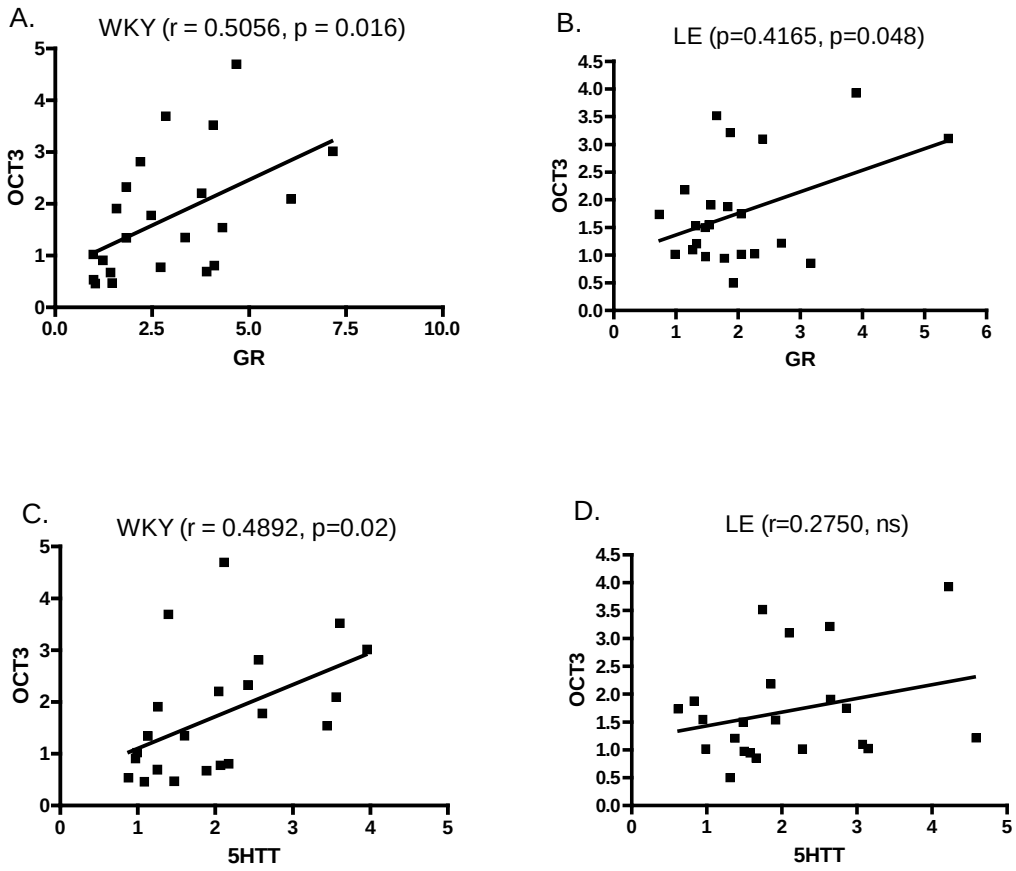

Figure 2-8. Bivariate correlation analysis for OCT3 x GR and OCT3 x 5HTT. Data points represent the RQ values for each individual sample.

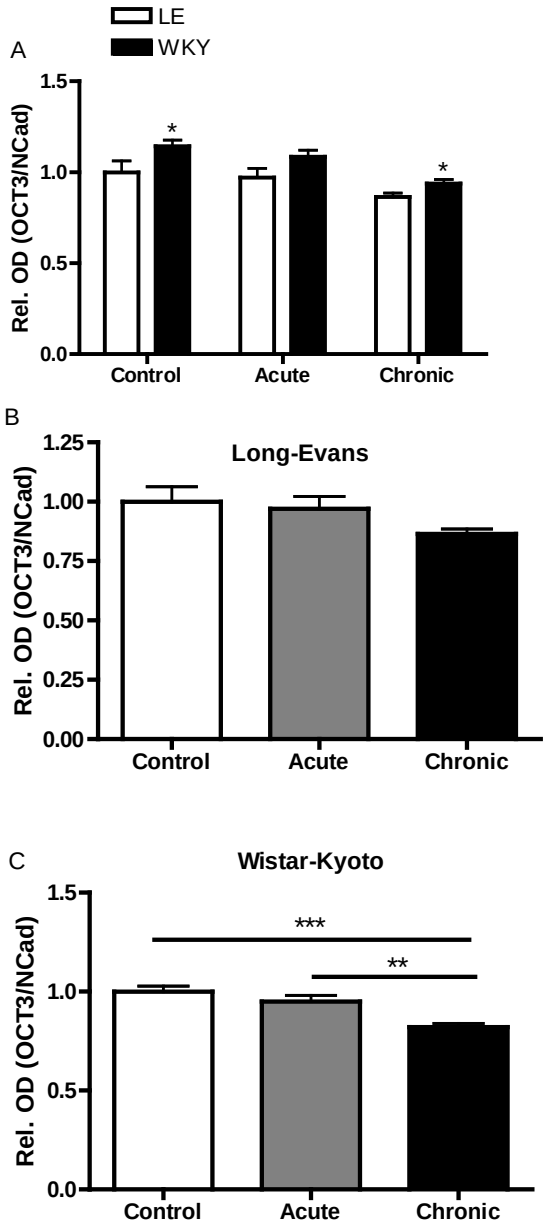

Figure 2-9. Effects of stress on plasma membrane expression of the OCT3 in the hippocampus. Data are expressed as relative optical density (OD) normalized to N-cadherin and values are mean ± SEM. (B) ** ($p<0.01$) denotes statistical significance relative to the LE group. (D) ** ($p<0.01$) and *** ($p<0.001$) denotes statistical significance compared to the indicated group.

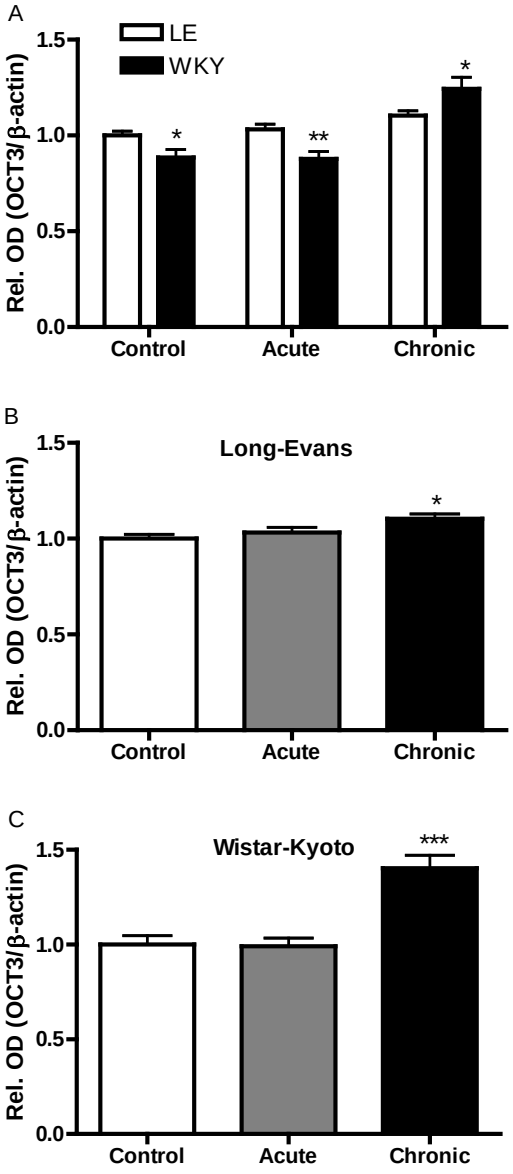

Figure 2-10. Effects of stress on cytosolic expression of the OCT3 in the hippocampus. Data are expressed as relative optical density (OD) normalized to β-actin and values are mean ± SEM. (A) * ($p<0.05$) denotes statistical significance relative to the LE group in each stress category. (C) * ($p<0.05$) denotes statistical significance compared to the control group and (D) *** ($p<0.001$) denote statistical significance relative to both control and acute stress groups.

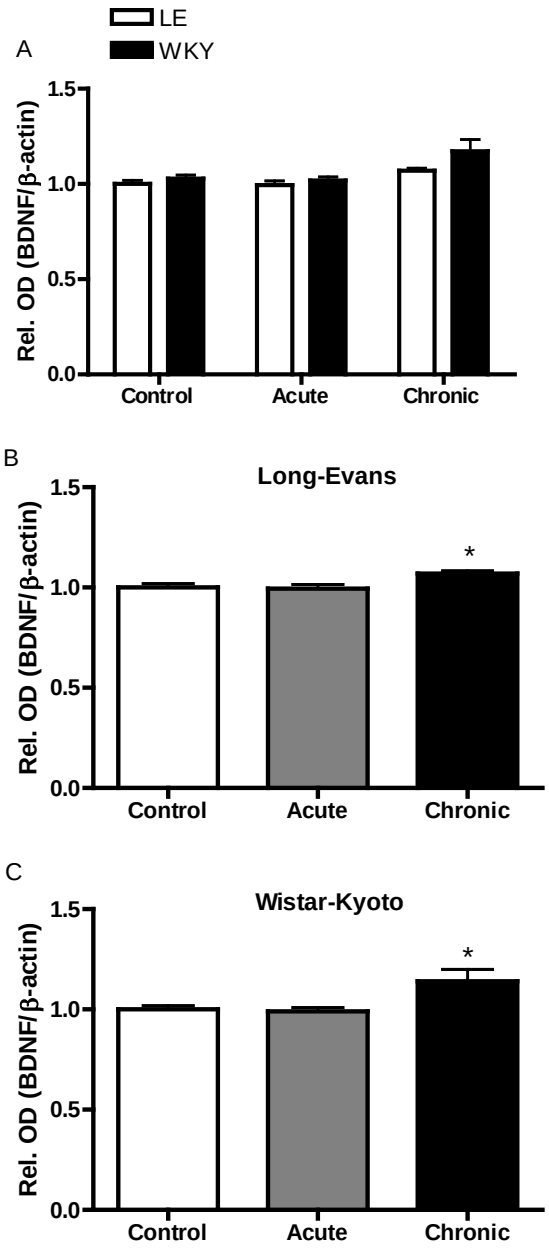

Figure 2-11. Effects of stress on BDNF protein expression in the hippocampus. Data are expressed as relative optical density (OD) normalized to β-actin and values are mean ± SEM. (C-D) * ($p<0.05$) denotes statistical significance compared to all other groups.

CHAPTER 3
ANTIDEPRESSANT EFFECTS OF AN OCT3 ANTAGONIST IN THE WISTAR-KYOTO RAT

Introduction

The goal of these studies was to assess whether the OCT3 may be a useful target for antidepressant intervention in an animal model that is resistant to the effects of conventional SSRI antidepressants. Individuals possessing one or more copies of the s allele of the 5HTT typically exhibit a poor response to SSRIs, especially when this polymorphism is combined with the 5HT1A -1018 C/G polymorphism, otherwise known as the g allele of the $5HT_{1A}$ receptor (Arias et al., 2005).

The WKY rat has been extensively characterized as an animal model of depressive behavior and heightened stress responsiveness that may shed new light on the neurobiological underpinnings of Major Depression and its pharmacological treatment in humans (Paré and Redei, 1993;Redei et al., 1994;De La Garza and Mahoney, 2004; De La Garza, 2005;Schmidt and Duman, 2007). Compared to most other strains, the WKY rat exhibits low levels of activity in the forced swim test, which reveals a poor adaptive response to stress and a tendency toward depressive-like behavior (Paré and Tejani-Butt, 1996;Griebel et al., 1999;Lopez-Rubalcava and Lucki, 2000;De La Garza and Mahoney, 2004;Tejani-Butt et al., 2003). The forced swim test is commonly used to screen new compounds for their ability to reduce immobility and increase active swimming and climbing behaviors, which is positively correlated with their clinical efficacy as antidepressants (Porsolt et al., 1978).

The WKY rat also displays an attenuated response to SSRIs in the forced swim test that is reminiscent of the antidepressant resistance observed in a significant

number of depressed individuals (Lahmame and Armario, 1996;Griebel et al., 1999;Lopez-Rubalcava and Lucki, 2000;Tejani-Butt et al., 2003). Furthermore, 5HTT binding in the hippocampus is reduced in WKY rats after stress compared to other strains, but without any concomitant changes in 5HT clearance rates in the hippocampus (Pollier et al., 2000). These data suggest that some other transporter may be playing a compensatory role in 5HT clearance in the WKY rat, much as it does in 5HTT knockout mice (Schmitt et al., 2003;Baganz et al., 2008). The OCT3 is upregulated in the hippocampus of WKY rats (see Chapter 2), which may underlie the reduced efficacy of antidepressant drugs in reversing helplessness behavior in the forced swim test. The relatively low expression of 5HTT combined with high expression of the $5HT_{1A}$ receptor resembles the genetic profile of depressed individuals with the ss/gg genotype who exhibited the poorest outcome during a 12-week trial with the SSRI citalopram (Arias et al., 2005). This led us to hypothesize that acute administration of decynium 22, a specific inhibitor of the OCT3, will mitigate depressive-like behavior on the forced swim test in WKY rats.

Methods

Experimental Design

WKY rats were exposed to a 15 minute swim or “pretest” 24 hours before the test day. On the test day, WKY rats were randomly assigned to receive 0, 1 or 10 ug/kg decynium 22 1 hour before testing on the forced swim test for 5 minutes during which swimming, climbing and floating behaviors were recorded with an overhead camera. Rats were terminated by rapid decapitation 30 minutes after the beginning of the swim session for measurement of plasma corticosterone concentrations.

Experimental Animals

Thirty-four adult male WKY rats (Charles River Laboratories International, Inc., Wilmington, MA) weighing between 250-300 g at the start of experiments were pair-housed in standard polycarbonate cages (43 x 21.5 x 25.5 cm) in a climate-controlled vivarium with a 12-hour light/dark schedule (lights on at 7 am daily). The rats were allowed *ad libitum* access to standard laboratory chow (Lab Diet 5001) and tap water. The rats were allowed 7 days to adapt to the housing facility before the experiments began. At the end of the experiment, the rats were terminated by rapid decapitation for blood and tissue collection.

Drugs and Injections

Decynium 22 (Sigma Aldrich) was dissolved in ethanol to achieve a concentration of 20 ug/ml, and 200 ug/ml, respectively. Each of these solutions was diluted 1:20 in isotonic saline to a final concentration of 5% ethanol and 1 ug/ml and 10 ug/ml of decynium 22. An intraperitoneal injection of 5% ethanol in isotonic saline with the appropriate concentration of decynium 22 (0, 1, or 10 ug/kg) was administered 1 hour prior to behavioral testing on the forced swim test.

Forced Swim Test

This method is based on a modified version of that described by Porsolt (Porsolt et al., 1978). Briefly, rats were placed individually into circular containers (45 cm height; 20 cm diameter) containing 30 cm of water at 23°C for 15 minutes during a “pretest” session. Twenty-four hours later, a test session was conducted for 5 min and swimming, climbing, and floating were recorded with a video camera mounted over the test apparatus. At the end of the test session, each rat was dried with a clean towel, placed in a heated cage for 15 minutes, and then returned to its home cage for an additional 15

minutes before decapitation. Fresh water was used for each rat and the cylinder was cleaned thoroughly with 4% bleach between sessions. A trained observer, blind to the treatment, scored the videotapes for time spent floating (immobile) (Figure 3-2). A rat was considered immobile when floating and making only the necessary movements to keep its nostrils above the water surface.

Plasma CORT analysis

Trunk blood (6 ml) was collected in chilled polystyrene tubes filled with 600 µl ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1000 x g for 5 minutes at 4ºC to separate the plasma layer from the cellular layer. The supernatant (plasma) was aliquoted and stored at -80ºC until further use. Plasma CORT concentrations were measured using a commercially available enzyme-linked immunosorbant assay kit (Immunodiagnostic Systems Ltd., UK) according to the manufacturer's protocol.

Statistical Analyses

A one-way ANOVA was used to analyze the effects of decynium 22 (0, 1, 10 ug/kg) on immobility behavior during the forced swim test and plasma CORT concentrations 30 minutes after the beginning of the test. Significant effects ($p<0.05$) were analyzed by Newman-Keuls post-tests to make pairwise comparisons between each dose.

Results

Behavioral data

Treatment with decynium 22 (1 and 10 ug/kg) produced a significant decrease in the time spent immobile on the forced swim test. ($F(2,31)=9.565$, $p=0.0006$) (Figure 3-3). Post-hoc analysis verified that both 1 ug/kg ($t=3.732$, $p<0.01$) and 10 ug/kg ($t=3.466$,

$p<0.01$) decynium 22 significantly attenuated immobility on the forced swim test relative to vehicle.

CORT data

Trunk blood was collected from decapitated animals 30 minute after stress onset on the same day as behavioral testing in rats treated with vehicle and 10 ug/kg decynium 22 only. The remaining rats treated with 1 ug/kg were used as controls (no stress) 1 week later. Swim stress significantly increased plasma corticosterone concentrations in WKY rats ($F(2,15)=120.9, p<0.0001$) (Figure 3-4). Post-hoc analysis revealed a significant increase in plasma corticosterone levels between stressed rats treated with 10 ug/kg D22 compared to those receiving vehicle.

Discussion

This is the first study to report that decynium 22, a specific inhibitor of the OCT3, has an antidepressant effect in the WKY rat, a putative animal model of both behavioral depression and antidepressant resistance. Our findings are consistent with studies in mice which also demonstrate a selective antidepressant effect of decynium 22 (1 ug/kg) on the tail suspension test in animals lacking at least one copy of the 5HTT (Baganz et al., 2008), which is sometimes used as a proxy for the low efficacy *s* allele that has been associated with a higher risk of depression and low responsiveness to antidepressants targeting the 5HTT. The WKY rat also exhibits a poor antidepressant response to SSRI drugs on the forced swim test, but respond well to drugs that block reuptake of norepinephrine and dopamine (Tejani-Butt et al., 2003). Decynium 22, which shows pharmacological activity against the OCT3, a polyspecific monoamine transporter, effectively increases synaptic concentrations of 5HT, dopamine and norepinephrine which may be required for antidepressant action in the WKY rat.

Furthermore, we have shown that the OCT3 is constitutively upregulated in the WKY rat compared to other strains, making the OCT3 an ideal target for antidepressant intervention as is the case with 5HTT knockout mice (Baganz et al., 2008). The failure of SSRIs to elicit an antidepressant response, combined with the robust antidepressant effect of decynium 22 in these same animals, suggests that drugs targeting the OCT3 may have utility in the treatment of depressed patients who are refractory to SSRIs.

The WKY rat is described as having a depressive phenotype based upon its low level of activity in the forced swim test. Studies have shown that corticosterone, which peaks shortly after presentation of a stressor, has a profound “antidepressant” effect in the forced swim test in mice (Stone and Lin, 2008). We postulated that in WKY rats, a blunted corticosterone response to swim stress may account for their relative inactivity in the forced swim test. As mentioned earlier, corticosterone elicits a rapid 5HT response, possibly through its inhibitory actions at the OCT3. However, we observed a robust increase in corticosterone levels in the WKY rat after swim stress, in agreement with previous studies which compared the HPA response of the WKY rat with the Sprague-Dawley rat. In this study, WKY rats actually had an enhanced corticosterone and ACTH response to swim stress relative to the response of the Spague-Dawley rats (Rittenhouse et al., 2002). These data suggest that behavioral dysregulation of the stress response in WKY rats occurs at some other level of the glucocorticoid signaling pathway, perhaps involving the interaction between corticosterone and the OCT3. In this case, decynium 22 essentially substitutes for corticosterone and reduces immobility behavior down to normal levels (~40%).

In conclusion, this study provides evidence that inhibition of the OCT3 can act as an antidepressant in our animal model and suggests that further investigation of the use of OCT3 antagonists as therapeutic agents is warranted, either alone or in combination with other antidepressants (Schildkraut and Mooney, 2004;Mooney et al., 2008).

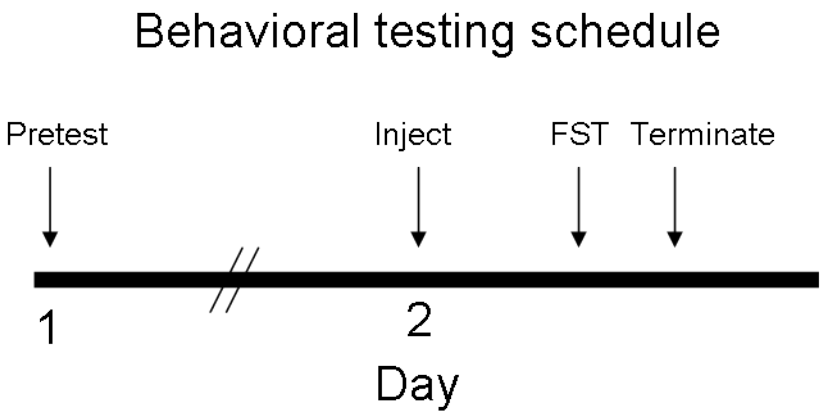

Figure 3-1. Schematic of experimental design. Behavioral testing schedule which consisted of a pretest on day 1 and the forced swim test on day 2. In Experiment 2, rats treated with vehicle or 10 ug/kg D22 were terminated 30 min after the beginning of the forced swim test for assessment of plasma corticosterone levels.

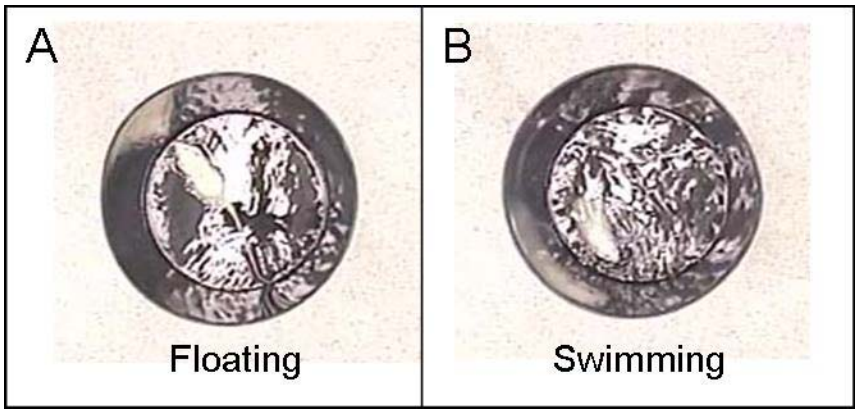

Figure 3-2. Pictorial representation of the forced swim test depicting (A) floating and (B) swimming behaviors.

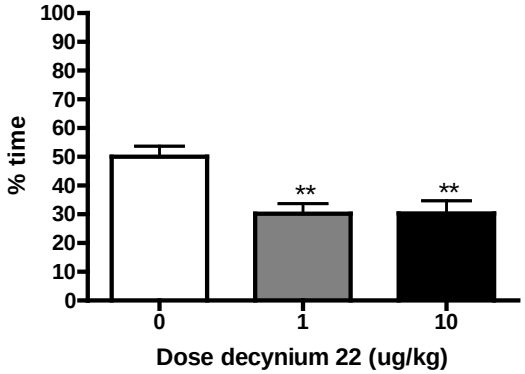

Figure 3-3. Antidepressant effects of decynium 22 in the forced swim test. As indicated, rats injected with 1 ug/kg and 10 ug/kg decynium 22 displayed reduced immobility on the forced swim test compared to vehicle. Data are expressed as the % time spent immobile over a 5 min test session. Values are expressed as mean ± SEM. *** ($p<0.001$) represents statistical significance over vehicle.

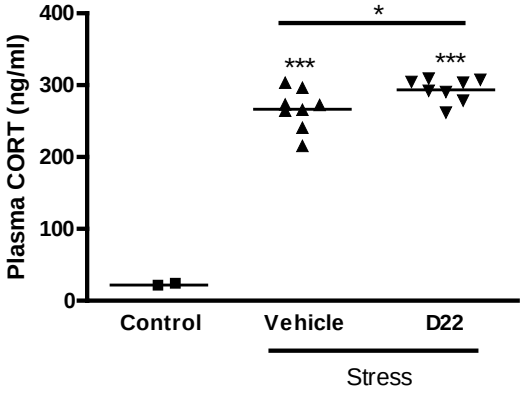

Figure 3-4. Effect of forced swim stress on plasma corticosterone levels. Swim stress causes a significant increase in plasma corticosterone levels that is unmodified by drug treatment with decynium 22 (5 ug/kg). Swim stress induces a significant increase in plasma corticosterone levels that is slightly augmented by pretreatment with decynium 22. Data are expressed as plasma corticosterone concentrations in ng/ml and all values are expressed as mean ± SEM. * ($p<0.05$) and *** ($p<0.001$) represents statistical significance over stress-free controls or vehicle-treated rats as indicated.

CHAPTER 4
CONCLUSIONS AND FUTURE DIRECTIONS

A Working Model of the OCT3 as a Molecular Switch that Regulates Stress Responsiveness and Vulnerability to Psychopathology

The OCT3 is a corticosterone-sensitive monoamine transporter which has been previously shown to play a role in depressive- and anxiety-like behavior in animal models (Wieland et al., 2000;Kitaichi et al., 2005;Baganz et al., 2008;Vialou et al., 2008;Wultsch et al., 2009). Unlike the high-affinity transporters such as 5HTT, NET, and DAT, the OCT3 is located on postsynaptic membranes where it participates in the bidirectional transport of 5HT, NE, DA and histamine (Gründemann et al., 1998;Gründemann et al., 1999;Schomig et al., 2006). This low-affinity, high-capacity transporter is recruited to remove extracellular monoamines when the high-affinity transporters are saturated or in low abundance (Schmitt et al., 2005;Baganz et al., 2008), which typically occurs under stress conditions. Stress triggers 5HT release in the hippocampus which saturates the high-affinity 5HTT, such that the OCT3 becomes a key player in determining the amplitude and duration of 5HT signaling. Corticosterone, which inhibits the transport function of the OCT3, results in local accumulation of extracellular 5HT and the potentiation of 5HT signaling (Lowry et al., 2001;Gasser et al., 2006;Feng et al., 2009). Thus, the temporal and spatial pattern of OCT3 expression and its functional interactions with corticosterone may play significant roles in emotional and behavioral regulation under stress conditions. The importance of hippocampal 5HT, and hence the OCT3, in stress regulation is illustrated by the fact that lesioning of 5HT projections to the hippocampus results in increased anxiety-like behavior and poor stress coping (Robertson et al., 2005).

Feedback inhibition of the HPA response is achieved in part by activation of GRs in the hippocampus by corticosterone (Herman et al., 1989;Whitnall, 1993;Herman and Cullinan, 1997), and previous studies have demonstrated that GRs are upregulated in the hippocampus after a single exposure to social defeat stress (Marini et al., 2006). These parallel actions of corticosterone on OCT-mediated 5HT signaling and GR activation in the hippocampus, and the subsequent upregulation of OCT3 and GR in this same region, may act in synergy to promote more rapid recovery from and long-term habituation to stressful stimuli.

We demonstrated differential expression of the OCT3 gene in WKY compared to LE rats under basal, acute and repeated stress conditions. Given the role of the OCT3 in regulating extracellular 5HT concentrations in the presence of corticosterone, these differences in genetic expression of the OCT3 may help to explain the abnormal neurochemical and behavioral responses of the WKY rat to stress. There is a marked elevation of OCT3 gene expression in the hippocampus and mPFC of WKY rats compared to LE rats, two limbic regions that have been implicated in feedback regulation of the HPA axis. Plasma membrane expression of the OCT3 is also higher in the hippocampus of the WKY relative to LE rats under basal and repeated stress conditions, with a trend toward higher OCT3 in WKY rats in the acute stress condition. Higher levels of OCT3 membrane expression may enable the WKY rat to maintain high levels of 5HT clearance under stress conditions, which in turn may hinder appropriate coping responses (Figure 4-1). This stress-independent upregulation of the OCT3 in WKY rats might be attributed to the relatively low levels of 5HTT gene expression in the

WKY rats, as other studies have established a negative correlation between the expression patterns of these two genes (Schmitt et al., 2005;Baganz et al., 2008).

Acute stress induces a robust upregulation of OCT3 gene expression in the hippocampus of LE rats, although this elevated mRNA expression in the LE rats was still well below that of the WKY rats (Figure 2-3). We did not observe a corresponding increase in OCT3 protein expression at the plasma membrane in the LE rats after acute stress, so it in unknown whether this upregulation in OCT3 gene expression translated to an increase in 5HT clearance. It is possible that we collected tissues for OCT3 protein expression assays too quickly (~ 3hrs) to observe the insertion of newly synthesized OCT3 protein into the membrane. In order to resolve this issue, we would have to collect tissues at a later time point (e.g. 24 hrs after the acute stress), in order to determine if the increase in gene expression eventually translate to an increase in plasma membrane expression.

The increase in OCT3 gene expression in LE rats in response to acute stress exposure is blunted in rats that have a history of stress, indicating that whatever was driving the increase in OCT3 transcription after acute stress may have also returned to equilibrium. Conversely in WKY rats, the OCT3 is upregulated in rats with a history of repeated stress. This may be a result of impairments in stress recovery which would result in excessive bombardment of hippocampal neurons with corticosterone during repeated stress.

Bimodal Regulation of OCT3 Gene Expression: A Hypothesis

We propose that OCT3 gene expression is differentially regulated under low and high stress conditions. In the low stress state, OCT3 gene expression may be primarily 5HT-driven; that is, low levels of 5HTT expression in the WKY rat triggers a

compensatory increase in OCT3 expression. This is supported by the observation that OCT3 gene expression is upregulated in the hippocampus of 5HTT knockout mice, although it is possible that expression of these two genes is driven in parallel by an addition, indirect mechanism. In the high stress state, the OCT3 gene may be regulated by the dual actions of corticosterone and 5HT. The widespread effects of corticosterone on gene transcription are well-documented. Glucocorticoids bind to cytosolic receptors and are subsequently translocated to the nucleus where they can interact with glucocorticoid response elements (GREs) on target genes to activate gene transcription, and one of these genes may be the OCT3 gene. The interaction between corticosterone and the OCT3 also inhibits 5HT uptake and increases extracellular concentrations of 5HT, which may trigger a homeostatic upregulation of the OCT3. This is one potential mechanism by which corticosterone may *indirectly* regulate OCT3 gene expression.

Stress-dependent upregulation of the OCT3 in the hippocampus, but not in the mPFC or striatum, of LE rats may be partly explained by the fact that the hippocampus contains the highest density of GR receptors in the brain, since it is these receptors that tranduce the genomic effects of stress (Jacobson and Sapolsky, 1991). This finding also concurs with those of Schmitt and colleagues, in which OCT3 gene expression was upregulated in the hippocampus of 5HTT knockout mice but not in other brain regions including the cortex, striatum, cerebellum or brainstem (Schmitt et al., 2005).

We originally hypothesized that WKY rats may have a blunted corticosterone response to stress which would account for their failure to induce OCT3 gene expression after acute stress. However, we report a relatively normal corticosterone

response to acute swim stress in WKY rats (see Figure 3-4), which concurs with previous studies (Rittenhouse et al., 2002). Therefore, we postulate that the abnormal neurochemical and behavioral responses to stress in the WKY rat may occur at the level of the glucocorticoid receptor (GR), the glucocorticoid response element (GRE), or one of the many downstream targets of the glucocorticoid signaling pathway. The failure of acute stress to induce GR gene expression in WKY rats supports this hypothesis, since GR is a key modulator of stress-induced gene expression and may play a role in the induction of the OCT3. Also, the fact that OCT3 expression is many times higher in WKY compared to LE rats raises the possibility that the OCT3 competitively inhibits binding of corticosterone to GRs, which may also interfere with corticosterone-driven gene expression. The absence of any effect of acute stress on OCT3 expression in WKY rats may also be the result of an insufficient change in extracellular 5HT levels between basal and stress conditions (Figure 4-1).

Presumably, LE rats adapt fairly normally to repeated stress and both glucocorticoid and 5HT signaling are reduced accordingly. In contrast, glucocorticoid signaling in the WKY is slowly ramped up as a function of repeated stress without the mitigating effect of enhanced 5HT signaling, which may eventually drive increased expression of the OCT3.

Stress-induced Membrane Trafficking of the OCT3

We obtained discrepant results with regard to OCT3 gene and protein expression when protein was quantified at the plasma membrane. This led us to question whether the OCT3 was internalized when bound by CORT after stress. We quantified OCT3 protein expression in cytosolic extracts and found that the OCT3 was upregulated after chronic stress in both LE and WKY rats, whereas acute stress had no apparent effect

(Figure 2-10). This corresponded well with the observed downregulation of the OCT3 in plasma membrane extracts, leading us to conclude that the OCT3 is translocated to cytosolic vesicles after stress. The absence of any effect of acute stress on plasma membrane or cytosolic OCT3 expression is difficult to explain, but it may have to do with the timing of sample collection in our experiment. We collected brain tissues 3 hours after stress for protein analysis, which means that the internalized OCT3 may have already been metabolized at this point. Newly synthesized OCT3 is in the process of being trafficked to the plasma membrane so that there is no apparent reduction in OCT3 in plasma membrane fractions. After repeated stress, the reduction at the plasma membrane and increase in cytosolic OCT3 becomes more apparent, but is much more pronounced in WKY rats. By this point, OCT3 gene expression has returned to basal levels in LE rats, but in WKY rats there is a robust upregulation. Therefore, the observed increase in cytosolic OCT3 in WKY rats may reflect both CORT-bound, internalized OCT3 and newly synthesized OCT3.

OCT3 Antagonists as Putative Antidepressant Agents

Our studies also demonstrate that the OCT3 antagonist decynium 22 is a potent antidepressant agent in WKY rats, which suggests that drugs targeting the OCT3 may hold promise for antidepressant pharmacotherapy in humans, especially in individuals with two copies of the s allele of the 5HTT gene. WKY rats are an excellent model for this genotype since they not only have a depressive phenotype, but they are typically resistant to the effects of SSRI antidepressants and exhibit low levels of 5HTT expression (Griebel et al., 1999;Lopez-Rubalcava and Lucki, 2000;Tejani-Butt et al., 2003). While currently there are no published studies on OCT3 gene expression in humans with the ss genotype, we hypothesize that the OCT3 will be upregulated in

such individuals. Constitutive upregulation of the OCT3, such as that found in the WKY rat, may dampen 5HT activity and increase susceptibility toward depressive-like behavior. We further postulate that heightened expression of the OCT3 may underlie resistance to the antidepressant effects of SSRIs. SSRIs exert their effects by inhibiting the 5HT uptake via the 5HTT, causing the OCT3, if present in sufficient quantities, to usurp this function. For this reason, drugs that antagonize the OCT3 should theoretically have potent antidepressant effects in individuals with high levels of OCT3 gene expression, as they do in the WKY rat.

As stated earlier, OCT3 is a low-affinity, high-capacity transporter of all monoamines including 5HT and is highly expressed in the hippocampus. As such, its contribution to 5HT uptake becomes significant when the high-affinity 5HTT is saturated or in low abundance. The latter condition is met in the presence of antidepressants such as fluoxetine, which selectively inhibit 5HTT and provoke its subsequent downregulation after chronic treatment (Hrdina et al., 1993). We postulate that chronic treatment with SSRIs will induce expression of the OCT3, which may account for the reduced efficacy of SSRIs over time in certain individuals. As such, OCT3 antagonists could potentiate the antidepressant effects of chronic SSRI treatment by increasing synaptic concentrations of 5HT and other monoamines such as NE and DA. Previous studies have shown that OCT3 inhibition by normetanephrine, the O-methylated metabolite of norepinephrine, augments extracellular norepinephrine in patients treated with venlafaxine and may contribute to its antidepressant effects (Rahman et al., 2008).

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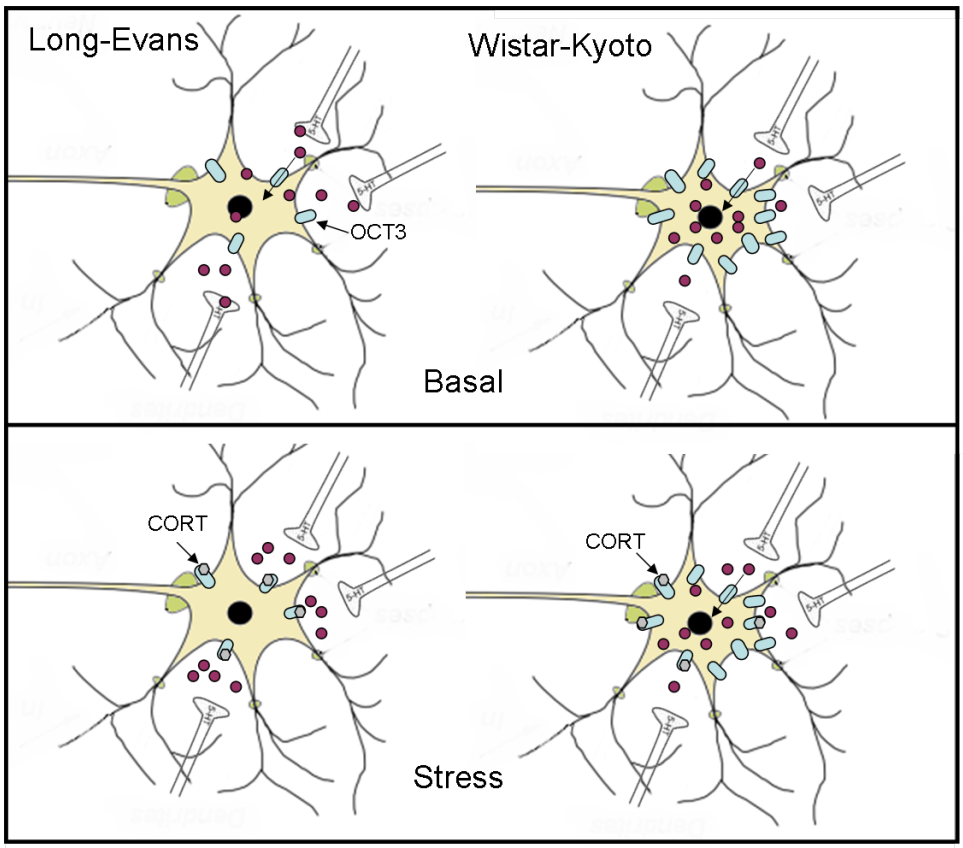

Figure 4-1. Proposed model for the effect of differential expression of the OCT3 on 5HT signaling in the hippocampus. Blue ovals represent the OCT3 protein and purple dots represent 5HT. Corticosterone (CORT) in this model is shown as gray hexagons. WKY rats have elevated basal OCT3 which results in reduced extracellular 5HT levels in the synapse. Corticosterone is released under stress conditions which inhibits 5HT uptake via the OCT3, causing local increases in extracellular 5HT in both LE and WKY rats. In WKY rats, however, OCT3-mediated 5HT clearance is only partially inhibited by corticosterone, resulting in lower levels of 5HT in the WKY relative to LE rats.

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BIOGRAPHICAL SKETCH

Catherine A. Marcinkiewcz was born in Point Pleasant New Jersey. Her family moved to Florida a few years later where she completed high school in St. Petersburg, Florida. She decided to pursue a career in science as an undergraduate at Johns Hopkins University where she completed her Bachelor of Science in biomedical engineering with a concentration in materials science. She returned to Florida to pursue a Master of Science in biomedical engineering before joining the Interdisciplinary Program in Biomedical Sciences in the fall of 2005 to resume her lifelong passion for neuroscience. She joined the laboratory of Darragh Devine in the fall of 2007 to study the neurobiology of stress and psychiatric disease and completed her dissertation work in the fall of 2010. She will pursue her postdoctoral work at the University of North Carolina in Chapel Hill starting in January of 2011.