IN PRESS

Book Chapter in "Experimental models in serotonin transporter research". Nova Science Publishers (NY). Allan V. Kalueff, Ed.

SERT Models of Emotional Dysregulation

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Abstract

The serotonin system plays a key modulatory role in central nervous system processes that appear to be dysregulated in psychiatric disorders. Specifically, the serotonin transporter (SERT) is thought to be critical to many aspects of emotional dysregulation, and has been a successful target for medications that treat several psychiatric disorders. Here, we narrowly focused on two psychiatric conditions, anxiety and depression, for which mice with SERT genetic manipulations has provided insight. Specifically, we suggest that dissecting syndromes according to a trait and state perspective may help us understand the complex and at times contradictory rodent results. The most compelling reason for this approach is provided by human studies in which increased trait neuroticism and stress-mediated vulnerability to develop depression were reported for subjects carrying the 5HTTLPR *s/s* allele of the SERT gene, thus placing the contribution of SERT to mood disorders in a gene x environment and trait/state context. Accordingly, current behavioral results in SERT KO mice are consistent with both increased trait and state anxiety-like behaviors, while evidence in support of a trait-based model of depression in SERT KO mice are inconsistent and mostly based on tests with limited relevance to human depression. However, comorbid symptoms associated with a wider definition of depression, such as altered gastrointestinal functions, lower pain threshold, and greater sensitivity to stress, have been reported in SERT KO mice, suggesting the presence of a pro-depressive state resulting from low SERT. Studies on SERT mutant mice, as putative genetic models of increased vulnerability to develop a depressive state in response to chronic challenges (i.e. paralleling the *s*-allele mediated vulnerability in humans), have only begun. Finally, studies will need to integrate trait/state features with gender specific approaches to fully recapitulate the risk factors that are known to influence the vulnerability to develop altered mood regulation in human subjects, namely genetic load, sex and environment. To this end, SERT mutant mice can provide a critical window into mechanisms leading to increased risk for mood disorders, with the potential to reveal new targets for antidepressant drug development.

Introduction

The serotonin (5-hydroxytryptamine, 5-HT) system plays a key modulatory role in central nervous system processes that appear to be dysregulated in many psychiatric disorders. These processes include: internal affective states such as anxiety, fear, depression, and aggression; control of sleep; modulation of digestive behaviors; and influence on reward circuits that mediate motivation, hedonic states, and the appetitive properties of drugs of abuse (LeMarquand et al. 1994; Linnoila and Virkkunen 1992; Murphy and Lesch 2008; Nestler et al. 2002; Spiller 2007) . The serotonin (5-HT) system has been a successful target for medications that treat many psychiatric disorders (Nemeroff and Owens 2002). The serotonin transporter (SLC6A4 gene; protein also characterized as 5-HTT or SERT) is thought to be critical to many aspects of emotional dysregulation in neuropsychiatric disorders in which serotonin is a key modulator. In addition to the comorbid psychiatric symptoms,

there are multiple neurological and physiological dysfunctions that are also comorbid with affective disorders, such as multiple sclerosis, dementia, epilepsy, gastrointestinal troubles and altered pain sensation (Esch et al. 2002; Ghaffar and Feinstein 2007; Ishihara and Brayne 2006; Kanner 2007; O'Brien 2005). As the scientific literature for all of these conditions is vast, we will be narrowly focusing on two psychiatric conditions within the larger panoply of SERT related emotional dysregulation, anxiety and depression, for which mice with SERT genetic manipulations have provided insight (Murphy and Lesch 2008). Focusing on these two affective disorders will illustrate in greater detail the important role, and possible mechanisms, of serotonin and SERT in emotional dysregulation.

Anxiety represents a serious and disabling psychiatric spectrum of disorders, including panic disorder and general anxiety disorder, with multiple comorbidities such as post traumatic stress disorder (PTSD) and depression. The greatest determinants of the development of anxiety disorders lie in individual environmental factors, although the genetic heritability of anxiety is estimated at 30-40% (Hettema et al. 2001). The rate of suicide with anxiety disorders is increased from the general population, by approximately 18 fold in panic disorder and significantly increased with comorbid general anxiety disorder (GAD) and depressive symptoms (Weissman et al. 1989). Selective serotonin reuptake inhibitors (SSRI) are an effective treatment for anxiety related disorders. The major target of SSRIs is SERT, and blockade of SERT is the mechanism by which excess anxiety or panic is relieved (Nemeroff and Owens 2008). Therefore, a better characterization of the role of key players in the serotonin system throughout life is crucial to the investigation of the neurobiology of anxiety as well as to identify new therapeutic targets for the treatment of this disabling psychiatric disorder.

Blockade of SERT function results in an anxiolytic effect in adult and adolescent patients. This pharmacologic anxiolytic response occurs with prolonged neurotransmission of serotonin by chronic SSRI block of its re-uptake from the synaptic cleft. Intuitively one would guess that constitutive genetic downregulation of SERT expression would result in a non-anxious phenotype throughout the life of the individual. However depending on the timing of its blockade, SERT blockade can have opposite and paradoxical behavioral outcomes. This paradox is illustrated in the context of a common promoter polymorphism of the human SERT gene (SERT gene-linked polymorphic region, 5HTTLPR). The 5HTTLPR polymorphism occurs in short (s) and long (l) allelic variants and has demonstrable effects on lymphoblast SERT messenger RNA (mRNA) and binding, and platelet serotonin reuptake; the (s) variant is a common 44-base pair insertion/deletion polymorphism associated with lower levels of SERT transcription, expression, function, and increased serotonin in the extracellular space in cell lines (Lesch et al. 1996). However, associations with reduced RNA or binding levels in the adult brain have been more difficult to establish (Lim et al. 2006; Mann et al. 2000; Parsey et al. 2006; Shioe et al. 2003), likely reflecting the presence of additional factors contributing to SERT regulation (Serretti et al. 2006).

Contrary to the effects seen with SERT blockade in patients on SSRI antidepressant treatment, the *s/s* polymorphism is associated with increased neuroticism and anxiety, and with increased vulnerability to affective disorders in concert with increased childhood stressful life events (Caspi et al. 2003; Collier et al. 1996; Gutierrez et al. 1998; Kendler et al. 2005; Lesch et al. 1996; Mazzanti et al. 1998). These contrasting reports highlight the complexity of the role of serotonin in mood regulation and emphasize the need to investigate putative neurobiological and neurodevelopmental mechanisms by which serotonin, SERT and the 5HTTLPR *s/s* polymorphism influences the vulnerability to develop anxiety-related and depressive states.

Several imaging studies have identified neural networks involved in anxious phenotypes that are influenced by the *s/s* 5HTTLPR polymorphism. Hariri et al report that individuals with one or two copies of the short allele of the serotonin transporter (5-HTT) promoter polymorphism exhibited greater amygdala neuronal activity, as assessed by blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (MRI), in response to fearful stimuli compared with individuals homozygous for the long allele (Hariri et al. 2002). BOLD fMRI identifies “active brain areas” through a process called the hemodynamic response. Blood releases oxygen to metabolically active neurons at a greater rate than to inactive neurons, and the difference in magnetic susceptibility between

oxyhemoglobin and deoxyhemoglobin and thus oxygenated or deoxygenated blood, leads to magnetic signal variation which can be detected using an MRI scanner. This study was independently confirmed, further implicating the amygdala, SERT, and the pathways that may inhibit or activate under fearful or depressive stimuli (Heinz et al. 2005). Pezawas et al have also reported functional consequences of the presence of the *s*-allele on the structure, function, and functional coupling of the perigenual cingulate cortex (rACC) and amygdala (Pezawas et al. 2005). The variance associated with the *s/l* polymorphism in amygdala-rACC functional connectivity was responsible for 30% of temperament variance in harm reduction subscale scores from the Tridimensional Personality Questionnaire. Moreover, the presentation of fearful stimuli revealed an uncoupling of the functional interaction of this regulatory circuit in *s/s* patients. Together, this study provided a rationale for the amygdale/rACC coupling as a feedback mechanism for amygdala regulation and a putative mechanism by which the 5HTTLPR *s/s* carrier was associated with a more neurotic and anxious phenotype.

Major depressive disorder is a significant psychiatric illness, contributing to death by suicide, as well as the 4th most common cause of disability per the World Health Organization (WHO) (Murray 1996). The lifetime prevalence for suicide attempts in individuals with unipolar depression is ~16%, about half the rate of those with bipolar disorder, and four times greater than those with any other axis I disorder (Chen and Dilsaver 1996). Significant clinical symptoms include sleep disturbances, anergia, anhedonia, changes in appetite, poor concentration and the appearance of suicidal ideation. Lifetime incidence reaches 20%, and is often found comorbid with PTSD, anxiety, and alcohol and substance use disorders. PTSD is characterized as development of characteristic symptoms following exposure to an extreme traumatic stressor involving direct personal experience of an event. This exposure results in intense fear and/or helplessness, persistent reexperiencing of the traumatic event, persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness, and persistent symptoms of increased arousal, that cause significant interference with normal daily social and psychological functioning. Multiple environmental and genetic factors act additively or synergistically to produce a depressive phenotype, with no one environmental or genetic factor contributing more than 5% of the variance between normal subjects and depressed subjects (Leonardo and Hen 2006; Mann and Currier 2006; Nestler et al. 2002). Depression is also a significant contributor to systemic organ diseases (Murray 1996), including potential shared etiology for numerous co-morbid symptoms and diseases, such as anxiety, increased pain sensitivity, irritable bowel syndrome (IBS) and overactive bladder. SSRIs were originally used as antidepressants and blockade of SERT is the mechanism by which the cluster of depressive symptoms is relieved (Nemeroff and Owens 2002). Similar to studies examining anxiety, the 5HTTLPR *s/s* polymorphism has both provided answers, and raised many additional questions regarding the role of SERT and serotonin in depression.

In a seminal study, Caspi et al identified reduced resilience for stressful life events in *s/s* carriers compared to *l/l* carriers, a strong clinically relevant and statistically significant direct correlation between number of stressful life events and depressive symptoms in *s/s* carriers, and an increased likelihood of suicide attempts or ideation with increased stressful life events (Caspi et al. 2003). The authors have speculated that 5HTTLPR *s/s* is a factor that predisposes individuals towards PTSD or depression through gene x environment interaction, rather than being a direct correlate of depression. This group also alluded to the gene x gene interaction that may work additively or synergistically to create depressive phenotypes. These findings have been replicated in some studies (Kendler et al. 2005), but not all (Gillespie et al. 2005). These results are nevertheless difficult to reconcile with the general assumption that depression is associated with decreased central serotonin levels, and anxiety with increased central serotonin levels (Handley; Lowry 2005]. How can it be that the *s/s* genotype is linked to both anxiety and depression? A potential explanation, which is partially supported by current pharmacological and genetic studies in mice (Ansorge 2004], could be that the acute consequence of the *s*-allele on the serotonin system may regulate anxiety levels, while changes in adult systems that are secondary to altered developmental events related to SERT genetic variations place the system at risk for developing depression (Sibille and Lewis 2006). These observations illustrate the need for more tractable models to investigate mechanisms that are downstream from SERT function, and that

can investigate additional gene x gene or environment x gene interactions, in order to provide a better understanding of the complexity of the genetics of depression (Gillespie et al. 2005). Indeed, while 5-HTTLPR studies have shed some light on the differential role of SERT in the neurobiology of anxiety and depression, there is a need for animal models, where experimental manipulation of SERT expression (such as transgenic and SERT KO rodents) can establish causality relationships and set the stage to investigate cellular and molecular mechanisms by which changes in SERT affect brain function, emotional regulation, and symptoms of neuropsychiatric disorders.

SERT Mutant Mice

SERT knockout mice (Bengel et al. 1998; Lira et al. 2003), and recently rats (Homberg et al. 2007), have provided useful models to investigate mechanisms by which SERT may regulate emotional behavior. SERT KO mice were independently derived by different groups (Ansorge et al. 2004; Bengel et al. 1998). The first SERT KO mouse was generated via homologous recombination, by replacing the second exon of the SERT gene with a neomycin cassette (Bengel et al. 1998). This alteration leads to a loss of the full-length, functional SERT protein in the brain. This mouse was back-crossed onto the C57BL/J6 (B6) strain. Mice homozygous for the null mutation ($SERT^{-/-}$ or KO) exhibit an absence of serotonin reuptake, resulting in a decreased rate of synaptic serotonin clearance and a 6-10 fold increase in basal levels of forebrain extracellular serotonin, and a compensatory 60-80% reduction in tissue levels of serotonin (Mathews et al. 2004; Montanez et al. 2003). Heterozygous mutant mice lacking one SERT allele (SERT HZ) show a deficiency in serotonin reuptake and clearance that is intermediate between that observed in SERT KO mice and WT controls (Mathews et al. 2004; Montanez et al. 2003). However, despite the 50% reduction in SERT expression, SERT HZ mice do not show the low locomotor activity phenotype that is characteristic of KO mice (Holmes, Yang et al. 2002; Kalueff, Ren-Patterson et al. 2007), and exhibit either more subtle behavioral differences compared to KO or, in the majority of studies, no differences in testing compared to their WT controls (Bengel et al. 1998; Kalueff, Ren-Patterson et al. 2007; Li et al. 1999). These results were confirmed in an independently derived SERT mutant line, where the first exon of SERT was replaced it with a neomycin cassette (Lira et al. 2003). This line has been mostly maintained on the 129S6/SvEv (S6) genetic background. Other SERT genetic manipulations have included the use of siRNA to downregulate SERT in adult male BALB/c mice, which resulted in a phenocopy of adult SSRI treatment in WT mice (Thakker et al. 2005). Finally, Zhao et al have recently trapped exon 14 of SERT (Zhao et al.), creating a nonfunctional truncated SERT protein at the C-terminus, rather than the N-terminus for the other two groups, with a phenotype similar to the other SERT KO strains (Bengel et al. 1998; Holmes, Lit et al. 2003; Lira et al. 2003).

A recent study investigated the neurochemical and behavioral effects of increased SERT expression by generating a novel transgenic mouse model that expresses huSERT from a yeast artificial chromosome construct (El Yacoubi and Vaugeois 2007) in addition to endogenous murine SERT (Jennings et al. 2006). huSERT consists of the human 5-HTT (h5-HTT) gene flanked by 150 kb of 5' and 300 kb of 3' sequences with the "short" allele of the 5-HTTLPR in the promoter region and the 10-repeat allele of the variable number tandem repeat in intron 2 inserted into the YAC construct, that was ultimately injected into fertilized eggs. These transgenic mice showed reduced tissue levels (~15 to 35%) of serotonin in the hippocampus, frontal cortex, hypothalamus, brainstem, and midbrain compared with wild-type animals. However in microdialysis experiments, SERT overexpressing mice demonstrated constantly lower (50 to 60%) basal extracellular levels of serotonin in both the prefrontal cortex and hippocampus compared with wild-type mice. This difference was stable and maintained over 3–4 hours of baseline sampling. Also, in both regions, the increase in serotonin evoked by local application of high potassium was significantly less (40–80%) in the transgenic mice compared with WT. Separate experiments showed that the low levels of extracellular serotonin in the prefrontal cortex of SERT-overexpressing mice could be reversed and normalized by local application of 1 μM paroxetine. While the genetic mutation is in some ways opposite to that in SERT KO mice, the two

lines share some unexpected similarities, such as reduced tissue levels of serotonin, thus prompting additional questions on the neurobiology of SERT expression in the brain, and suggesting differential possibilities for compensatory mechanisms following dysregulated SERT function (See Anxiety behavior later).

Studies in SERT mutants have also reported crucial adaptive mechanisms and compensations resulting from decreased or absent SERT function. SERT KO mice, and to a lesser extent HZ mice, displayed reduced dorsal raphe serotonergic neuronal firing, a reduction in serotonin neurons, and a desensitization and downregulation of somatodendritic 5-HT1A autoreceptors (Gobbi et al. 2001; Li et al. 2000; Lira et al. 2003; Mannoury la Cour et al. 2001). Binding density reductions of postsynaptic 5-HT1A receptors are seen in SERT KO mice in the frontal cortex, amygdala, septum, and hypothalamus, but not the hippocampus (Li et al. 2000; Mannoury la Cour et al. 2001). Changes in the expression of other serotonin receptor subtypes appear to be less profound and more region-specific. SERT KO mice show reduced 5-HT1B receptor binding density in the substantia nigra but not other brain regions (Fabre et al. 2000) and fail to respond to the locomotor-stimulating effects of 5-HT1B receptor activation (Holmes, Yang et al. 2002). The binding density of 5-HT2A and 5-HT2C receptors is increased in the hypothalamus and amygdala, respectively (Li et al. 2003), while 5-HT3 mRNA levels are reduced in the enteric nervous system of SERT KO mice (Liu et al. 2002). In SERT KO mice, modest residual serotonin reuptake into axons is accomplished by dopamine or norepinephrine transporter function (Montanez et al. 2003; Murphy et al. 2001) and by compensatory upregulation of the OCT3 transporter (Schmitt et al. 2003), although not as efficiently (Daws et al. 2006; Holmes, Yang et al. 2002; Mathews et al. 2004; Schmitt et al. 2003). Together, these neurochemical characterizations revealed significant downstream compensations due to altered expression of functional SERT, and highlight the complexity of changes and putative confounding effects that will need to be taken into consideration when investigating the contribution of SERT and SERT mutant models in anxiety and depressive disorders.

Anxiety

The constitutive silencing of SERT expression results in elevated anxiety-like behavior in adult mice (Holmes, Lit et al. 2003). These findings were replicated in different KO variants in mice (Lira et al. 2003) and rats (Olivier et al. 2008), but also appeared to be modulated by differences in genetic background (Holmes, Yang et al. 2002). Early blockade of SERT by chronic SSRI treatment recreates the adult SERT KO phenotype (Ansorge et al. 2004). Finally, SERT over-expression resulted in the opposite phenotype, a low anxiety- or anxiety-resistant phenotype (Jennings et al. 2006).

Holmes et al examined anxiety related behaviors in SERT KO and HZ mice using the original strain of SERT KO mice on the C57B6 genetic background in the light-dark exploration, emergence, open field, and elevated plus maze tests (Bengel et al 1998; Holmes et al. 2003). SERT KO mice spent less time in the aversive, open arms of the elevated plus-maze and made fewer entries into the open arm, thus exhibiting more inhibited, less explorative anxiety-like behavior compared to WT controls. Knockout mice showed less exploration of brightly lit areas in both the light/dark exploration and emergence tests, ~50-75% less time compared to WT controls. Knockout mice also showed a general reduction in exploratory locomotion and greater “wall-hugging” in a brightly lit open field arena. These findings suggest a robust increase in anxiety-like behavior in SERT KO mice. Interestingly, despite exhibiting a gene-dosage dependent alteration in serotonin neurotransmission (e.g., partially elevated basal synaptic serotonin), SERT HZ mice fail to exhibit clear behavioral abnormalities in these tests. No robust gender linked differences were observed. Together, SERT KO mice on the C57B6 genetic background were uniformly more anxious than their WT controls (Holmes, Yang et al. 2002).

In contrast, phenotypic abnormalities were not observed in SERT KOs that were bred onto a congenic 129S6 background (Holmes, Lit et al. 2003; Holmes, Murphy et al. 2002), as measured in

the elevated plus maze and light-dark exploratory tests. A possible explanation for these findings is that the naturally elevated anxiety-related behavioral baseline of 129S6 WT controls precluded the detection of further anxiety-like abnormalities caused by the SERT KO mutation, especially in tests that rely on locomotor activity and that suffer from known floor effects in high anxiety groups. This is especially relevant since SERT KO mice exhibit a hypoactive locomotor phenotype in a home cage environment (Holmes, Yang et al. 2002), which may have contributed to the lack of detectable abnormal behavior in exploration-based tests for anxiety-like behavior (Holmes 2001).

The absence of an increased anxiety-related phenotype in SERT KO mice on the 129S6 genetic background appeared to be confirmed in a separate line of SERT mutant (Lira et al. 2003). These mice exhibited no behavioral phenotypic differences related to anxiety measurements in the open field and elevated plus maze tests. In the elevated plus maze test, Lira et al observed no gender or genotypic differences in time spent or percent of entries to the open and closed arms; however, activity differed between the genotypes, with SERT KO mice showing less total arm entries and fewer head dips than their WT controls by ~25%. This is in contrast to the findings of Holmes et al which did not find activity related differences in elevated plus maze testing, but consistent with prior reports of lower activity of SERT-KO mice in open field (Holmes, Yang et al. 2002). More subtle behavioral differences have suggested differences in anxiety-like behavior in SERT KO mice on the 129S6 genetic background as well. Indeed, Ansorge et al reported no difference in the percentage of entries or time spent in the open arms of the elevated plus maze, as well as no difference in the percentage of time in the center of the test chamber using the open field test. However, SERT-KO mice displayed significant reductions, by ~33-50% depending on the test, in the total arm entries, total ambulatory time, as well as total vertical activity counts, which was interpreted by the authors as anxiety-like behavior, and not simply a matter of baseline reduced locomotor activity, since no differences in home cage activity were observed between KO and WT control mice.

A more compelling argument for elevated anxiety-like behaviors for SERT-KO mice on the 129S6 background may actually be provided by results from the same group in the novelty suppressed feeding (NSF) test. Although the NSF has been used recently to investigate the long term behavioral response to chronic antidepressant activity (Santarelli et al. 2003), the latency to feed in a threatening novel environment of the NSF chamber correlates with fearfulness and decreases after acute treatment with both anxiolytic drugs (Bodnoff et al. 1988) or chronic antidepressant exposure (Santarelli et al. 2003). This pharmacological response suggests that mechanisms underlying changes in the latency to start feeding involve both anxiety-like and antidepressant-like processes. Moreover, this test is mostly activity-independent, and thus less likely to be confounded by the low locomotor activity of SERT KO mice. In this test, 129S6 SERT KO mice displayed a significant increase in the latency to start eating (Ansorge et al. 2004), thus providing supporting evidence for a anxiety-related phenotype on that genetic background as well, which the authors characterized as “emotional” behavior. Alternatively, background genetic differences present in one strain (129S6), but not another, might partially protect against the anxiety promoting effects of the SERT KO mutation. Also of note there were no appreciable sex differences in C57B6 or 129S6 WT, HZ, or KO mice on anxiety-related behavioral testing, in contrast to the known sex differences in mood disorders observed in human subjects. Taken together, these findings underscore the utility of assessing mutants on multiple genetic backgrounds, and in using multiple tests that do not all rely on behavioral inhibition.

In a follow up study, Ansorge et al determined that early postnatal blockade of SERT in WT mice with fluoxetine and then normalization in adulthood mimicked the elevated emotional phenotype of constitutive SERT KO mice (Ansorge et al. 2004). They pinpointed the necessary period of SERT blockade for the emotional phenotype by parallel testing of KO mice and chronic early treatment of WT mice with fluoxetine (Ansorge et al. 2004). No difference were observed in the percentage of entries into the open arms or time spent in open arms, as well as no difference in the percentage of time in the center of the test chamber using the open field test. However, SERT KO mice and postnatally fluoxetine treated mice showed significant reductions in total arm entries, total ambulatory time, and total vertical activity counts. The developmental origin of the adult “emotional” phenotype

was clearly demonstrated in the NSF test as WT mice treated with fluoxetine between postnatal days 4 to 21 pheno-copied the increased latency to start feeding in the NSF test of SERT KO mice. The authors have recently replicated their findings and showed that this effect was specific to SERT compared to postnatal blockade of the norepinehrine transporter (Ansorge et al. 2008). In summary these studies demonstrated that early disruption of normal SERT function can result in a trait-based emotional phenotype, which may be relevant to anxiety-like, but also to a pro-depressive behavior due to the pharmacological validations of the NSF test by both anxiolytic and antidepressant drugs.

By shifting the time frame to earlier developmental events, these rodent studies may shed light on the apparent discrepancies between the similar biological effects of the 5HTTLPR *s* allele and SSRI treatment in human subjects (decreased serotonin uptake through either reduced SERT expression or pharmacological blockade, respectively) and the opposite clinical outcomes (greater risk for depression with the *s*-allele versus therapeutic improvement with SSRIs). Specifically, these studies suggest that serotonin, as a trophic factor, can influence the development and establishing of neural networks that later in life participate in mood regulation (Sibille and Lewis 2006). Accordingly, once established, these altered neural networks may mediate the increased vulnerability to depression in adulthood, independent of the current state of serotonin function.

Finally, additional evidence for a causal role for SERT in modulating anxiety-like behavior were provided by Jennings et al using a line of SERT over–expressing mice. C57B6- transgenic SERT over-expressor mice expressed a more resilient and low anxiety phenotype compared to normal controls (Jennings et al. 2006). Specifically, they displayed increased entries (2 fold) and a decreased latency (~50% reduction) to enter the open arms of the elevated plus maze, as well as a significant reduction in latency to eat in the NSF test (Jennings et al. 2006), contrasting with the increased latency observed in SERT KO mice (Ansorge et al. 2004). Interestingly, paroxetine treatment of SERT over-expressing mice reversed the low anxiety phenotype in the elevated plus maze test, suggesting that mechanisms responsible for the decreased anxiety-like phenotype were directly related to increased SERT function.

The Jennings et al study dovetailed with SERT KO mice studies, showing that constitutive lack of SERT expression, or early SERT blockade during development resulted in increased anxiety phenotype, suggesting altogether that SERT expression may act like a “temperamental thermostat,” with more SERT resulting in less anxiety, and vice versa. Thus, SERT genetic murine studies support the notion of emotional regulation by serotonin, where a decreased amount of serotonin uptake early in development (due to low SERT) results in adult animals that are more prone to affective extremes of anxiety and depression, and less able to modulate their affective state, compared to mice with normal or increased levels of SERT expression displaying normal or low anxiety levels. The importance of the time window of SERT downregulation (i.e. developmental versus constitutive blockade) and of multiple compensatory mechanisms in SERT KO mice also suggest that mechanisms supporting the increased anxiety phenotype may in some cases be remote from the original SERT disruption. Specifically, changes in SERT activity early in development suggest that developmental adaptations occurred, which in turn may disrupt the formation of neural networks that are critical for normal adult functions.

Nevertheless, potential discrepancies in anxiety-related phenotypes in SERT KO mice suggest the following question: Would an anxiety-like abnormality in SERT KO mice clearly manifest under high or chronic stress conditions? This would test for potential differences between an anxiety-like “state” rather than “trait” phenotype in SERT KO mice. Testing this hypothesis is only beginning in SERT KO mice. In a recent study, male SERT KO mice on the C57B6 background, but not HZ, were more susceptible to mild stress (cat odor), as measured by increased anxiety-like behavior in open arm entries in elevated plus maze testing and dark/light box testing (Adamec, Burton et al. 2006). Interestingly, the more handled the mice were, the less their basal differences were apparent (i.e. nurtured/handled SERT KO mice seem to be less anxious than naïve SERT-KO mice). These results parallel the effects of nurturing on the impact of the 5HTTLPR *s/s* polymorphism in nonhuman primates and humans (Bennett et al. 2002; Caspi et al. 2003; Champoux et al. 2002; Kendler et al. 2005). In this particular study, an anxious predisposition was unmasked in SERT KO mice with repeat

exposure to stress, echoing a variety of mammalian models demonstrating the important interaction of gene and environment in the resiliency, or lack thereof, to neuropsychiatric disorders under different environmental conditions (Bennett et al. 2002; Champoux et al. 2002; Wellman et al. 2007). Adamec et al previously demonstrated that female C57/B6 WT mice, but not male mice, showed an increased susceptibility to stress and anxiety after being exposed to cat odor (Adamec, Head et al. 2006). Thus, they postulated that they partially recreated a "female-like" susceptibility to anxiety and stress in male SERT KO mice by subcutaneous implantation of estradiol-β releasing pellets in, and that resultant downstream changes in 5HT1A and 5HT2A binding may have played a role in these adaptive differences in anxiety-like behavioral outcomes (Ren-Patterson et al. 2005). This study helps to define similarities and differences in state versus trait testing of anxiety, in addition to testing the role of critical factors in the expression of anxiety-related phenotypes, including differences based on the extent of nurturing and gender.

Neurotrophic factors such as brain derived neurotrophic factor (BDNF) can interact with SERT to affect the levels of serotonin in extraneuronal space as well as modulate anxiety-like behavior. Interestingly, SERT/BDNF double KO mice display gender differences, in which females, perhaps through estrogen expression, are more resilient to the effects of increased serotonin in the extraneuronal space in behavioral testing (Ren-Patterson et al. 2006). Female $SERT^{-/-} \times BDNF^{+/-}$ mice showed a protective effect, through increased TrKB receptor expression, and BDNF modulation (Ren-Patterson et al. 2006), in that significantly less reductions in serotonin concentrations were observed in hypothalamus and other brain regions than males, relative to controls. Likewise, in the elevated plus maze, female $SERT^{-/-} \times BDNF^{+/-}$ deficient mice also demonstrated no increases in the anxiety-like behaviors previously found in males $SERT^{-/-} \times BDNF^{+/-}$. Female $SERT^{-/-} \times BDNF^{+/-}$ mice did not manifest the ~40% reduction in the expression of TrkB receptors or the ~30% reductions in dopamine and its metabolites that male $SERT^{-/-} \times BDNF^{+/-}$ did. After estradiol implantation in male $SERT^{-/-} \times BDNF^{+/-}$ mice, hypothalamic serotonin was significantly increased compared to vehicle-implanted mice. These findings support the hypothesis that estrogen may enhance BDNF function via its TrkB receptor, leading to alterations in the serotonin circuits which modulate anxiety-like behaviors. Together, the sex effects were reversed by estrogen implants in males, or ovarectomy in females, identifying estrogen as a potential important modulator in the behavioral outcomes and altered serotonin levels in extraneuronal spaces.

In short, SERT KO mice exhibit a robust baseline difference in anxiety-like behaviors, although modified by the background strain of the KO mice, suggesting the presence of modifier genes interacting with SERT function. These findings parallel human studies with the 5HTLLPR polymorphism, in which the *s/s* allele can make individuals more susceptible to anxiety disorders, but anxiety disorders are still multifactorial, and not solely due to disruption of one gene. Comparisons between constitutive deletion and pharmacological manipulations have been instrumental in determining the critical developmental time-window for the long-lasting effects of the lack of SERT expression on adult anxiety-like behavior. Studies in SERT/BDNF double KO have started to investigate interactions of SERT with other key genetic (BDNF) or neuroendocrine (estrogen) modulators that act in concert with SERT to establish levels of anxiety-like behaviors. Together, the somewhat conflicting results in gender and strain differences illustrate the complex nature of gene and environment interactions even for a "pure" affect-related syndrome such as anxiety, in contrast to depression, which is characterized by clusters of symptoms, each of those potentially supported by different neurobiological systems and potentially acting with relative autonomy.

Depression

SERT plays an important role both in putative mechanisms underlying the pathophysiology of depression, as well as in its pharmacological treatment. Interestingly, current studies suggest that the constitutive lack of SERT expression may result in some depression-like behaviors. Since SERT pharmacological blockade relieves depression, these results are in contrast to what was initially

hypothesized, which was that the lack of serotonin reuptake would result in a mouse that if anything, would be more resistant to develop depression-like behavior. Remarkably, behavioral studies in SERT KO mice suggest just the opposite phenotype, with more anxious-like behavior and potential increased susceptibility to stress and depression.

However numerous limitations to modeling a depressive syndrome may mitigate these conclusions and must be addressed first. The difficulties associated with studying depression in rodents could be framed by the following facts: (1) the lack of a model to induce a validated depressive pathology, (2) an almost exclusive focus on tests for antidepressant mechanisms, rather than on the primary pathology of the disorder, and (3) the heterogeneity of a disorder with multiple symptom dimensions. Thus, to establish a rodent model of depression, as a cluster based syndrome (i.e. paralleling the definition of the human syndrome in DSM-IV and ICD-10), several criteria would need to be fulfilled, including: (1) good face validity (close ethological counterpart for emotion-related and anhedonia-like behaviors), (2) good construct validity (for instance, unpredictable psycho-social stress mimics real-life stress etiology and recruit equivalent neuroendocrine and biological systems), and (3) good predictive validity (antidepressant reversal respects the time-courses for mechanisms of disease and drug reversal).

It is important to note that the majority of paradigms that are frequently used either to induce or characterize depressive-like states (social defeat, learned helplessness), or to predict antidepressant activities (forced swim and tail suspension tests), follow at best 1 or 2 of these criteria or do not model epidemiological characteristics of human depression and neurobiological mechanisms. Thus, conclusions drawn from these tests have only limited relevance to the human depressive syndrome. In this section, we first review results from some of these behavioral tests, which have been used mostly for predictive values of antidepressant effects. Then, in the absence of a current validated model with good face and construct validities for the full depressive syndrome, we argue that reviewing sets of symptoms that are commonly found co-morbid with depression and that are affected by the lack of SERT would in the meantime contribute to our understanding of the role of SERT in depression. Indeed, some or all of the following overlapping symptoms may be found along with depressive-like behavior, and are believed to share some genetic and environmental components: i) increased alcohol and/or substance use, ii) anxiety, iii) increased or decreased aggression, and iiii) additional physiological symptoms, - potentially mediated by peripheral serotonin and SERT - such as irritable bowel syndrome (IBS) and/or chronic pain (See Introduction). In other words, the goal is to assess multiple features of a depressive-like phenotype in SERT KO mice, as a means to help us understand the overall role of SERT in this syndrome.

Holmes et al assessed the presence of a depressive-like phenotype in SERT KO mice through forced swim test (FST) and tail suspension test (TST) for inescapable stress-related behavior (Holmes, Yang et al.). In the FST, SERT KO mice backcrossed onto a 129S6 genetic background spent significantly more time in passive immobility than WT controls. Because reduced immobility in this test is opposite to the effects of antidepressants, such a profile could be indirectly interpreted as a “depression-like” response to inescapable stress. However, SERT KO mice on the C57B6 background did not show any differences in FST or TST testing (Holmes, Yang et al. 2002) while reduced immobility in TST testing for 129S6 SERT KO strain was reported in the same study, just the opposite of what would be expected for a “depressive” phenotype. Limitations of the TST and FST tests and present results at addressing potential molecular or cellular mechanisms of depression include paradoxical responses in the two tests, and importantly a time course for pharmacological validation of these tests that rely on minutes, rather than weeks, as observed in depression. Therefore, these studies provide only weak and contentious evidence for a putative trait–based depression-like phenotype (Holmes, Yang et al. 2002).

As mentioned above, Ansorge et al using the 129S6 SERT KO strain reported heightened “emotional” behavior in the activity-independent NSF test. SERT KO mice and postnatally fluoxetine-treated WT mice both showed increased latency to feed in the NSF test compared to HZ and WT control mice. Again, the pharmacological validation of this test by chronic exposure to antidepressant (Santarelli et al. 2003) suggests that increased latency in that test may also be consistent with a

depressive-like phenotype. Similar results were reported with the foot shock avoidance test, where both fluoxetine treated mice and constitutive SERT KO mice showed an increased latency to avoiding shock, considered “learned helplessness” behavior. Activity was controlled for by observing intershock activity and did not differ across groups. Together, these studies suggest evidence for trait-based depressive-like behavior in SERT KO mice, although they highlight the difficulty of clearly separating depressive-like from anxiety-like behaviors. Alternatively, these tests may be viewed as assessing an emotional dimension of behavior that is present in both syndromes, and that should be interpreted in the context of additional behavioral dimensions for conclusive evidence supporting a depressive-like phenotype. Importantly, these studies only assessed trait-like behaviors and did not address the possibility of SERT KO mice state-based depressive behavior, as induced by interactions with the environment. Stress has been most often used to induce higher emotion-related states. Here, in support of impaired stress responses resulting from the lack of functional SERT, SERT KO mice show exaggerated plasma adrenocorticotropin and catecholamine responses to 15 minutes of immobilization or saline injection (Li et al. 1999; Tjurmina et al. 2002), deficits on active avoidance in the shock avoidance test (Lira et al. 2003).

In face of these conflicting results and lack of all encompassing tests for depression, how then should SERT KO mice be evaluated as a potential animal model of depression? What additional evidence has been provided by studies performed in SERT KO with regard to other putative co-morbid “symptoms”? We suggest that the presence of these symptoms (i.e. including aggression, chronic pain, irritable bowel syndrome and fear conditioning) should be included and considered in a wider definition of a depressive syndrome, and that they should help us defining the role of SERT in potentially modulating multiple symptom dimensions that are either co-morbid or interactive with depression. We suggest that the overall pattern of changes observed reflects a potential trait-like pro-depressive and cluster-based behavioral phenotype in SERT KO mice, and suggests that induced state-based behavioral paradigms should be investigated.

Changes in *aggression* levels and *agonistic behaviors* are often co-morbid with human depression. Holmes et al have reported reduced aggression levels in SERT KO mice (C57B6 background) using the resident-intruder test (Holmes, Murphy et al. 2002). SERT KO mice took longer to initiate the first attack and, generally, attacked less frequently and less intensely than WT controls. In a rare example of intermediate phenotype, SERT HZ mice showed an intermediate aggressive phenotype, attacking as quickly as WT controls but generally attacking with lower frequency. To test whether aggression in SERT KO mice would emerge under further provocation, mice were exposed to an intruder for a second time. Repeated testing increased aggression in SERT HZ and WT control mice, while aggressive behavior in SERT KO mice remained low. The amount of time SERT mutant mice spent in non aggressive, social investigative behavior was similar to that of the other genotypes, thus suggesting a specific inhibition of aggressive responses, rather than a more general social deficit, in SERT KO mice. However, some recent studies have shown global social deficits in SERT KO mice (Kalueff 2007; Kalueff, Fox et al. 2007; Kalueff, Ren-Patterson et al. 2007). This decreased aggression phenotype in SERT KO parallels clinical findings in some studies in human depression (Holmes, Murphy et al. 2002; Linnoila and Virkkunen 1992). Interestingly, this study not only evaluated aggression as a baseline trait, but also as an induced state over time. With increasing exposure to intruders, SERT KO mice remained more docile, thus displaying a consistent trait and state decreased aggressive behavior phenotype.

Chronic pain often is comorbid in individuals experiencing depression. In a recent study, Vogel et al examined changes in pain perception in SERT KO mice (Vogel et al. 2003). In SERT KO mice, reduced serotonin levels in the injured peripheral nerves correlate with diminished behavioral signs of thermal hyperalgesia, a pain-related symptom caused by peripheral sensitization. In contrast, bilateral mechanical allodynia (“other pain”, pain from stimuli which are not normally painful), a centrally mediated phenomenon, was associated with decreased spinal serotonin concentrations in SERT KO mice and may possibly be caused by a lack of spinal inhibition. In short, lack of serotonin doesn’t activate heat hyperalgesia at local site, but lack of serotonin causes a lack of central inhibition in the spine and an increase in mechanical pain, making it bilateral rather than remaining unilateral, through

a lack of 5HT2A and 5HT3 activation or downregulation of these receptors. This study finds increased pain in SERT KO mice to stimuli that otherwise do not elicit pain, demonstrating the presence of another analogous pro-depressive characteristic of trait-based depression, paralleling observations in some depressed human subjects (Jann and Slade 2007).

Other comorbid peripheral conditions often observed in depressive disorders include *irritable bowel syndrome* (IBS) and *overactive bladder* (Chen et al. 2001; Cornelissen et al. 2005). Gastrointestinal and bladder functions are mediated by peripheral serotonin that is synthesized in the gastrointestinal tract and transported back into the cell by SERT as well. Gastrointestinal and bladder dysfunction were reported in SERT KO mice (Chen et al. 2001; Cornelissen et al. 2005). Stool water and colon motility were increased in most SERT KO animals; however, the increase in motility (diarrhea) occasionally alternated irregularly with decreased motility (constipation) (Chen et al. 2001). The watery diarrhea is probably attributable to the potentiation of serotonergic signaling in SERT KO mice, whereas the transient constipation may be caused by episodes of enhanced serotonin release leading to serotonin receptor desensitization. Cornelissen et al found this attribute more robust in female mice, leading to a clinical correlation mimicking the comorbidities found in human subjects with depression, bladder dysfunction, and IBS (Cornelissen et al. 2005). These studies were performed in SERT KO mice back-crossed on a C57B6 background strain.

Importantly, these studies did not directly evaluate depressive behavior, but rather addressed comorbid conditions associated with depression observed in human studies, providing additional evidence for potential links between central and peripheral symptoms related to a more encompassing definition of a depressive syndrome, and which may help our overall understanding of depression (Zorn et al. 1999). This study illustrates peripheral mechanisms of serotonin dysregulation, increased serotonin release resulting in alternate diarrhea and transient constipation, similar to human IBS, in which SSRI treatment improves global quality of life by acting on central CNS targets, but does not affect peripheral serotonin receptor activation, or improve gut pathophysiolgy (North et al. 2007).

Human imaging studies have reported and characterized a link between the *s/s* 5HTTLPR polymorphism, rACC-amygdala coupling and increased avoidance, and amygdala activation with fearful stimuli (Hariri et al. 2002; Pezawas et al. 2005). These results provide network-based evidence for a role of SERT in emotional regulation, and additional evidence in support of the link between fear, depression, and SERT function. One recent study has evaluated the effects of *fear conditioning* and repeated exposure to FST on *fear extinction* and depressive-like behavior (FST) resulting from stress exposure, a potential model of “state-based” depressive behavior in mice (Wellman et al. 2007). The fear memory was then extinguished by repeatedly presenting the fear-associated stimulus in the absence of aversive outcome. During the first session of extinction learning, SERT KO and WT mice exhibited similar progressive reductions in conditioned freezing. In contrast, whereas both genotypes showed an expected spontaneous recovery of the fear response when tested for recall of the extinction memory 24 hours later, the response was markedly higher in SERT KO mice than in WT mice. SERT KO mice were subsequently able to (re)extinguish to WT levels with additional repeated exposure to the conditioned stimulus. This has parallels with individuals with PTSD, as well as depressed individuals with the 5HTTLPR *s/s* polymorphism (Caspi et al. 2003; Kendler et al. 2005; Lee et al. 2005).

The results of this study identified stronger spontaneous recovery of the fear response to the conditioned stimulus, a PTSD-like behavior, in SERT KO mice, supporting the potential use of this model to investigate neuropsychiatric disorders, such as PTSD, in addition to anxiety and depression. One of the limitations of this mouse study is that only male mice were used, and that sex differences were not assessed. Again, the use of the FST as a test of depressive-like behavior, rather than antidepressant predictability has been questioned (Cryan and Holmes 2005; Kalueff, Fox et al. 2007; Kalueff et al. 2007; Lucki 2001; Mayorga and Lucki 2001), and another test such as the sucrose preference test or NSF may have strengthened the results. Nevertheless, this is one of the first studies to address “state-based” or evoked depressive-like behavior in SERT KO mice after exposure to repeated environmental stress. Similar studies need to be performed to examine trajectories and dynamics involved in these behavioral, genetic, and state/trait interactions. Together this study

provides insight into more clinically relevant scenarios of rodent models for anxiety, depression, and PTSD.

In summary, we postulate that unlike anxiety and similarly to the way it is diagnosed in human population, investigating “depression” in rodents may benefit from the inclusion of a “cluster of symptoms” rather than a more limited focus on affect-related behaviors. Numerous studies have addressed components of this cluster. In some cases (i.e. depressive-like behavior) the results have been conflicting, while for other symptoms or comorbid conditions (i.e. anxiety and IBS), the effects of SERT KO are more robust. Although it is far from conclusive at this point, taken together, these studies provide some level of evidence suggesting the presence of a “trait-based” depressive phenotype in SERT KO mice, as manifested by elevated anxiety, altered agonistic behavior, higher susceptibility to stress, decrease in fear extinction, IBS and overactive bladder, and increase in pain experience. Also, few studies (Avgustinovich et al. 2005; Kudryavtseva et al. 1991; Mineur et al. 2006; Wellman et al. 2007; Willner et al. 1992) have examined “state-based” model of depression, evoking depressive-like behavior after exposure to environmental stressors. Here, one study suggests that SERT KO mice may be useful as a potential state-based model of depression (Wellman et al. 2007), although little is currently known about state-based changes in depressive behavior in SERT KO mice.

Limitations / Future directions

The limitations of current studies using SERT KO mice at addressing potential mechanisms involved in the neurobiology of depression and anxiety can be summarized along four general lines.

The *first limitation* is inherent to the constitutive nature of the genetic manipulation, in that SERT KO mice are an artificial construct, where lifelong constitutive lack of functional SERT expression has no parallel with depressed or anxious patients. Depression and anxiety are complex diseases, and most likely involve a complex series of mild genetic alterations that contribute to these conditions, thus SERT HZ may be more valuable as a putative and clinically relevant research model (Kalueff, Ren-Patterson et al. 2007). It is also important to note that the current studies do not directly model the role of the commonly investigated *s/l* SERT polymorphisms in human anxiety and depression, as SERT levels do not necessarily correlate with *s/l* polymorphism in the adult human brain (Mann et al, 2000; Shioe et al, 2003; Lim et al, 2006; Parsey et al, 2006), rather these studies are investigating the impact of variable SERT levels to anxiety-like and depression-related behaviors. In view of the critical role of low SERT during development (Ansorge et al. 2004), it will be essential to develop new lines of mutant mice with better temporal control of SERT expression, in order to genetically confirm and further investigate early time-windows of selective vulnerability in establishing neural networks that later in life regulate emotion-related behaviors. The facts that adult knockdown of SERT recapitulates the pharmacological blockade of SERT (Thakker et al. 2005), and not the KO or HZ phenotype, and that SSRIs reduce the low anxiety phenotype of SERT-overexpressing mice (Jennings et al. 2006), further emphasizes this latter point. In view of the considerable biological adaptations and developmental compensations that take place in constitutive SERT KO mice, such more fine-tuned genetic manipulations will be valuable in identifying changes that are critical to mood regulation and to segregate relevant biological events from epiphenomena.

The *second categorical limitation* relates to background strain variance and limitations of tests at assessing depression-related mechanisms. Indeed, variable anxiety- and depression-related results were obtained depending on the behavioral tests and genetic background of SERT KO mice (Holmes, Yang et al. 2002). In addition to a reduced locomotor activity phenotype in SERT-KO mice (Holmes, Yang et al. 2002), the 129S6 strain itself is hypoactive, together leading to potentially false positive results on locomotion-based test for anxiety measurements, such as the elevated plus maze and open field, and on depression-like behavioral tests, such as the FST and TST. Moreover, while these latter behavioral tasks demonstrate good predictive validity for the acute effects of

antidepressants, it is unclear how they relate to the complex neurobiology of human depression (Cryan and Holmes 2005). Thus in view of behavioral differences due to genetic background variability, and due to inconsistent results in SERT KO mice in tests that are similar in construct (FST and TST) but that are more limited in face validity for putative implication on depression mechanisms, caution is needed when interpreting data on altered depression-like behavior in SERT KO mice.

Taken together, this suggests that a depression phenotype in SERT KO mice cannot be established only by 'despair' tests such as the FST and TST, and would benefit from re-evaluation using other depression paradigms. Accordingly, the absence of difference between WT, HZ and KO mice in the activity-independent sucrose preference test (Kalueff et al. 2006), a model for anhedonia that has better face and construct validities for studying depressive characteristics, further suggests that SERT KO mice may not be "depressed" (Kalueff, Ren-Patterson et al. 2007), or at least do not display trait-based depression-like behaviors. These conclusions are only mitigated at this point by the results from Ansorge et al (2004) in the NSF test, due to the dual assessment of depressive-like and anxiety-like features of this test, and by the presence of several additional related symptoms that are often co-morbid, and potentially related to a depressive phenotype, as described earlier.

The *third major limitation*, relates to the lack on in-depth assessment of sex differences. Indeed, in view of the well-characterized increased prevalence of mood disorders in female subjects, it is surprising that sex differences in behavioral assays have not been more systematically investigated in SERT KO mice. Some sex specific effects have been identified, female $BDNF^{+/-}/SERT^{-/-}$ (sb) appear to be more adaptive to a lack of SERT expression, having significantly diminished reductions in serotonin concentrations in hypothalamus and other brain regions compared to males (Ren-Patterson et al. 2006). Likewise, in the elevated plus maze, female sb mice demonstrated no increase in anxiety-like behaviors and did not manifest the ~40% reduction in the expression of TrkB receptor observed in male sb mice. The authors suggest that estrogen may exert a protective and moderating effect on compensatory changes occurring in SERT KO mice (Ren-Patterson et al. 2006). Together, these studies represent only the beginning of investigations aimed at understanding and identifying the potential biological substrates of sex by serotonin interactions in mood regulation.

Finally, the *last major limitation* of the current studies is the lack of evocative testing and modeling, or lack of comparisons between trait- and state-based models of anxiety and depression in SERT KO mice. The most compelling reason suggesting that such studies are needed is provided by studies in human subjects carrying the 5HTTLPR *s/s* polymorphism, where prior exposure to some form of stress is necessary to reveal the genetic contribution of SERT to the development of depressive episodes, thus clearly identifying a gene x environment interaction (Caspi et al. 2003; Kendler et al. 2005). Also, there is significant cormorbidity of affective disorders, with other disorders affected by stress, including PTSD, multiple sclerosis, chronic pain, and fibromyalgia (Buskila and Cohen 2007; Esch et al. 2002; Fietta et al. 2007; Ghaffar and Feinstein 2007; Lee et al. 2005). Thus, projecting back to rodent models, how would SERT KO and HZ respond to unpredictable chronic mild stress or to a social defeat paradigm, two models used to induce depressive-like states?

Trait-based studies in SERT KO mice on one hand, and evoked or state-based studies in normal WT mice on the other hand, have been missing out on important factors in the gene x environment model of depression. In other words, while SERT KO mice may represent, at best, a poor model a trait-based depression, does the lack of SERT function result in an increased vulnerability to develop a depressive–like state? Studies using fear-conditioning and repeated exposure to stressful stimuli (Wellman et al. 2007) have suggested increased stress reactivity and impaired fear extinction, thus representing first attempts at investigating evoked states in SERT KO mice. Preliminary studies from our lab indicate that unpredictable chronic mild stress induces robust increases in emotional behavior in the NSF test in all genotype groups [WT, HZ and KO; C57B6 background (Bengel et al. 1998)] and that maximal evoked behaviors are achieved in SERT-KO mice, with female SERT KO mice displaying highest emotional behavior compared to all other groups (Edgar et al. 2007), thus suggesting the presence of an increased vulnerability to develop elevated emotional behavior in SERT KO mice, and mimicking the increased prevalence of mood disorders in female subjects.

Together, these preliminary results suggest that SERT KO mice may not yet be ruled out as a model, if not of trait-depression, of increased vulnerability to develop depressive states.

Summary

Now that we have briefly addressed the limitations of the knocking down, out and over-expressing SERT in modeling aspects of anxiety-like and depression-related behaviors, what can we say about SERT and emotional dysregulation? In short, SERT KO may represent a good model of trait-anxiety, a poor model of trait-depression, and putatively a better model for evoking state-depression. However, much work remains to be done to assert these conclusions, especially with regards to evoked anxiety-like and depression-related states. Studies on gene x gene, and gene x environment interactions have only begun, and will need to be integrated with trait/state studies and with gender specific studies to fully recapitulate risk factors that are known to influence the vulnerability to develop altered mood regulation, namely genetic load, sex and environment (especially early-life and chronic stress). These new studies will benefit from completing the baseline and evoked characterization of constitutive SERT mutants. If HZ-SERT mutant ended up representing an intermediate phenotype in evoked states, as suggested by few studies performed to date (Kalueff, Ren-Patterson et al. 2007), this would validate the use of SERT KO mice to investigate relevant changes in neural networks, as an extreme phenotype, yet still relevant to mechanisms of altered mood regulation.

So how will SERT mutant models help us in identifying cellular and molecular mechanisms that are proximal to an altered mood phenotype? And could a SERT mutant be useful in identifying true new pharmacological targets, beyond the monoamine neurotransmitters systems? Interestingly, adult SERT downregulation from normal or artificially elevated levels have so far suggested a lack of compensatory mechanisms beyond SERT, as RNAi mimicked SSRI application (Thakker et al. 2005) and since SSRI reversed the low anxiety phenotype of SERT overexpressors (Jennings et al. 2006). On the other hand, early SERT blockade have clearly identified a postnatal developmental window that appears to be critical for establishing emotion-related behavior in adulthood (Ansorge et al. 2004). Together, these observations point to two potential directions to focus on: *first*, identifying developmental switches affected by changes in SERT function, and *second*, since early developmental periods are unlikely to be realistically targeted for the prevention of mood disorders, identifying the molecular, cellular, and circuitry changes in adult systems that are secondary to altered developmental events related to SERT and that place the system at risk for developing mood disorders (in concert and in addition to already characterized compensations in monoamine systems). To this end, SERT mutant mice provide a critical window into mechanisms leading to increased risk for mood disorders. In turn, this latter set of studies has the potential to reveal new targets for antidepressant drug development. Moreover, it is likely that identified mechanisms underlying adult vulnerability to mood disorders will be subject to modulation through other etiological pathways and may thus represent common mechanisms conferring risk for anxiety and depressive disorders.

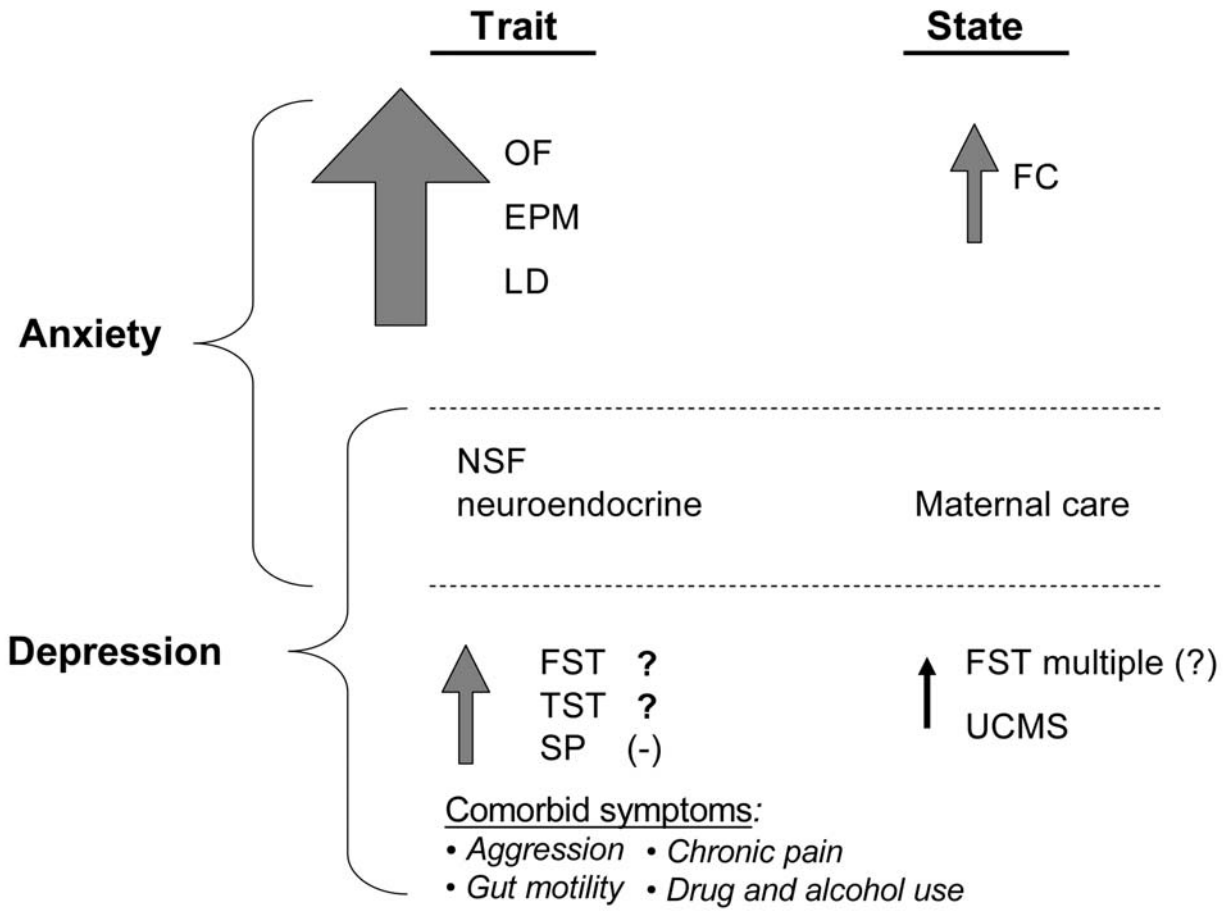

Figure 1. Summary of evidence supporting anxiety- and depression-like phenotypes in SERT KO mice.

The model illustrates the respective levels of support from behavioral and physiologic testing for anxiety- and depression-like phenotypes in SERT KO mice, according to an anxiety-depression continuum, and under baseline trait- or induced state-conditions. In short, SERT KO may represent a good model of trait-anxiety, a poor model of trait-depression, and putatively a better model for induced states of emotional dysregulation. ↑ denotes evidence supporting increased anxiety- or depressive-like phenotypes. OF, open field; EPM, elevated plus maze; NSF, novelty suppressed feeding; TST, tail suspension test; FST, forced swim test; SP, sucrose preference; FC, fear conditioning; UCMS, unpredictable chronic mild stress.

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