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Modeling SERT × BDNF interactions in brain disorders: single BDNF gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities in serotonin transporter knock-out mice

ABSTRACT

There is growing clinical evidence that many psychiatric illnesses have overlapping genetic mechanisms. Understanding these mechanisms is important to the improvement of psychiatric treatment and preventions of the disorders, and animal genetic models continue to be a critical avenue of research towards these ends. As serotonin is a key neurotransmitter with important roles in normal behavioral processes and has been implicated in the pathogenesis of psychopathological conditions such as depression, anxiety, and addiction, it is a prime target for investigation in behavioral neurogenetics. The serotonin transporter (SERT) is a key brain protein that regulates the amount of serotonin that can activate the receptor. It is becoming evident that SERT interacts with brain-derived neurotrophic factor (BDNF), an important modulator of dopaminergic, cholinergic, and serotonergic neurons, which has been linked to memory function, activity, eating behavior, depression and anxiety. The pivotal roles played by these two brain molecules have resulted in the development of numerous mutant animal models that have reduced function of SERT, BDNF, or both. Interestingly, SERT × BDNF mutant mice show numerous different behavioral phenotypes that are distinct from either SERT mutant of

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BDNF mutants alone, displaying phenotypes that are highly relevant to human clinical scenarios and bringing them added validity. This chapter will provide data from numerous experiments utilizing these rodent models, and will explain their relevance and validity for research into the genetics of neuropsychiatric disorders.

INTRODUCTION

Various genetic animal models are used in neuroscience research for screening psychotropic drugs, testing neurobiological hypotheses and finding candidate genes for stress-related brain disorders (Crawley, 1999; Kaiser *et al*., 2001; Kalueff and Tuohimaa, 2004; Van Meer and Raber., 2005; Vetter *et al*., 2002). Mounting data indicate that many brain disorders represent overlapping pathogenetic pathways with common genetic determinants and clinical manifestations (Kalueff and Nutt, 1996; Kalueff and Tuohimaa, 2004; Kalueff *et al*., 2007b), raising the possibility that several distinct but interacting domains may contribute to clinical and experimental phenotypes. This also implies that a closer in-depth analysis of different domains may stimulate new, clinically relevant genetic experimental modeling of neuropsychiatric disorders. This chapter will focus on two key brain molecules – serotonin transporter (SERT) and brain-derived neurotrophic factor (BDNF) – that have been implicated in multiple neuropsychiatric disorders, and discusses how their genetic animal models may optimize further experimental research in this field.

Serotonin (5-HT) is a key brain neurotransmitter (Adayev *et al*., 2005; Aghajanian, 1990; Aghajanian and Marek, 1997, 1999; Lauder, 1990; Turlejski, 1996; Whitaker-Azmitia, 1991, 2001). Clusters of serotonergic cell bodies are located along the midline of the brain stem known as the raphe nuclei, and their axonal projections are distributed throughout the central nervous system (CNS). The dorsal and median raphe nuclei send their projections to diverse regions including the cortex, hippocampus, limbic structure, striatum, thalamus, midbrain, and hypothalamus. Although found in only a small percentage (about 1–2%) of neurons in the brain, 5-HT is an important morphogenetic contributor to the developing brain (Ansorge *et al*., 2004; Bonnin *et al*., 2006, 2007; Vitalis *et al*., 2007; Vitalis and Parnavelas, 2003; Whitaker-Azmitia, 1999, 2001; Whitaker-Azmitia *et al*., 1996). Disrupted signaling of this neurotransmitter during early development produces enduring changes in the morphology and function of the CNS (Gross and Hen, 2004a, 2004b; Gross *et al*., 2002).

Altered developmental and postnatal 5-HT effects numerous facets of cognition and emotional regulation (Graeff *et al*., 1996; Gross and Hen, 2004a, 2004b; Gross *et al*., 2000, 2002; Lucki, 1998; Owens and Nemeroff, 1994), as evidenced by its implication in the pathogenesis of many brain disorders such as anxiety, depression, mania, addiction, schizophrenia, autism and obsessive compulsive disorder (OCD) (Firk and Markus, 2007; Kennedy *et al*., 2003; Lesch, 2005a, 2005b; Lesch and Mossner, 2006; Lesch *et al*., 2003; Meyer, 2007; Senkowski *et al*., 2003; Shiah and Yatham, 2000; Yatham *et al*., 2000).

The uptake of synaptic 5-HT into nerve terminals – the most important mechanism of serotonergic regulation – is mediated by SERT, a high-affinity plasma membrane serotonin transporter (Lesch, 2005b; Murphy *et al*., 2001, 2003, 2004; Rudnick, 2006a, 2006b; Zhou *et al*., 2002). In humans, a common SERT polymorphism in the promoter region, a variable-number tandem repeat in intron 2, and a coding region mutation have been reported to be associated with a variety of neuropsychiatric diseases, including anxiety, autism, OCD and depression (Firk and Markus, 2007; Glatt *et al*., 2001; Glatt and Reus, 2003; Hariri and Holmes, 2006; Holmes and Hariri, 2003; Kalueff *et al*., 2007a; Murphy *et al*., 2003; Ozaki *et al*., 2003).

Previous studies have demonstrated that BDNF is the most abundant brain neurotrophic factor and that reduced expression of BDNF in mice can affect brain synaptic vesicle function, synaptic plasticity, and can lead to specific alterations in hippocampus-based spatial learning as well as hypothalamus-regulated eating behavior and motor activity (Angelucci *et al*., 2000, 2005; Berton *et al*., 2006; Bonhoeffer, 1996; Kernie *et al*., 2000; Ren-Patterson *et al*., 2006). In addition, loss of the BDNF receptor, TrkB, leads to more severe changes through neuronal loss and cortical degenerative abnormalities (Vitalis *et al*., 2002; Xu *et al*., 2000).

Similarly, decreased serum levels of BDNF have been found in patients under stress and in patients with mood disorders (Karege *et al*., 2002; Licinio and Wong, 2002; Martinowich and Lu, 2008; Martinowich *et al*., 2007; Nestler *et al*., 2002). A recent study found that only the BDNF gene was identified as a potential risk gene out of 76 candidate genes studied in a bipolar disorder sample (Sklar *et al*., 2002), supporting the hypothesis that BDNF plays a primary role in mood disorders. In addition, a val66met BDNF human gene variant has been shown to be associated with changes in memory and abnormal hippocampal activation assessed by fMRI (Egan *et al*., 2003). This variant of the BDNF gene is also associated with several neuropsychiatric disorders

(Hall *et al*., 2003; Kim *et al*., 2007; Lohoff *et al*., 2005; Neves-Pereira *et al*., 2005). BDNF has also been found to directly affect the brain serotonergic system. For example, intracortically administered BDNF produces localized increases in serotonin axon density (Mamounas *et al*., 1995). Several cultured cell models indicate that BDNF enhances the differentiation of a serotonergic phenotype (Eaton and Whittemore, 1996; Galter and Unsicker, 2000a, 2000b; Rumajogee *et al*., 2002, 2004, 2005, 2006). BDNF also modulates serotonin transporter (SERT) function in cultured cell lines (Mossner *et al*., 2000; Ren-Patterson *et al*., 2005b). Pre-administration of BDNF prevents the formation of serotonergic axonal lesions produced by the serotonin neurotoxin parachloramphetamine (Mamounas *et al*., 1995, 2000). Moreover, abnormal thalamocortical axon overgrowth, which is a consequence of excess serotonin availability during certain stages of brain development in mice with a targeted deletion of the MAO-A gene, is exacerbated by inter-breeding these MAO-A gene-deleted mice with mice lacking the BDNF receptor, TrkB (Vitalis *et al*., 2002).

To extend these studies of brain plasticity and of specific interactions between BDNF and the serotonergic and dopaminergic systems (Berton *et al*., 2006; Lyons *et al*., 1999; Ren-Patterson *et al*., 2005a), we investigated whether an endogenous BDNF gene difference might play a role in the consequences of a serotonin transporter deficit found in SERT knock-out mice.

Notably, BDNF is an important modulator of dopaminergic, cholinergic, and serotonergic neurons, implicated in synaptic vesicle function and synaptic plasticity. Leading to specific alterations in behaviors, including memory, activity, eating behavior, depression and anxiety (Aloe *et al*., 2000; Angelucci *et al*., 2000, 2004, 2005; Bartoletti *et al*., 2002; Berton *et al*., 2006; Bonhoeffer, 1996; Chourbaji *et al*., 2004; Kernie *et al*., 2000; Kuipers and Bramham, 2006; McAllister, 1999; Minichiello *et al*., 1999; Murphy *et al*., 2004; Pozzo-Miller *et al*., 1999; Yamada *et al*., 2002). Prominent physiological changes have been observed in a double SERT−/− BDNF+/− knock-out mouse model (Ren-Patterson *et al*., 2005a, 2005b, 2006), strongly supporting the importance of SERT–BDNF interactions.

Loss of BDNF single gene exacerbates 5-HT deficiencies in male SERT × BDNF (SB) double-mutant mice, but not in female mice

Serotonin (5-HT) concentrations in the four brain regions for both genders and four genotypes (SB = SERT+/+ BDNF+/+; Sb = SERT+/+

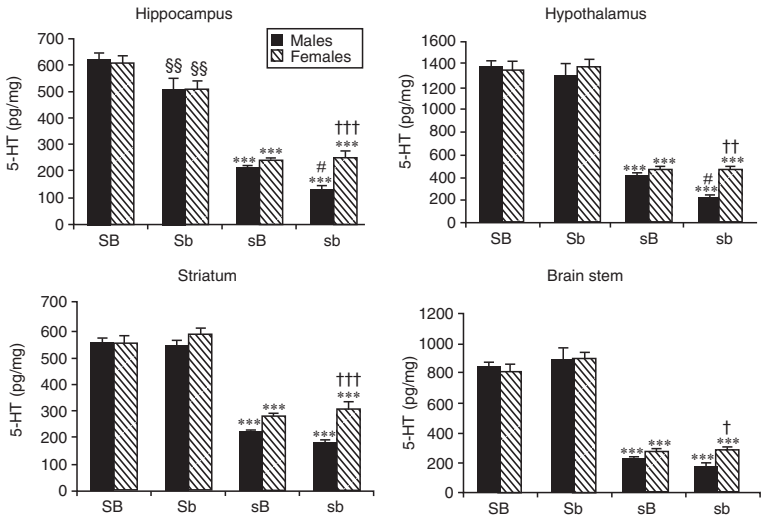

Figure 9.1 5-HT concentrations in different brain regions were significantly reduced (pg/mg protein, mean ± SEM, $n = 5$–6) in sb double-mutant mice compared to SB and Sb mice (SB = SERT+/+ BDNF+/+; Sb = SERT+/+ BDNF+/−; sB = SERT−/− BDNF+/+; and sb = SERT−/− BDNF+/− mice). Hippocampus −79%, Hypothalamus −80%, Brain stem −79%, Striatum −69%. A significant further serotonin reduction of 37% in hippocampus and 43% in hypothalamus was observed in sb mice compared to sB mice. In addition, male compared to female sb mice had a significant reduction of 5-HT concentrations in hippocampus and striatum, †††$p<0.001$, in hypothalamus, ††$p<0.01$, in brain stem, †$p<0.04$. Furthermore, both genders of sB mice had significant reductions in all four brain regions (***$p<0.001$) relative to SB mice. Sb mice compared to SB controls had significant reductions of 5-HT in only the hippocampus (§§$p<0.008$), but not in other brain regions.

BDNF+/−; sB = SERT−/− BDNF+/+; and sb = SERT−/− BDNF+/−) are presented in Figure 9.1A–D. Significant gender × genotype interactions were found in hippocampus ($F_{3,46}=4.2$, $p<0.01$) and striatum ($F_{3,46}=4.6$, $p<0.006$). Significant genotype-related 5-HT reductions were found in sB and sb mice relative to the SB controls in multiple brain regions: hippocampus ($F_{3,46}=189.9$, $p<0.001$), striatum ($F_{3,46}=177.3$, $p<0.001$), hypothalamus ($F_{3,46}=224.5$, $p<0.001$) and brain stem ($F_{3,46}=200.5$, $p<0.001$). Post-hoc analyses revealed that significant serotonin reductions of 37% in hippocampus ($p<0.01$) and 43% in hypothalamus ($p<0.02$) were observed in male sb double-mutant mice compared to male sB SERT knockout mice (Ren-Patterson *et al.*, 2005a). In contrast,

female sb mice had no further significant reductions in serotonin concentrations compared to female sB mice. Serotonin concentrations in these female sb mice differed significantly from male sb mice in all four brain regions studied (Figure 9.1).

DOPAMINE AND METABOLITES SHOW GENDER-BASED DIFFERENCES IN SERT × BDNF (SB) DOUBLE-MUTANT MICE IN STRIATUM

A significant gender × genotype interaction for dopamine concentrations was found in striatum ($F_{3,46}$=5.07, p<0.004). Significant genotype-related reductions in dopamine were observed ($F_{3,46}$=3.0, p<0.04). While male mice had significant reductions of dopamine of 32% (Figure 9.2) relative to SB mice (p<0.001) and Sb mice (p<0.001), and of 25% relative to sB mice (p<0.004), female sb mice had no reductions of dopamine in striatum. Thus, male sb mice compared to sb female mice had significantly reduced striatal dopamine (p<0.001). Dopamine concentrations were unaltered in other brain regions.

Furthermore, both DOPAC (a primary dopamine metabolite) and HVA (a major catecholamine metabolite) concentrations in striatum were altered, with significant gender × genotype interactions: DOPAC ($F_{3,46}$=4.2, p<0.01); HVA ($F_{3,46}$=4.1, p<0.01). For both DOPAC ($F_{3,46}$=8.2, p<0.001) and HVA ($F_{3,46}$=12.7, p<0.001), significant reductions were observed only in male sb relative to male SB mice, but not in female mice. Significant genotype-related DOPAC and HVA reductions were found in sB (p<0.004) and sb (p<0.001) mice relative to the Sb controls. However, significant gender differences were only found in double-mutant mice: both DOPAC and HVA (p<0.001) were significantly different in post-hoc comparisons of sb male with sb female mice.

ANXIETY-LIKE BEHAVIORS ARE GENDER-DEPENDENT IN SERT × BDNF-DEFICIENT MALE, BUT NOT FEMALE, MICE

Behavioral changes observed in a double sb (SERT−/− BDNF+/−) knock-out mouse model further strongly support the importance of SERT–BDNF interactions. Several results based on this model reflect SERT–BDNF interplay and illustrate the utility of dissecting individual domains and studying them as a system of interacting endophenotypes. For example, the elevated plus-maze (EPM) data (Figure 9.3 A–C) show a significant gender × genotype interaction for the percentage of time the animal spent in the open arm areas ($F_{3,91}$=2.67, p<0.05). While male

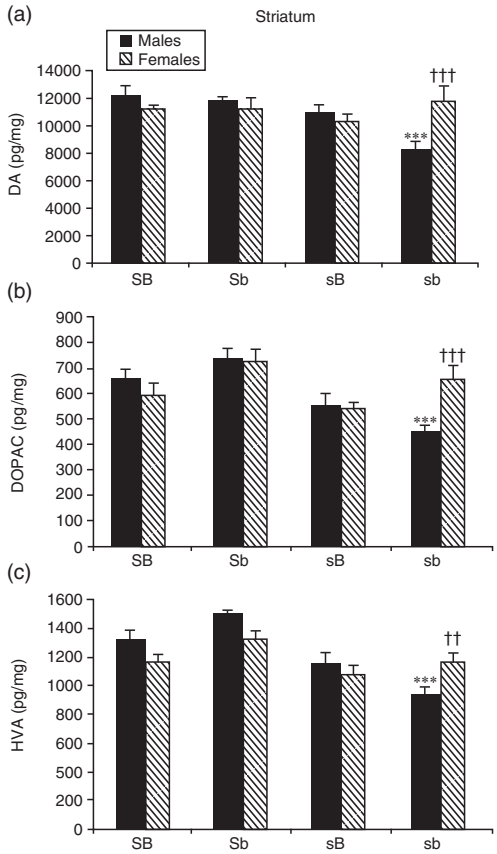

Figure 9.2 Dopamine (DA) concentrations were significantly reduced by 32% (***$p<0.0001$) only in male sb double-mutant mice compared to SB, Sb, and sB mice. DA concentrations in female sb mice were significantly different compared to sb male mice (†††$p<0.0004$). DOPAC and HVA concentrations were also significantly reduced by 32% (***$p<0.001$) and 30% (***$p<0.001$) only in male sb mice compared to SB, Sb, and sB mice. In contrast, DOPAC (†††$p<0.001$) and HVA (††$p<0.01$) concentrations in female sb mice were significantly different compared to male sb mice.

sb mice spent less time on the open arms than male SB mice ($p<0.006$; Figure 9.4A) and also made fewer open entries compared to SB mice ($F_{3,91}=5.35$, $p<0.001$; Figure 9.4B), female sb double-mutant mice showed no differences from their littermate controls on either open arm time or open arm entries percentages. Thus, there were significant differences between the male and female sb mice on percent open arm time ($p<0.001$), but not on open arms entries.

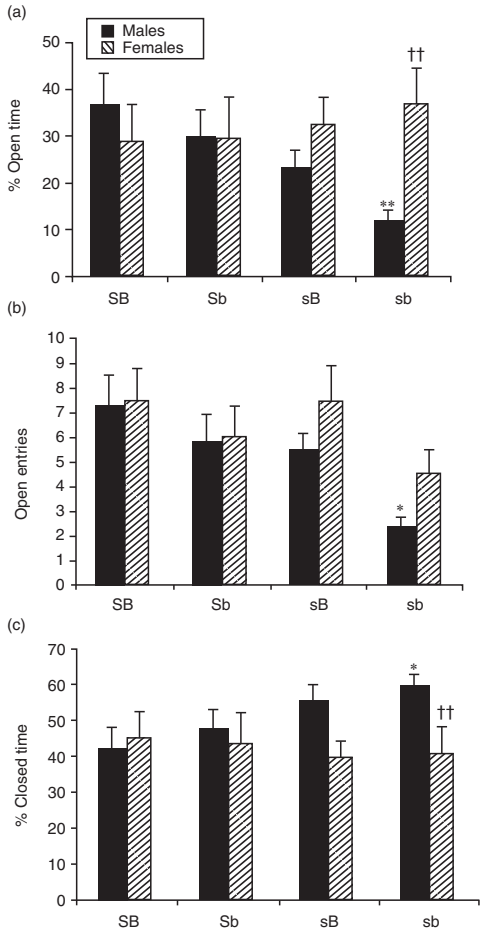

Figure 9.3 Male sb double-mutant mice showed heightened anxiety-like behavior in the elevated plus-maze test relative to SB controls. sb double-mutant male mice spent less time on the open arms ($^{**}p<0.006$) and made fewer open arm entries ($^{*}p<0.003$) compared to SB or Sb controls. Female sb double-mutant mice were not different from their littermate controls, but female sb double mutant mice were significantly different from male sb mice in percent open time ($\dagger\dagger p<0.001$). Male sb double-mutant mice spent more time on the closed arm than male SB mice ($p<0.05$). In addition, sb male mice were significantly different from female sb mice ($p<0.02$). Data (also in Figures 9.4–9.5) are means ± S.E.M., $n = 12$–15 males and $n = 10$–12 females per genotype.

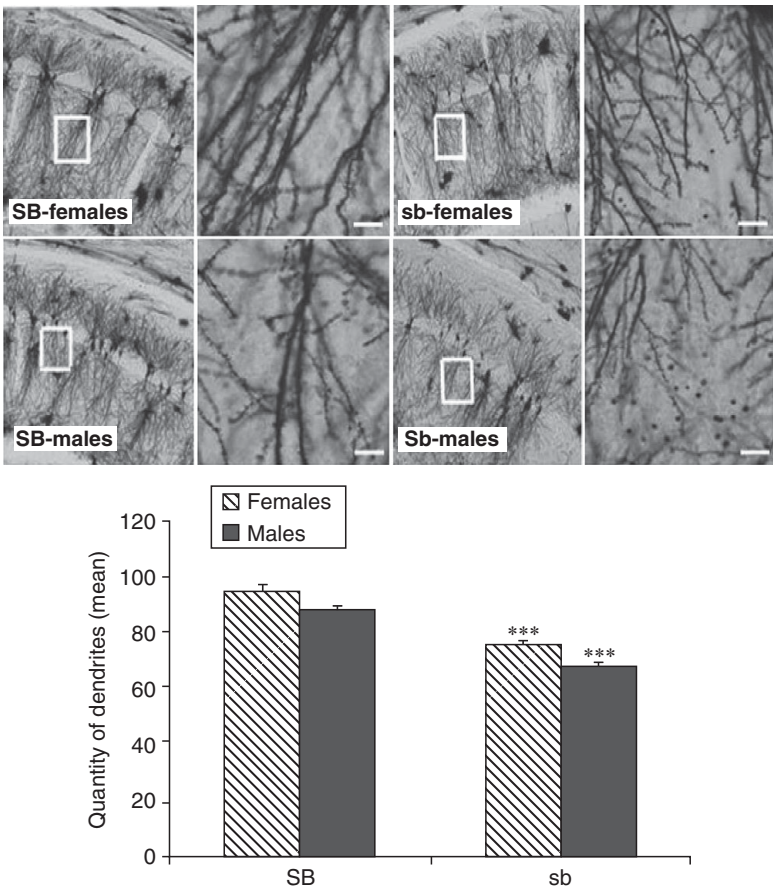

Figure 9.4 Reductions of 5-HT and BDNF affect development of neuronal dendritic branches in sb mice. The morphology of brain neuronal hippocampal near dentrate gyrus dentrities and spines was evaluated in 20 fields. (Scale bars = 10 μm). The quantity of dendrites in brain sections with Golgi impregnation. Both genders had significant reductions in sb mice ($p<0.0001$) compared to SB mice using two-way ANOVA test (see legend for Figure 9.3 for details).

On the other hand, as can be seen in Figure 9.4C, a significant gender difference was found for percentage of time spent in the closed arm ($F_{1,91}=4.5$, $p<0.04$), but no genotype or gender × genotype interaction was found. Thus, male sb mice spent more time on the closed arm than female sb mice ($p<0.01$). For the closed arm entries endpoint,

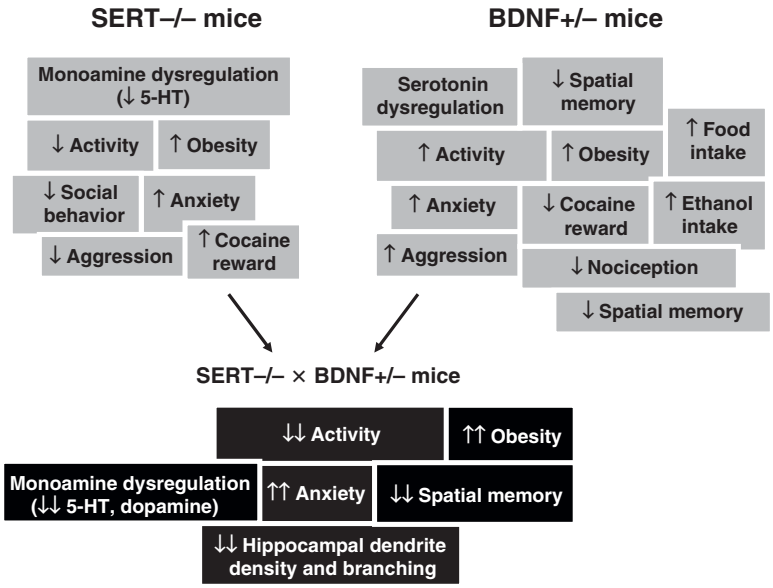

Figure 9.5 Domain architectonics in 5-HT transporter knock-out (SERT−/−), brain-derived neurotrophic factor heterozygous knock-out (BDNF +/−), and double mutant (SERT−/− BDNF+/−) mice. Note that only selected disordered domains are presented (gray) for each genetic model (↑, increased, ↓, decreased profile). Exacerbation of the respective known domains in the double knock-out (SERT−/− BDNF+/−) model, as a result of genetic interactions, is marked with black color and double arrows (see legend for Figure 9.3 for details).

a significant genotype difference was found ($F_{3,91}$=7.71, p<0.001), but no gender or gender × genotype interaction. Thus sb mice of both genders made significantly fewer closed arm entries than SB mice (p<0.01). In contrast, female sb double-mutant mice showed no differences from their littermate controls on either closed arm time or closed arm entries percentages.

TARGETING SERT- AND BDNF-MEDIATED BRAIN DISORDERS

Figure 9.5 compares several altered domains in SERT−/− and BDNF+/− gene-targeted mice, outlining their possible interplay in SERT−/− BDNF+/− double-mutant mice. For example, SERT−/− BDNF+/− mutant mouse data show that reduced BDNF availability during development exaggerates the consequences of absent SERT

function, leading to increased anxiety and obesity (Murphy *et al*., 2003; Ren-Patterson *et al*., 2005a). Interestingly, using neonatal models, Garoflos *et al.* (2005) examined the effects of early developmental experience on spatial learning and memory, food intake, hippocampal glucocorticoid, mineralocorticoid and 5-HT1A receptors, and BDNF. They found that neonatal handling has a beneficial effect in the male mice, improving their cognitive ability, accompanied by increased hippocampal gluco/mineralocorticoid receptors ratio and BDNF. Another pathway causing the anti-stress effects of handling may involve upregulated 5-HT1A receptors that prevent stress-induced hyperphagia, obesity and resistance to leptin (Garoflos *et al*., 2005; Panagiotaropoulos *et al*., 2004).

These findings are consistent with our observations that SERT × BDNF double-mutant mice have larger stress-induced increases in plasma adrenocorticotropic hormone (than any single-knock-out mice) (Murphy *et al*., 2003), confirming that the multiple gene interactions affect many systems (including the neuroendocrine system) co-modulating the animal behavioral and physiological phenotypes. Importantly, BDNF, SERT and 5-HT are present not only in the brain, but also in peripheral tissues involved in metabolic functions and responses to stress (Tjurmina *et al*., 2002; Tonra *et al*., 1999). Thus, both central and peripheral 5-HT/BDNF-mediated mechanisms are affected in the double-mutant SERT × BDNF mice. One of the mechanisms for this may be mediated by corticotropin-releasing hormone that originates from hypothalamus paraventricular nucleus, which in turn results in the release of adrenocorticotropic hormone from the pituitary into general circulation. Furthermore, stress-induced obesity (Bjorntorp, 2001; Bjorntorp and Rosmond, 2000; Rosmond *et al*., 1998) is believed to be associated with glucocorticoid-induced resistance to leptin (Solano and Jacobson, 1999), although other important neuroendocrine mechanisms (Dutton *et al*., 2006; Kuo *et al*., 2007a, 2007b), potentially associated with 5-HT, SERT and BDNF, have recently been reported.

As BDNF plays a central role in the development and plasticity of neuronal circuits in the central nervous system, analysis of neuronal morphology showed that hypothalamus and hippocampus neurons exhibited 25–30% reductions in dendrites (especially in multiple, highly ordered dendrites branches) in double-mutant mice compared with BDNF+/− mice (Figure 9.4). These morphological changes imply that the deletion of BDNF × SERT genes significantly affects the development and growth of dendrites – the structural elements that are crucial for synaptogenesis. Furthermore, a more focused examination of the

dendrites and spines in the hippocampus near the dentate gyrus in female and male sb mice compared to SB mice (Figure 9.4). Our results revealed a significant 20–23% genotype-related reduction in spines relative to SB wildtype. Also, these double-mutant mice showed poorer performance in the radial arm maze (compared with any single-mutant mice; R. Ren-Patterson *et al*., unpublished data). This may indicate aberrant hippocampal memory caused by irregular hippocampal morphology (but also does not exclude other hippocampal abnormalities, such as impaired navigation and exploration). Clearly, a further dissection of diverse domains may be possible in this model, elucidating the role of the two genes in their regulation and co-modulation.

CONCLUDING REMARKS

In addition to the fundamental roles that SERT and BDNF play independently of each other, it is clear that SERT and BDNF interact at numerous levels and play an integral part in the regulation of physiological and behavioral functions as seen in both clinical and experimental studies (Berton *et al*., 2006; Kaufman *et al*., 2006; Ren-Patterson *et al*., 2005a, 2005b, 2006). This evidence, as summarized in this chapter, effectively demonstrates that this related involvement allows for the effective co-modulation of a range of neural mechanisms. However, genetic interactions also play an active part in this regulatory process, adding another interesting dimension to the interplay between SERT and BDNF. The elucidation of such mechanisms offers encouraging potential for novel avenues of investigation into the pathogenesis of common and devastating brain maladies. With the possibilities for inventive exploration, there arises the obligation for developing relevant animal models that foster treatment-oriented research. Given the importance that genetic interactions have on the development and perpetuation of many disorders, genetic models based on mutant or transgenic mice are ideal candidates for this task. As clearly summarized in this chapter, SERT, BDNF, and SERT × BDNF mutant mice emerge as particularly promising models pertinent to many prevalent human disorders.

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