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Impaired spatial working memory and decreased frontal cortex BDNF protein level in dopamine transporter knock out mice

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Abstract

Brain-derived neurotrophic factor (BDNF), one of the key brain neurotrophins, has been implicated in neuronal plasticity and memory. Recent studies document the importance of BDNF for normal long-term memory functions. However, there are few studies of the roles of BDNF in short term memory. Dopamine is likely to play important roles in BDNF gene expression in specific brain regions, including frontal cortical regions that are implicated in short term working memory processes that include spontaneous alternation. We have thus tested spatial working memory in dopamine transporter knockout (DAT KO) and wild-type mice. Spontaneous alternation in the Y-maze, an index of short-term spatial working memory in mice, was significantly decreased in DAT KO mice compared to wildtype mice. BDNF protein was significantly decreased in frontal cortex, though not in striatum or hippocampus, of the DAT KO mice. The data support the hypothesis that impaired spatial working memory in DAT KO mice may be related to decreased frontal cortical BDNF in these animals, and document apparent roles for BDNF in a short term memory process.

Keywords

dopamine transporter knock out (DAT KO) mouse; Brain-derived neurotrophic factor (BDNF); Y-maze; frontal cortex

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein that is widely expressed in the adult mammalian brain (Murer et al., 2001). There is abundant evidence that BDNF is involved in synaptic plasticity and memory processes (Poo et al., 2001; Tyler et al., 2002), particularly as BDNF relates to hippocampal dependent memory. BDNF has been suggested to be essential for normal persistence of long-term memory storage (Bekinschtein et al., 2008) and endogenous BDNF is required for long-term memory formation in the rat parietal cortex (Alonso et al., 2005). Lower levels of frontal cortical BDNF have been associated with impaired working memory performance in Ts65Dn mice, which are considered to be an animal model of Down's syndrome (Bimonte-Nelson et al., 2003). Reducing BDNF expression through intracerebroventricular infusion of BDNF antisense impairs performance in radial arm maze tests (Mizuno et al., 2000). BDNF has

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been implicated in long-term potentiation, an electrophysiological concomitant memory acquisition (Korte et al., 1998; Lessmann 1998).

There is less data concerning the effects of BDNF on spatial working memory, although BDNF has been closely related to dopamine pathways and implicated in dopaminergic function (Berton et al., 2006; Fumagalli et al., 2003; Li et al., 2006; Li et al., 2007a,b). Lesions and other manipulations of mesocortical dopamine pathways can change performance in spontaneous alternation paradigms (Pioli et al., 2008) and alter BDNF gene expression in frontal cortical regions (Fumagalli et al., 2003). Dopamine has also been shown to directly regulate BDNF expression in striatal cells *in vitro* (Küppers and Beyer, 2001). These data therefore collectively implicate a dopamine mediated regulation of BDNF in prefrontal cortex dependent memory function.

We have produced and extensively characterized a line of DAT KO mice that display hyperlocomotion (Sora et al., 1998; 2001; 2009) as well as increased extracellular dopamine levels (Shen et al., 2004). These and other lines of DAT KO mice display altered performance in 8-arm radial maze testing, reduced prepulse inhibition and increased prefrontal cortical BDNF levels (Gainetdinov et al., 1999; Yamashita et al., 2006; Fumagalli et al., 2003). Each of these results suggests that DAT KO mice might also display alterations in spatial working memory due to increased dopaminergic tone, perhaps mediated by alterations in BDNF function. We thus now report results of spatial working memory Y-maze testing and evaluation of BDNF expression in DAT KO mice. We discuss ways in which this data, taken together, is consistent with the idea that direct and indirect effects of this knockout, including the altered BDNF expression, could contribute to the altered performance in this index of short term memory function (Krejcová et al., 2004).

2. Materials and methods

2.1. Animals

DAT KO mice were produced as described (Sora et al, 2001), bred at the Animal Laboratory Institute of Tohoku University Graduate School of Medicine and maintained on a mixed genetic background combining C57BL/6 and 129Sv/J mouse strains. Offspring from heterozygote crosses were weaned at 28 days postnatal and housed in groups of two to five (segregated by sex), in an animal room maintained under a 12 h/12 h light/dark cycle with lights on from 8:00. Food and water were available *ad libitum*. Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tail biopsies, as previously described (Shen et al, 2004). Behavioral testing was conducted in 8–11 week old mice. All animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of Tohoku University Graduate School of Medicine.

2.2. Y-maze test

The Y-maze consisted of 3 arms ($14 \times 4.5 \times 40$ cm). Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The total number of arm entries (locomotor activity) and alternation behavior were recorded using a video camera. The percentage of alternation was calculated as (total of alternation/total arm entries – 2). This measure is considered to reflect short-term memory in mice (Mamiya and Ukai, 2001). Additionally, the number of total arm entries was calculated as an index of locomotor activity (Ma et al., 2007).

2.3. ELISA for measuring BDNF protein concentration

Animals were sacrificed by decapitation. The brains were quickly removed and dissected on ice. Samples taken from the frontal cortex, caudate putamen and hippocampus were frozen

at – 80 °C before homogenization. Brain samples were diluted (hippocampus, 1:30; frontal cortex and striatum, 1:20) and homogenized in a lysis buffer (137 mM NaCl, 20 mM TRIS, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml aprotinin, 1 µg/ml leupeptin, 0.5mM sodium vanadate. The homogenates were centrifuged at 10,000 g for 20 min, and the supernatants were collected and processed for quantification of BDNF by ELISA using a BDNF Emax Immuno Assay kit (Promega, Madison, Wis., USA) according to the manufacture's instructions (Schaaf et al., 1998) and carried out as described previously (Li et al., 2006 and 2007a; Amano et al., 2007). Nunc Maxisorp 96-well immunoplates were coated with 100 µl/well of anti-BDNF Monoclonal antibody (mAb) and incubated overnight at 4 °C. The plates were incubated in a block and sample buffer at room temperature for 1 h. Then, the samples were added to the coated wells (100 µl) and shaken for 2 h at room temperature. Following this the plates was incubated with an anti-Human BDNF polyclonal antibody (pAb) for 2 h at room temperature with shaking and then incubated with an anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature. The plates were then incubated with tetramethylbenzidine solution for 15 min and 1 M hydrochloric acid was added to the wells. The colorimetric reaction product was measured at 450 nm. BDNF standards ranging from 7.8 to 500 pg/ml were used for quantification. Standard curves were plotted for each plate (correlation coefficient; $r=0.99$). Detection limit was 15.6 pg/ml, and cross-reactivity with other related neurotrophic factors was less than 3 %.

2.4. Statistical analysis

The significance of the data was analyzed using unpaired T-tests. $P<0.05$ was considered significant.

3. Results

3.1. Behavioral results in the Y-maze test

Fig. 1 shows spontaneous alternation in Y-maze testing of homozygous DAT KO and wildtype littermate mice. Spontaneous alternation was decreased in DAT KO mice compared to wild-type mice ($P<0.05$). Despite these differences, there were no significant differences in number of total arm entries between DAT KO mice and wild-type mice.

3.2. Changes of BDNF protein

Fig. 2 shows changes of BDNF protein in DAT KO mice compared to wildtype littermates. The concentration of BDNF protein was significantly decreased, by approximately 50 %, in the frontal cortex of DAT KO mice compared to wild-type mice ($P<0.05$). However, there were no significant changes in BDNF level in the caudate putamen or hippocampus of DAT KO mice.

4. Discussion

Observation of decreased Y-maze spontaneous alternation in DAT KO mice is potentially consistent with the idea that these animals have impaired working memory function. Since the number of total arm entries was not significantly different between the two genotypes, confounding influences of locomotor hyperactivity in DAT KO mice seems unlikely. Documentation of significantly decreased frontal cortical BDNF in the DAT KO mice provides a plausible potential mechanism for impairments in spontaneous alternation.

However, although it would seem most parsimonious to attribute differences performance in DAT KO mice to differences in working memory function, it is possible that other behavioral changes primarily underlie these differences. Although prefrontal cortex lesions

impair spontaneous alternation (Mogensen and Divac, 1993), manipulations of several other brain regions also affect spontaneous alternation (Lalonde, 2002), and performance in spontaneous alternation tests is open to attentional and motivational confounds (Hughes, 2004). Attentional impairments have already been noted in DAT KO mice (Yamashita et al., 2006) as well as differences in motivational function (Hironaka et al., 2004). In particular the latter study demonstrated normal operant responses for food under many conditions, but impaired extinction behavior. One way to interpret such changes is as a perseverative response. Similar perseveration of dominant or initial response tendencies have been suggested to underlie alterations in DAT KO behavior in the forced swim test (Perona, 2008), and delayed acquisition of the Morris Water Maze (Hall, Sora, and Uhl, unpublished observations). Perseverative behavior has been associated with dopamine function and is enhanced by amphetamine in a manner that is dependent on the baseline probability of a particular response (Evenden and Robbins 1983). Furthermore, locomotor sensitization induced by repeated DA agonist administration is also associated with reduced spontaneous alternation (Einat and Szechtman 1995). This circumstance might be considered to apply also to DAT KO mice that have enhanced extracellular dopamine function in the striatum and nucleus accumbens (Shen et al, 2006), although it must be considered to what extent these differences are not just mediated by differences in striatal dopamine function, but altered balance of dopamine function in the nucleus accumbens and prefrontal cortex.

There is substantial support for the idea that intact prefrontal dopamine function is important for certain mnemonic functions. Blocking dopaminergic transmission in rat the mediodorsal cortex degrades spatial choice performance in Y-maze testing (Kozlov et al., 2001), and the prefrontal cortex has been postulated to play key roles in short-term memory (Goldman-Rakic, 1996; Kesner and Rogers, 2004). Dopamine agonists can improve short-term spatial memory in human volunteers (Mehta et al., 2001), in ways that are postulated to involve frontal cortex (Egan et al 2002). These postulated prefrontal mnemonic roles thus add to traditional roles for dopamine transmission in the prefrontal cortex that include influences on higher motor functions, motivation, and cognition (Egan and Weinberger, 1997; Lewis et al., 1998; Yang et al., 1999).

At least some of these dopaminergic effects may involve D₁ receptors. In the prefrontal cortex of rodents and monkeys, both the amount of receptor mRNA and the number of receptor-binding sites are significantly greater for the dopamine D₁ receptor than for the other dopamine receptor subtypes (Lidow et al. 1991; Gaspar et al., 1995; Goldman-Rakic et al., 1992). Disrupting dopamine transmission in the prefrontal cortex caused by infusions of dopamine D₁ receptor antagonists or by excitotoxic lesions impairs working memory in nonhuman primates (Sawaguchi and Goldman-Rakic, 1991, 1994). Although extracellular dopamine levels in the prefrontal cortex are not affected by DAT knockout (Shen et al., 2006), further studies are needed to determine whether there are postsynaptic differences in dopaminergic function, and in particular whether differences in D₁ receptor function might contribute to differences in spontaneous alternation in DAT knockout mice that are reported here.

There is also substantial support for dopamine effects on BDNF. Dopaminergic agonists can regulate BDNF mRNA and protein levels (Küppers and Beyer, 2001). BDNF mRNA expression is also reduced in the frontal cortex of another line of DAT KO mice (Fumagalli et al., 2003). Influences in the opposite direction may be more modest; Chourbaji et al (2004) reported that tissue content of dopamine was unchanged in the frontal cortex of BDNF heterozygous mice. These data, combined with our current results, suggest that dopamine could contribute to reduced synaptic formation and impaired spatial working memory, in part, through reductions in neurotrophin expression. Associations between lower frontal cortical BDNF protein levels and impaired working memory in Ts65Dn Down's

syndrome mice are also consistent with this idea (Bimonte-Nelson et al., 2003). However, this study also showed that effect of BDNF on working memory is maybe related to cholinergic degeneration. However, whether impaired spatial working memory in DAT KO mice is related to cholinergic degeneration need further study. Another study revealed that performance in the complex maze was better in wild-type than APP23 animals. This difference is maybe related to decreased hippocampal BDNF levels on training in APP23 animals (Hellweg et al., 2006). This brain region differs from ours (frontal cortex). However it still confirms that change of BDNF level play a role in maze behavior like our study.

Our current data found that BDNF levels were reduced by approximately 50 % in the frontal cortex of our DAT KO mice, extending results obtained in other strains of DAT KO mice (Fumagalli et al., 2003). While extracellular dopamine levels in the frontal cortex of DAT KO mice are similar to those of wild-type mice (Shen et al., 2004), their frontal cortical dopamine content is approximately 50 % of wildtype levels (Sora et al., 2001) which may indicate changes in synaptic content that may relate directly to both differences in BDNF levels and spontaneous alteration.

While finding spatial working memory deficits and frontal cortical BDNF deficits in DAT KO mice is consistent with the possibility that these two observations are linked, there is no direct evidence for such a linkage. The hippocampus, for example, expresses abundant BDNF (Li et al., 2006) and is closely tied to spatial working memory (Luine et al., 1994). Conceivably, the trend toward decreased BDNF in this region might contribute to the behavioral observations made here. It is possible that the changes in dopamine alone, by affecting working memory or some other function as discussed above, may make large contributions to the behavioral phenotype in ways that make the BDNF findings coincidental. However, it seems unlikely that the robust changes in BDNF levels that are observed here would be without behavioral consequences.

In conclusion, spontaneous alternation in the Y-maze was impaired in DAT KO mice compared to wild-type mice. Concomitant changes in the expression of BDNF protein were observed in the frontal cortex but not in the caudate putamen or hippocampus of DAT KO mice. Taken together, these observations are at least consistent with the hypothesis that impaired working memory in the Y-maze in these mice may receive contributions from the decreased frontal cortex BDNF found in DAT KO mice.

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Fig. 1. Comparison of spontaneous alternation between DAT (+/+) and DAT (-/-) mice in Y-maze test. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Spontaneous alternation but not number of total arm entries was decreased in DAT (-/-) mice compared to DAT (+/+) mice ($P<0.05$). Columns represent the mean $\pm$ S.E.M, $n=10-11$, *: $P<0.05$, unpaired T-test.

Fig. 2.

Changes of BDNF protein in DAT (+/+) and DAT (-/-) mice. BDNF level was significantly decreased in frontal cortex of DAT (-/-) mice compared to DAT (+/+) mice ($P < 0.05$).

However, there were no changes in caudate putamen and hippocampus in DAT (-/-) mice. FC, frontal cortex; CPU, caudate putamen; HP, hippocampus. Columns represent the mean $\pm$ S.E.M, $n=8$, *: $P < 0.05$, unpaired T-test.