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Research report

Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice

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ABSTRACT

It is widely accepted that brain derived neurotrophic factor (BDNF) plays a crucial role in mediating changes in learning and memory performance induced by environmental conditions. In order to ascertain whether BDNF modulates environmentally induced changes in exploratory behaviour, we examined mice carrying a deletion in one copy of the BDNF gene.

Young heterozygous male BDNF knockout mice (BDNF+/−) and their wild-type (WT) controls were exposed to the enriched environment condition (EC) or the standard condition (SC) for 8 weeks. Exploratory behaviour was assessed in the open-field (OF) and hole-board (HB) test. Brains from EC and SC reared animals were processed for Golgi-Cox staining and the dendritic spine density in the dentate gyrus (DG) and CA1 hippocampal regions were examined.

We found behavioural differences both due to the genetic modification and the environmental manipulation, with the BDNF+/− mice being more active in the OF whereas the EC mice had increased exploratory behaviour in the HB test. Environmental enrichment also led to an increase in dendritic spines in the hippocampal CA1 region and DG of the wild-type mice. This effect was also found in the enriched BDNF+/− mice, but was less pronounced.

Our findings support the critical role of BDNF in behavioural and neural plasticity associated with environmental enrichment and suggest that besides maze learning performance, BDNF dependent mechanisms are also involved in other aspects of behaviour. Here we provide additional evidence that exploratory activity is influenced by BDNF.

1. Introduction

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family which is abundantly expressed in the mammalian hippocampus and was previously shown to be important for learning and memory. BDNF binds to the TrkB receptor, [25] thus triggering a series of downstream events that regulate hippocampal long-term potentiation (LTP) [11] and gene transcription via CREB [27]. In rodents hippocampal expression of BDNF increases after testing in the Morris water maze [12] and other spatial learning tasks [28,29]. Also environmental factors such as physical exercise, dietary restriction and housing conditions have been shown to affect BDNF levels. The expression of hippocampal BDNF mRNA and protein levels is up-regulated in rats with access to running wheels permitting physical exercise [1,7,32]. Moreover exercise was shown to improve spatial learning performance in mice [21,49]. Similarly, dietary restriction which is known to extend lifespan in rodents [45] and to improve cognitive functions [18] has been shown to lead to an increased expression of BDNF in the hippocampus when compared to *ad libitum* feeding [8,22,23].

Previous studies in rats have shown that exposure to the enriched environmental condition (EC) elevates hippocampal expression of NGF and BDNF mRNA [47] as well as neurotrophin protein levels [17,36,37] which is correlated with an improved performance in learning and memory tasks [14,15,35].

While a wealth of data is compatible with the view that BDNF is critically involved in learning and memory, the role of BDNF in regulating emotional behaviour is less clear. Clinical studies suggest an involvement of BDNF in the pathophysiology of depression

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and in the mechanisms triggered by antidepressant treatments [4,20]. Both antidepressants and electroconvulsive shocks increase the expression and signalling of BDNF in the hippocampus and cortex [33,43]. A single bilateral infusion of BDNF into the rat dentate gyrus (DG) produces an antidepressant effect in two tests used as behavioural models of depression (i.e. learned helplessness and forced swim test). A comparable magnitude of effect is obtained after repeated systemic administration of a chemical antidepressant [44]. Furthermore there is evidence showing the behavioural effects typically produced by antidepressants are abolished in the absence of a normal TrkB signal in mice [43].

Conflicting results in the elevated plus maze, a test used to measure emotional behaviour, hinder the interpretation of EC effects on mouse behaviour [6,42]. We have previously shown in adult C57BL/6J mice that EC leads to decreased reactivity in the open-field (OF). In the elevated plus maze the male EC mice showed increased locomotor activity, performed more visits to the closed arms of the maze, and engaged in more risk assessment behaviours [52]. These findings were interpreted as reflecting anxiety, but they could indicate a different strategy for extracting information about the environment in the enriched male mice as previously demonstrated for golden hamsters reared in enriched conditions [46]. The aim of the current study was to further investigate which aspects of environmentally induced changes in exploratory behaviour are BDNF dependent in male mice.

2. Materials and methods

2.1. Animals

Fifty 8-week-old male mice were employed in this study. Twenty-four mice heterozygous for a targeted deletion in the BDNF gene (BDNF+/−) and twenty-six of their wild-type (WT) littermates were used as controls. The mice were obtained as previously described by Ernfors et al. [10].

2.2. Housing conditions

Two weeks after the arrival at the animal facility, at the age of 10 weeks, the mice were assigned to the experimental groups.

One group ($n = 23$) was housed in standard cages, Macrolon® type III (40.5 cm × 25.5 cm × 14.5 cm; B&K Universal, AB, Sweden)—standard condition (SC). In each cage 3–4 animals were housed.

The second group ($n = 27$) was transferred to larger enrichment cages for 8 weeks. The enriched condition (EC) consisted of housing 13/14 mice per cage, in large wire mesh cages (100 cm × 60 cm × 35 cm) containing ladders, shelves, tunnels and additional diverse toy objects. The EC mice were provided with three different objects at a time, which were changed twice a week. A particular combination of different toys was used only once during the whole enrichment period.

All the cages contained wood chip bedding and were cleaned once a week. The mice had *ad libitum* access to food (standard laboratory rodent pellets, Beekay Diets, B&K Universal, AB, Sweden) and tap water. Room temperature was maintained at 22 °C, humidity at 45–55% and a 12/12 light–dark cycle with lights on at 07:00 am was used during the experiment. All behavioural experiments were run between 09:00 and 15:00 h.

All experimental procedures used in this study followed the guidelines of the Swedish animal protection legislation and were approved by the Southern Stockholm Animal Ethical Committee.

2.3. Behavioural studies

2.3.1. Open-field test with zone monitoring

All the mice were tested in the open-field at the age of 5 months. Each mouse was placed in the center of the arena and the activity was automatically recorded for 60 min, divided in six 10 min bins. The setting allowed for 4 cage mates to be singly tested in separate arenas at the same time.

The OF consisted of a Plexiglas square arena (35 cm × 35 cm × 18 cm) with grey bottom and transparent walls covered by additional grey surrounding walls. The activity of the mice was recorded by sensitive photocells (disposed into 2 rows) connected to a computer. The interruption of infrared beams on the lower layer of photocells was translated into horizontal activity herein referred to as 'overall locomotion', whereas the interruptions detected by the upper level of photocells were recorded as 'rearing' counts. One light bulb (25 W) provided the illumination for each arena. Between trials the arena was cleaned with alcohol and then water, in order to eliminate odour cues left by the previous subject. All the animals were tested in the OF twice, at 24 h interval.

For the OF test the following parameters were analyzed: overall locomotion (count), rearing (count) and the number of fecal boli deposited during the trial period [16].

2.3.2. Hole-board exploration

The test takes advantages of the natural tendency of mice to explore small, dark spaces. The hole-board (HB) apparatus consisted of a Perspex board (40 cm × 40 cm × 27 cm; Ugo Basile 6652, Comerio, VA, Italy), with 16 round holes (3 cm diameter) placed in 4 rows of 4 on the floor of the arena. Each mouse was placed in the center of the arena and left to explore it. The mice were tested for two 9-min episodes, 24 h apart.

Four behavioural variables were recorded for this test situation.

1. *Hole-dipping (probing)*: An automatic device counted beam breaks recorded by photocells in each hole. Any probing by the mouse's snout that interrupted a beam would add a single count to the total score.
   The mouse's behaviour on the HB was also filmed by a VHS video camera from above the board, and the resulting VHS tapes were digitized for observational analysis using "The Observer 5.0" software (Noldus Information Technology).
   The analysis was confined to locomotory movement around the apparatus.
2. *Hole visits*: Movements within the boundaries of the board were logged by recording the times when the mouse moved near a specific hole from somewhere else and sniffed at it, whether or not the mouse stopped near it or passed by.
3. *Peering*: This measure was added to include periods when the mouse extended its snout beyond an edge of the board.
4. Number of fecal boli deposited during the trial period.

One observer coded all the mice using the above scheme, after having well established both interobserver and intraobserver agreement between early and late repetitions of the observer's own observations on a proportion of the mice (data not shown).

2.4. Brain morphology

An additional set of behaviourally naïve mice (12 in total) received similar environmental conditions (EC or SC) for 10 weeks. At the end of the differential housing conditions, they were deeply anaesthetized intraperitonealy with pentobarbital (Nembutal) overdose (60 mg/kg) and then decapitated, their brains were removed and cut in the midsagital direction. The left hemisphere was further cut in a coronal plane into 3 blocks of tissue and processed for Golgi-Cox staining [3,48,50].

Tissue blocks were placed in Golgi-Cox solution for 3 weeks, with one change of solution every 3 days [19,48]. After impregnation, the tissues were dehydrated, embedded in celloidin and sectioned coronally at 180 μm. To develop staining, the sections were immersed in 20% ammonium hydroxide solution for 5 min and then transferred to a 15% solution for 25 min. After rinsing they were further processed through 1% sodium thiosulfate for 7 min, dehydrated in alcohol, cleared in Histoclear (National Diagnostic) and covered with Histomount (National Diagnostic) mounting media [19,48].

2.5. Statistical analyses

Statistical analyses were conducted with StatView for Windows (version 5.01) and STATISTICA (v8, StatSoft Inc., Tulsa, OK, USA). Between groups comparisons for data with normal distribution were assessed by 2 way (genotype × housing) analysis of variance (ANOVA) with repeated measures (2 days of test). Mann–Whitney test was conducted for data not normally distributed. The results were considered significant when $p < 0.05$. All data are presented as mean ± standard error of the mean.

3. Results

3.1. Behavioural analysis

3.1.1. Open-field test

One mouse was found to be abnormally hyperactive in the open-field (horizontal beam interruptions on OF day 2 >mean + 3S.D. of the group), thus its data were excluded from the analysis.

3.1.1.1. Locomotion (Fig. 1a and b). Significant main effects of genotype and housing on overall locomotion counts for 2 days were revealed. The BDNF+/− mice had significantly higher locomotor activity than the WT mice ($F(1, 45) = 10.5$, $p < 0.01$), and the EC mice were less active ($F(1, 45) = 6.8$, $p < 0.05$) than the SC mice. There was