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Research report

Neurotrophin levels and behaviour in BALB/c mice: Impact of intermittent exposure to individual housing and wheel running

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Abstract

This study assessed the effects of intermittent individual housing on behaviour and brain neurotrophins, and whether physical exercise could influence alternate individual-housing-induced effects. Five-week-old BALB/c mice were either housed in enhanced social (E) or standard social (S) housing conditions for 2 weeks. Thereafter they were divided into six groups and for 6 weeks remained in the following experimental conditions: Control groups remained in their respective housing conditions (E-control, S-control); enhanced individual (E-individual) and standard individual (S-individual) groups were exposed every other day to individual cages without running-wheels; enhanced running-wheel (E-wheel) and standard running-wheel (S-wheel) groups were put on alternate days in individual running-wheel cages. Animals were assessed for activity in an automated individual cage system (LABORAS) and brain neurotrophins analysed. Intermittent individual housing increased behavioural activity and reduced nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) levels in frontal cortex; while it increased BDNF level in the amygdala and BDNF protein and mRNA in hippocampus. Besides normalizing motor activity and regulating BDNF and NGF levels in hippocampus, amygdala and cerebellum, physical exercise did not attenuate reduction of cortical NGF and BDNF induced by intermittent individual housing. This study demonstrates that alternate individual housing has significant impact on behaviour and brain neurotrophin levels in mice, which can be partially altered by voluntary physical exercise. Our results also suggest that some changes in neurotrophin levels induced by intermittent individual housing are not similar to those caused by continuous individual housing.

Keywords: Intermittent individual housing; Social housing; Environmental enrichment; Running-wheel; Mice; BDNF; NGF

1. Introduction

Long-term exposure to individual housing in rodents is frequently used to analyse particular aspects of psychiatric disorders, like schizophrenia [20,58] and depression [13,29]. Our previous studies in rats have shown that social isolation in the form of non-physical contact with other conspecifics can induce changes on behaviour and brain neurotrophic factors [27,43]. Other studies report that long lasting social isolation affects a number of physiological variables, including plasma corticosterone concentration [9], brain corticotrophin releasing hormone [47] and brain opiate systems [28]. In spite of these findings, little is known about the impact of intermittent exposure to individual housing on behaviour and neurobiology. It is important to define terms as (social) isolation and individual housing in order to clarify what is meant. Social isolation implies animals are isolated from their conspecifics in terms of visual, auditory, olfactory and tactile stimuli. Individual housing refers to the animals being singly housed and still be able to see, smell and/or hear other animals.

Brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are two members of the neurotrophin family known to be important for survival and maintenance of function of neurons in the central and peripheral nervous systems [34,49]. These neurotrophins are abundantly expressed in hippocampus [14], where they are critically involved in spatial learning [16,25,37,45] and activity-dependent synaptic plasticity, such as

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long-term potentiation (LTP) [15,21,30,33,35]. BDNF and NGF participate in psychological and physical stress-induced effects in human and rodents (see review [1]) and are reported to regulate emotional behaviours [2]. For example, BDNF-deficient mice develop enhanced inter-male aggressiveness [36], and abnormal neurotrophin levels are observed in psychiatric and neurodegenerative conditions, such as Alzheimer's disease (AD), bipolar disorders, depression and schizophrenia [19,31,48].

Different environmental factors can induce alterations in gene expression and protein levels of hippocampal BDNF and NGF [7,11,38]. For example, rodents with access to running-wheel have elevated levels of BDNF mRNA and protein in hippocampus [7,11,17,40,56]. Exercise is beneficial for the cardiovascular system, and improves the stress response mechanism, and can even be beneficial in the treatment of depression in both human and animal model of depression [4,7]. In addition to its effects on neurotrophins, wheel-running has other neural and behavioural effects in rodents, such as to increase the number of neurons in hippocampus [26,53], enhance LTP [12], promote learning and memory [52], influence recovery of function following brain damage (for review see [46,57]), and change the neurochemistry in brain reward pathways in a similar way as addictive drugs [54,55]. That wheel running could alter the neurochemistry in brain regions involved in learning, memory, motivational drive and addiction illustrates the complexity in the responses of wheel running, and calls for a better understanding of the use of running-wheel as an enrichment device for the animals.

Female mice are more socially interactive than males indicating that female mice could be more sensitive to social instability, such as alternate individual housing. Also the anxious BALB/c mouse strain is susceptible to environmental manipulation [3,44,51]. Thus, in the present study female BALB/c mice were used and were assigned to two types of social housing, i.e. enhanced social (E) or standard social (S) housing, which were determined by presence of absence of nesting and shelter materials. We hypothesized that the effects of intermittent exposure to individual housing with or without running-wheel on E and S mice would show different patterns of changes in behaviour and brain neurotrophin levels.

With regard to the environmental conditions, it should be pointed out that terms as "environmental enrichment" and "environmental enhancement" are not synonymous. "Environmental enrichment" is often used in the field of neuroscience, to refer mainly to social housing in a large, complex cage comprising different toys that are changed frequently in order to induce changes in the brain and behaviour. "Environmental enhancement", on the other hand, focuses on specific needs of the animals such as nest building, hiding and gnawing in order to improve the well being of the animals.

2. Materials and methods

2.1. Animals

Forty-eight female BALB/c/BKI mice (B&K Universal, AB, Sweden) at 5 weeks of age at start of the experiment were used.

2.2. Housing conditions

After arrival, the mice were randomly assigned to four mice per cage over 12 cages in standard transparent elongated (40.5 cm × 25.5 cm × 14.5 cm) Macrolon® type III (B&K Universal, AB, Sweden). 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 h. Cages were cleaned once a week. One group of mice ($n = 24$) was housed under standard social condition (S), whereas another group ($n = 24$) was housed under enhanced social condition (E). S consisted of standard social housing in Macrolon® type III cage with 50 ml of wood chips bedding. Under E-condition, the animals were provided with a Shepherd Shack (egg-box carton 15 cm × 9 cm × 6 cm, Shepherd Specialty Papers, Kalamazoo, MI, USA), two Kleenex® tissues (Kimberly Clark Corp., Sweden) and two gnawing sticks were provided (aspen wood, 1 cm × 1 cm × 5 cm, Finn Tapvei, Finland), which were renewed as necessary (approximately once per week).

After 2 weeks of acclimatization to the animal facility in either S- or E-housing conditions, at the age of 7 weeks, the mice were randomly assigned to six different experimental groups with eight mice per group as follow.

2.2.1. Experimental groups

- E-condition in home cage (E-control).
- S-condition in home cage (S-control).
- E and alternate days in individual housing (E-individual).
- S-condition and alternate days in individual housing (S-individual).
- E-condition and alternate day access to running-wheel cage (E-wheel).
- S-condition and alternate day access to running-wheel cage (S-wheel).

2.2.2. Procedures

Fig. 1 illustrates the experimental design.

Individual-housing condition was set in the Macrolon® type II cage (25.5 cm × 19.5 cm × 13.5 cm). The E- and S-individual mice were exposed on alternate days to individual cages for 24 h. Running-wheel cage condition consisted of a wheel (diameter of 12.4 cm) in same size Macrolon® type II cage. The E- and S-wheel mice were housed in individual cages with running-wheels on alternate days for 24 h. The running-wheel and individual-housing groups returned every other day to their respective E- or S-conditions. The control groups (S- and E-control) remained in E- or S-conditions with their cage mates throughout the study (for 6 weeks).

2.3. Behavioural parameters

A behavioural profile of the mice was assessed in the automated behaviour registration system LABORAS (Laboratory Animal Behaviour Observation, Registration and Analysis System, Metris, The Netherlands) when mice were at 13 weeks of age. With this system, the following behaviours can be assessed: locomotion, distance travelled and velocity, grooming, climbing immobility, drinking and eating. In this study we focused on locomotor activity.

Four mice from different groups were tested simultaneously on four sensing platforms for 30 min. Each mouse was placed individually in a clean Macrolon® type II cage with the wood chips bedding, food and water as in their home cages. Each cage was put on a sensing platform. The animals' movements are transformed via the sensing platforms into electrical signals. Each movement has its own unique frequency, amplitude and pattern, which can be separated into behavioural categories and automatically registered by a computer. Movement signals not recognized by the system are registered as 'undefined' behaviour.

2.4. Sample preparation and protein assay

Two days after the completion of behavioural test, the animals were killed by decapitation. The brains were extracted and separated in two hemispheres. The left hemispheres were dissected for the following brain regions: frontal cortex, amygdala, hippocampus and cerebellum, and the dissected brain tissues were stored at −70 °C until time of neurotrophins protein analyses. The right hemi-

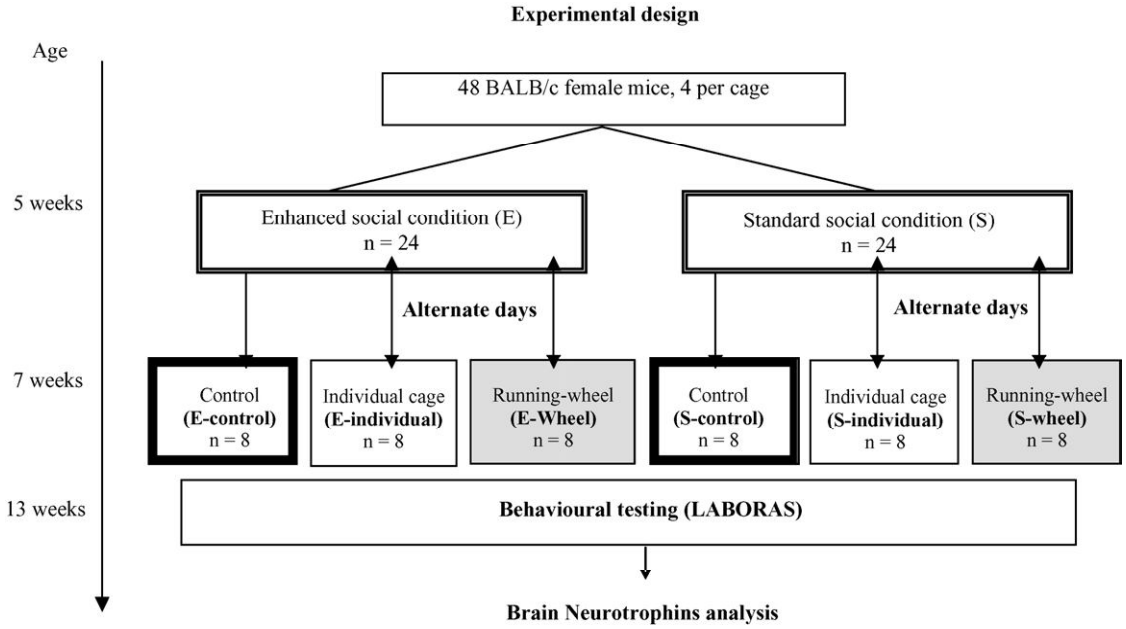

Fig. 1. Schematic representation of the experimental design.

spheres were immediately frozen at −70 °C until time of in situ hybridization study.

Brain tissue samples were homogenized in ice-cold lysis buffer, containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF 10 μg/ml aprotinin, 1 μg/ml leupetin, 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged with 14 000 × *g* for 5 min at 4 °C. The supernatants were collected and used for quantification of total protein and neurotrophin levels.

The total protein concentration of supernatants was measured by using the BCA Protein Assay Reagent kit (Peirce, Sweden).

2.5. Enzyme immunoassay for BDNF and NGF

NGF and BDNF levels were assessed in the selected brain regions using ELISA assay kits (Promega, Sweden). Briefly, Standard 96-well flat-bottom NUNC-Immuno maxisorp ELISA plates were incubated with the corresponding captured antibody, which binds the neurotrophin of interest, overnight at 4 °C. The next day the plates were blocked by incubation for 1 h at room temperature (RT) with a 1× Block and Sample buffer. Serial dilutions of known amount of NGF and BDNF ranging from 0 to 500 pg/ml were performed in duplicate for the standard curve. Wells containing the standard curves and supernatants of brain tissue homogenates were incubated at RM for 6 or 2 h, as specified by the protocol. They were then incubated with second specific antibody overnight at 4 °C or for 2 h at RT, as specified by the protocol. A species-specific antibody conjugated to horseradish peroxidase (HRP) as a tertiary reactant for 2.5 or 1 h at RT followed this incubation step. A TMB One Solution was used to develop color in the wells. This reaction was terminated with 1N hydrochloric acid at a specific time (10–15 min) at RT, and the absorbance was then recorded at 450 nm in a plate reader within 30 min of stopping the reaction. The neurotrophin values were evaluated by comparison with the regression line for each proposed neurotrophin standard. Using these kits, NGF and BDNF can be quantified in the range of 7.8–500 pg/ml. For each assay kit, the cross-reactivity with other trophic proteins is ≤2–3%.

2.5.1. In situ hybridization for BDNF mRNA

Coronal brain sections (30 μm) were cut on a cryostat at −20 °C and thawed on glass slides. The hybridization cocktail contained 50% formamide, 4× SSC (1× SSC is, in M, NaCl, 0.15; sodium citrate, 0.015, pH 7.0), 1× Denhardt's solution, 1% Sarcosyl, 0.02 M Na_3PO_4, pH 7.0, 10% dextransulphate, 0.06 M dithiothreitol and 0.1 mg/ml sheared salmon sperm DNA. Single-stranded oligonucleotide 48-mer DNA probes specific for BDNF (250–298) [34] mRNA were used. The probes were 3′-end labeled with α-^{33}P-dATP (Dupont NEN, Wilmington, DE) using terminal deoxynucleotidyl transferase (Gibco) to a specific activity of approximately 1×10^9 cpm/mg. Hybridization was performed for 18 h in a humidified chamber at 42 °C. Following hybridization, the sections were rinsed 4 × 20 min each in 1× SSC at 60 °C. Finally, the sections were rinsed in autoclaved water for 10 s, dehydrated in alcohol and air-dried. Thereafter, the slides were exposed to film (Kodak Biomax MR Film, Kodak, Rochester, NY) for 4 days and developed. Films were scanned and optical density values quantified using appropriate software (NIH image analysis program, version 1.62). A ^{14}C step standard (Amersham, Buckinghamshire, UK) was included to calibrate optical density readings and convert measured values into nCi/g.

2.6. Statistical analyses

Statistic analyses were conducted with StatView for Windows (version 5.01). The behaviour data and the levels of NGF and BDNF were analysed by analysis of variance (ANOVA) using 2 × 3 (housing × treatment) randomized block design. Separate ANOVA on the two housing conditions was also conducted. Statistical significance was set at a probability level of $p < 0.05$ for all tests. Fisher's post hoc test was used when ANOVA showed significance.

All experimental procedures in this study were approved by the Northern Stockholm Animal Ethics Committee.

3. Results

3.1. Behavioural analysis

Results of behavioural analysis are shown in Fig. 2.

3.1.1. Distance moved (Fig. 2A)

ANOVA (housing × treatment) revealed significant effects of housing ($F(1, 42) = 5.582$, $p < 0.05$) and treatment ($F(2, 42) = 71.681$, $p < 0.0001$) on total distance moved. There was an interaction effect ($F(2, 42) = 21.694$, $p < 0.0001$) between housing and treatment. Fisher's post hoc test showed that mice exposed intermittently to individual housing (both E- and S-

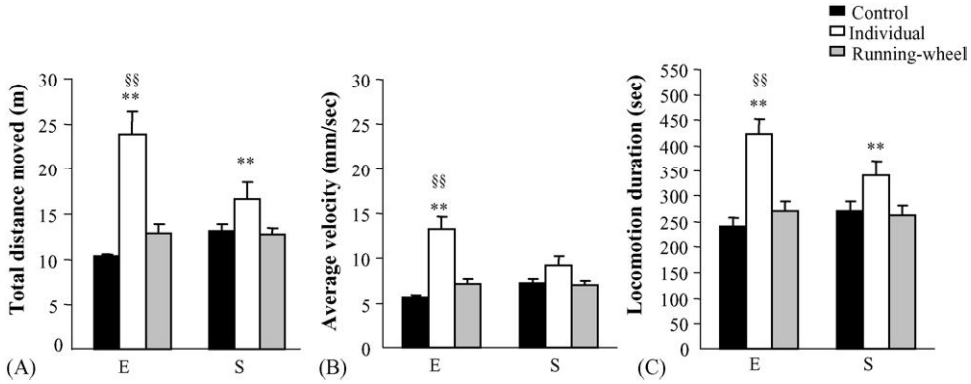

Fig. 2. The effects of housing conditions (E and S) on intermittent exposure to individual and wheel running in BALB/c mice. Behaviours were recorded by LABORAS during 30 min observation. Data is presented as group mean ± S.E.M. on each behavioural category. Black bars show data of the control in home cage (E/S-control $n = 8$); white bars show the data of exposure to individual cage without running-wheels (E/S-individual, $n = 8$); grey bars show results of exposure to individual cages with running-wheels (E/S-wheel $n = 8$). (A) Total distance moved, (B) average speed of moving and (C) total locomotion. $^{**}p < 0.01$ compared with control and running-wheel groups; $^{§§}p < 0.01$ compared with S-individual group.

individual) moved significantly more than mice in control and running-wheel groups. E-individual animals travelled longer distances than S-individual animals.

3.1.2. Velocity (Fig. 2B)

There were also significant housing ($F(1, 42) = 5.59$, $p < 0.05$), treatment ($F(2, 42) = 71.61$, $p < 0.001$) and their interaction ($F(2, 42) = 21.69$, $p < 0.0001$) effects on average velocity. Post hoc test showed that mice exposed to individual housing had significantly higher velocity than mice in control and running-wheel groups. E-individual mice moved faster than S-individual mice.

3.1.3. Locomotion (Fig. 2C)

The locomotion pattern was similar to that of distance moved. There were significant effect of housing ($F(1, 42) = 3.78$, $p < 0.05$), treatment ($F(2, 42) = 60.50$ $p < 0.0001$) and their interaction ($F(2, 42) = 10.13$, $p < 0.0001$) in time spent on locomotion. Post hoc test showed mice intermittently exposed to individual housing (E- and S-individual) spent significantly more time on locomotion than mice in control and running-wheel groups. This was true for both the E- and S-conditions. E-individual animals spent longer time on locomotion than S-individual animals.

3.2. Neurochemical analyses

3.2.1. NGF protein levels (Fig. 3A and B)

There were significant housing ($F(1, 41) = 16.25$, $p < 0.01$) and treatment ($F(2, 41) = 4.70$, $p < 0.01$) effects on hippocampal NGF level (Fig. 3A). Separate ANOVA and Fisher's post hoc test showed that running-wheel groups in both E- and S-conditions had significantly higher NGF levels than the individual and control groups.

There were also significant housing ($F(1, 41) = 5.55$, $p < 0.01$) and treatment ($F(2, 41) = 20.658$, $p < 0.001$) effects on NGF level in the frontal cortex (Fig. 3B). In both E- and S-conditions control animals had significantly higher NGF levels in frontal cortex than mice exposed to individual housing and running-wheel.

S groups showed a statistically non-significant trend to an effect of housing on NGF levels in cerebellum ($F(1, 41) = 4.034$, $p = 0.0512$).

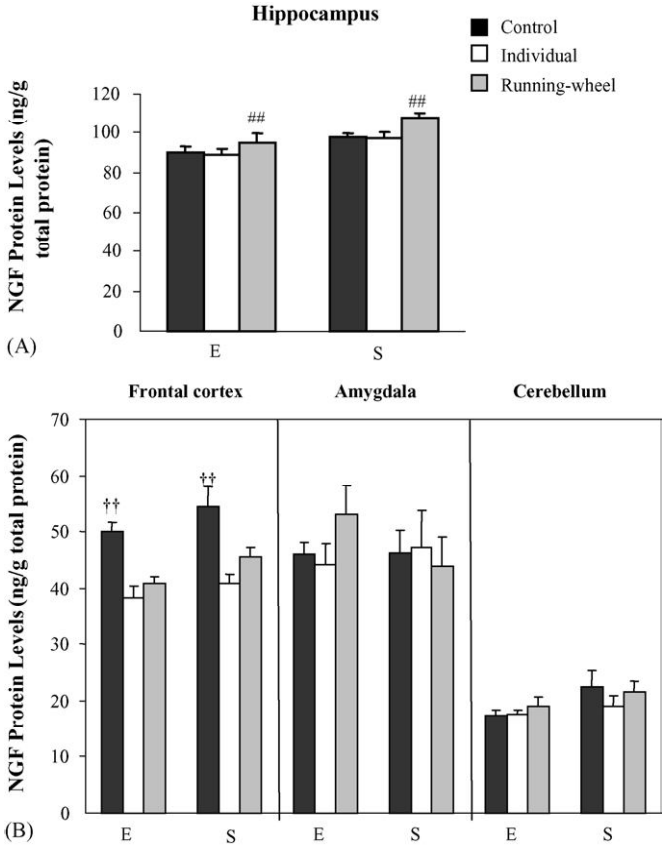

Fig. 3. NGF protein levels in selected brain regions measured with ELISA. The BALA/c mice were housed in two social conditions (E and S) and were intermittently exposed to control, individual or running-wheel treatment ($n = 7$ S-control, $n = 8$ per other groups). Results are presented as group mean ± S.E.M. $^{##}p < 0.01$, compared with control and individual-housed mice; $^{††}p < 0.01$, compared with individual and running-wheels groups.

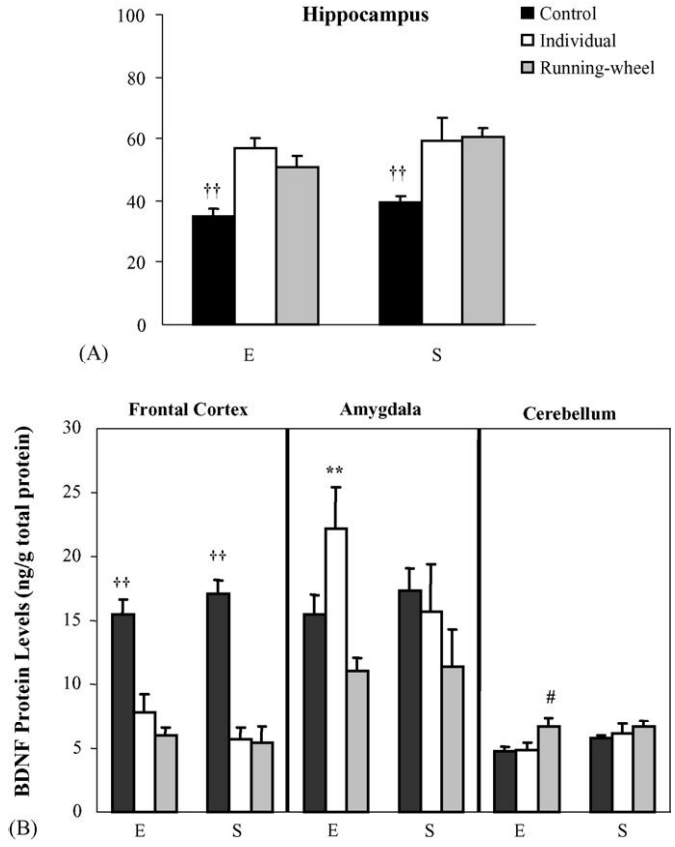

Fig. 4. BDNF protein levels in selected brain regions measured with ELISA. The BALA/c mice were housed in two social conditions (E and S) and were intermittently exposed to control, individual or running-wheel treatment ($n = 7$ S-control, $n = 8$ per other groups). Results are presented as group mean ± S.E.M. $^{\dagger\dagger}p < 0.01$, compared with individual and running-wheels groups; $^{**}p < 0.01$, compared with control and running-wheel groups; $^{\#}p < 0.05$, compared with control and individual groups.

3.2.2. BDNF protein levels (Fig. 4A and B)

There was a significant treatment effect on BDNF level in hippocampus ($F(2, 41) = 15.23$, $p < 0.01$). Fisher's post hoc test showed that mice intermittently exposed to running-wheels and individual cage had significantly higher levels of BDNF in hippocampus than control mice remaining in their home cages (Fig. 4A). This was true in both E- and S-conditions.

There were significant treatment differences of BDNF levels in frontal cortex ($F(2, 41) = 60.865$, $p < 0.001$) in amygdala ($F(2, 41) = 4.777$, $p < 0.01$) and in cerebellum ($F(1, 41) = 3.83$, $p < 0.05$). Fischer's post hoc test showed that E- and S-control groups had significantly higher BDNF levels in the frontal cortex than the individual (E- and S-individual) and running-wheel (E- and S-wheel) groups (Fig. 4B). Post hoc test further revealed that E mice exposed to individual-housing condition had significantly higher BDNF levels in the amygdala than the other groups. Fischer's post hoc test also showed that in E-condition, wheel-running group had significantly higher cerebellar BDNF levels than the other groups.

Taken together, the neurotrophin results showed: NGF levels in selected brain regions were influenced by both housing condition (E, S) and subsequent treatment (control, wheel, individual), whereas BDNF levels were mainly affected by treatment. No interaction between housing and treatment effect was found, implying that the factors of housing and treatment are independently affecting changes of neurotrophin protein levels.

3.2.3. BDNF mRNA levels (Fig. 5A and B)

There were significant treatment ($F(1, 38) = 7.73$ $p < 0.01$) and interaction effect between housing and treatment ($F(2, 38) = 3.41$, $p < 0.05$) on BDNF mRNA levels in region of dentate gyrus (DG). Individual and running-wheel groups showed significantly higher levels of BDNF mRNA in DG as compared to control groups. This was in agreement with our finding of BDNF protein levels in hippocampus (Fig. 4A). Separate ANOVA and Fisher's post hoc test showed that mice intermittently exposed to individual housing (E-individual) had significantly higher level of BDNF mRNA compared to control and running-wheel groups in E-housing condition. Fig. 5B illustrates that the E-individual group had greatest density of BDNF mRNA than E-control and E-wheels groups, whereas E-control showed lowest density of BDNF mRNA than other the two groups.

No significant housing or treatment effect was found in CA1-3, hilus and amygdala.

4. Discussion

We have recently investigated the influence of alternate access to running-wheel in enhanced and standard social housed BALB/c mice, and found that alternate individual housing caused changes in behavioural reactivity, and these changes could be normalized by providing running-wheel in the individual cages [42]. In the present study, we further examined the effects of intermittent individual housing on behaviour and neurotrophins in the brain; investigated how physical exercise influences intermittent individual-housing-induced effects.

The behavioural test results in this study clearly showed that intermittent individual housing had a strong impact on motor activity in BALB/c mice. Regardless of the housing condition (E or S), both E- and S-individual groups demonstrated significantly increased motor activity as compared to the control groups maintained in stable social conditions and the groups exposed to intermittent individual housing with running-wheels (Fig. 2A–C). Moreover, the increased activity was more pronounced in E-individual mice than in S-individual mice, as shown by total distance travelled, velocity and locomotion duration, indicating that E-individual animals might be more alert or more aroused, possibly due to the more pronounced impact of the change in housing conditions (from E to individual-housing) on the E animals than on the S animals. This phenomenon could be counteracted, however, by providing a running-wheel. This is consistent with our previous finding [42], which showed that wheel running could counteract some behavioural effects such as hyperactivity induced by individual housing. Long-term social isolation causes increased locomotion in an open field situation [39], which could reflect increased reactivity and anxiety. Our results showed that intermittent individual housing could induce similar effects as long-term social isolation does, and physical exercise can counteract the behavioural changes induced by individual housing.

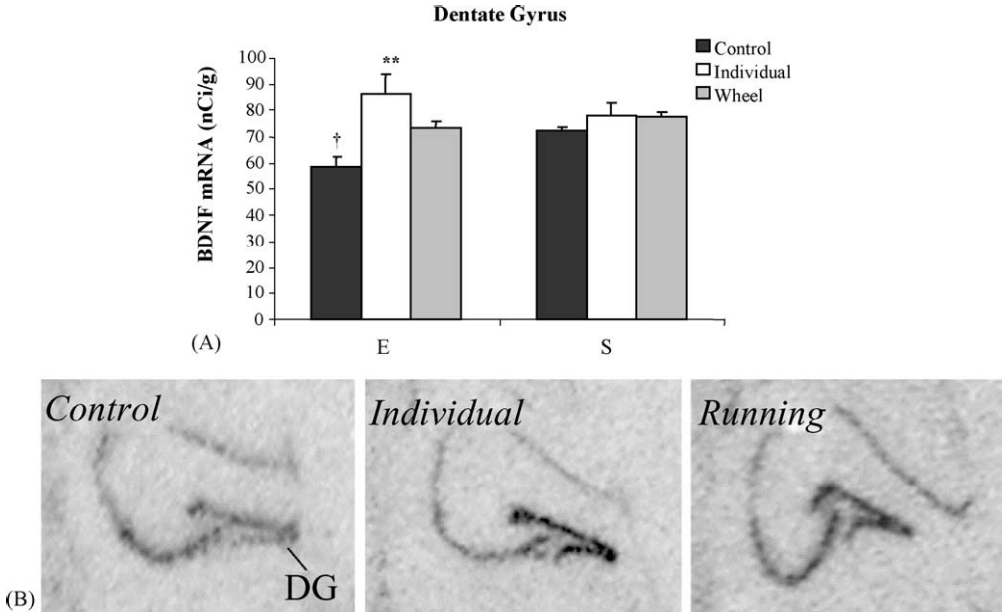

Fig. 5. (A) BDNF mRNA levels in hippocampal dentate gyrus measured with in situ hybridization. The BALA/c mice were housed in two social conditions (E and S) and were intermittently exposed to control, individual or running-wheel treatment ($n = 7$ S-control, $n = 8$ per group in other groups). Results are presented as group mean ± S.E.M. Note under E-condition, $^{\dagger}p < 0.05$, compared with individual and running-wheels groups; $^{**}p < 0.01$, compared with control and running-wheel groups. (B) BDNF mRNA expression in hippocampus of mice under enhanced condition and intermittently exposed to control, individual or running-wheel treatment. Note the differences in BDNF mRNA levels in the dentate gyrus, where the strongest expression is found in the individual group and the least expression is found in the control group.

In the present study, we showed that changing environmental conditions had great impact on neurotrophin protein and mRNA levels in the frontal cortex and hippocampus. The groups that were on intermittent individual housings with or without running-wheel had lower protein levels of NGF and BDNF (Figs. 3B and 4B) in the frontal cortex as compared to control animals, which remained group housed in their home cages. This effect is comparable to that reported by Ickes et al. [27], who showed a similar decrease in brain neurotrophins levels following long-term individual housing in rats. It further demonstrates that intermittent individual-housing experience can also cause neurotrophin down regulations in the frontal cortex, a brain region found to play a key role in locomotor responses in an unfamiliar environment [6]. It is possible that the observed locomotor changes of E- and S-individual mice (Fig. 2A–C) may be a consequence of altered neurotrophin levels in this critical brain region. Moreover, voluntary exercise could not attenuate the neurochemical changes in this region, although it could counteract increased motor activity and affect neurotrophin levels in other brain regions like amygdala.

While in an earlier study we observed a decrease in brain neurotrophin levels following long-term individual housing [27], in the present study we found that mice exposed to intermittent individual housing with or without running-wheel had increased hippocampal BDNF protein and mRNA levels as compared to control mice (Figs. 4A and 5A and B). Moreover, in line with the increased levels of BDNF in hippocampus there is also an increased cell proliferation in the sub granular zone of the dentate gyrus in the groups of animals that were subjected to intermittent individual housing (unpublished findings). Thus, together these findings indicate a possible increased neuronal activity in hippocampus, which may be elicited by frequent alteration in housing conditions. Unlike in long-term social isolation, animals exposed to intermittent social deprivation had the chance to interact with their cage-mates during certain periods. Consequently a neurobiological compensatory pathway might be provoked by the intermittent social and physical contacts, resulting in enhanced neurotrophin secretion. Compared with other brain regions, the hippocampus is known to be a sensitive region and responsive to a variety of internal and external stimuli [32]. Interestingly, the effect on BDNF upregulation was specifically found in the hippocampus.

BDNF is a neurotrophic factor potentially involved in stress responses and antidepressant-induced effects on the brain (for review see [24]). We show that withdrawal from physical and social contact-induced changes in behaviour, which are associated with regulation of BDNF protein levels in amygdala. This could support the notion that BDNF has a role in this region in modulating emotionality. Furthermore, mice intermittently exposed to individual cages also had higher levels of BDNF protein and mRNA in the hippocampus (Figs. 4A and 5). In addition to its involvement in learning and memory the hippocampus is also involved in anxiety-related behaviour [2,5,23]. This would be in agreement with our recent findings [59] which showed that regional contents of specific neurotrophins correlated with anxiety-like behaviour in mice, and hippocampal BDNF protein level correlated positively with anxiety level. Our findings of elevated BDNF levels in hippocampus and amygdala in individually housed animals in conjunction with the increased activity shown in the behavioural tests would be an indication of increased emotional reactivity. As our results showed, when individual housing was combined with running-wheel,

their motor activity levels were similar to those of control animal, and amygdala BDNF levels were not elevated (Figs. 2 and 4B).

In the present study, higher BDNF levels were detected in hippocampus and cerebellum of running-wheel groups as compared to control groups (Fig. 4A and B). The involvement of cerebellum in regulating motor activity and learning is well established (for review see [8]), and our finding of increased BDNF affecting runners suggests a possible involvement of cerebellar neurotrophins in motor learning. We also found that the running-wheel groups had higher NGF and BDNF levels in hippocampus as compared to control groups (Figs. 3A and 4A). The relocation from the home cages to individual cages with running-wheels together with voluntary exercise induced higher hippocampal levels of NGF and BDNF in the E- and S-wheel mice.

The different housing conditions revealed limited effects on behaviour and brain neurotrophins compared to other studies [38,43], where environmental enrichment increased NGF and BDNF levels in the hippocampus. However, our enhanced housing condition differed considerably in terms of, e.g. cage size and novel objects, compared with the environmental enrichment in these studies.

Variations in levels of brain neurotrophins have been related to a number of behaviours. For example, neurotrophin levels in hippocampus have been related to learning [45] and emotion [24]; in hypothalamus to aggressive behaviours [18]; in the striatum to stereotypic behaviour [50]; and in amygdala to playfulness in juvenile animals [22]. While higher levels of neurotrophins in hippocampus have in many cases been found to be beneficial, it does not imply that higher neurotrophin levels in other brain regions are always indicative of salutary effects. For example, higher NGF levels can result in induction of abnormal neuronal connectivity with resultant cognitive impairment [10]. Indeed abnormal high levels of BDNF have been linked to the psychiatric conditions, as schizophrenia [48] and autism [41]. A requirement for normal behavioural function could therefore be optimal levels of neurotrophins in some critical brain regions. These optimal levels might be disrupted by changing the environment, especially from a social environment to individual housing, with resultant behavioural consequences, such as increased activity and arousal. However, voluntary exercise could partially modify these alterations.

Acknowledgements

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