Journal of Developmental Origins of Health and Disease

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Amygdala – and serum – neurotrophic factor levels depend on rearing condition in male rats

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Brief Report

Cite this article: Babri S, Mohaddes G, Mosaferi B. (2018) Amygdala – and serum – neurotrophic factor levels depend on rearing condition in male rats. *Journal of Developmental Origins of Health and Disease* **9**: 377–380. doi: 10.1017/S2040174418000144

Received: 27 June 2017
Revised: 21 December 2017
Accepted: 20 February 2018
First published online: 27 March 2018

Key words:
amygdala; brain-derived neurotrophic factor; early life experiences; environmental enrichment; psychiatric disorders

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Abstract

Early life experiences could determine brain and behavioral development. Neurotrophic factors are likely to mediate the effects of the experience on brain structures and function. Brain-derived neurotrophic factor (BDNF) plays a central role in psychiatric disorders. To investigate the effects of early rearing condition on the amygdala – and serum – BDNF levels, we reared male Wistar rats from weaning (postnatal days 21) to adulthood (postnatal days 119) in three different rearing conditions: (1) enriched, (2) standard and (3) isolated. We found that long-term post-weaning environmental enrichment leads to lower amygdala – and serum – BDNF levels as well as lower brain weights. Grouped rearing in standard laboratory cages enhanced body weight. Thus, early rearing condition might play a crucial role in adult healthiness by predetermining individual BDNF profiles.

Introduction

Early life experiences could affect brain and behavioral development.[1] An impoverished/stressful environmental condition during early life periods could predispose to myriad dysfunctions, such as psychopathologies.[2] In experimental studies, the environment is enriched in order to provide positive experiences and improve the animals' quality of life.[3] Neurotrophic factors are likely to mediate the effects of the experience on brain structures and function.[4] Brain-derived neurotrophic factor (BDNF) is the most widespread neurotrophic factors in the mammalian central nervous system[5] that is produced in a neuronal activity-dependent manner and protects neural connections.[6] BDNF has a central role in psychiatric disorders.[7]

Amygdala is associated with several neurodevelopmental disorders.[8] The amygdala in vulnerable animals shows stronger BDNF responses to stressful condition.[9] The effect of the early experiences on the amygdala basal BDNF levels are rarely investigated. In a recent study, post-weaning environmental enrichment enhanced central amygdala BDNF levels of trait anxiety rats.[10]

Serum BDNF levels are considered as a possible biomarker for brain health[11] and psychiatric disorders.[12] In clinical depression, serum BDNF levels show a positive association with improved quality of life, and successful antidepressant treatment raises serum BDNF.[13] Moreover, physical activity increases messenger RNA for BDFN in rat brain.[14] We have recently demonstrated a stronger serum BDNF responses following inescapable foot shocks in enriched rats, 14 weeks after cessation of manipulation period.[9] Just after manipulation period, the effect of rearing condition on the serum BDNF levels has not yet been investigated.

Here, we have investigated whether differential rearing condition from weaning to adulthood alters amygdala – and serum – BDNF levels in male rats.

Methods

Animals

In total, 32 male Wistar rats from six different litters obtained from our own laboratory breeding colony were weaned at postnatal days 21 and semi-randomly assigned to one of three rearing conditions: (1) isolated condition (IC), (2) standard condition (SC) in laboratory cages and (3) enriched condition (EC). It is noteworthy that post-weaning period in rodents is relevant to human childhood which is emphasized more than infancy in the literature.[15]

The differential rearing condition was the same as our previous study.[16] Briefly, IC composed of a singly housed rat in a plastic translucent small cage ($13.5 \times 22.5 \times 16.5$ cm). Animals of SC were kept in plastic translucent standard laboratory cage ($20 \times 45 \times 30$ cm) under group housing (four rats per cage). Animals in the EC lived in a large cage ($88 \times 82 \times 63$ cm) in a group of five (see environmental enrichment). They were maintained in a noise-isolated, air-conditioned animal room with constant temperature (22 ± 2°C) under a

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regular 12 h light/dark cycle (lights on at 0700 h) until the end of the experiment (young adulthood: postnatal days 119). Bedding was changed once weekly for all animals. IC rats were kept in the upper slides of the racks, and they received minimal contact. The minimal number of animals were used and particular care was taken to reduce their suffering. The protocol and procedures of this work were approved by the Regional Ethics Committee of Tabriz University of Medical Sciences (No. 91/2-2/3) and are in accordance with the guide for the care and use of laboratory animals of the National Institute of Health (NIH; Publication No. 85-23, revised 1985).

Environmental enrichment

The procedure for environmental enrichment was as we have previously described.[16] Briefly, the EC cages were equipped with two running wheels, two food dispensers and two water bottles, and were enriched with a variety of toys (Fig. 1). The internal configuration of the cages was changed every week; creating different spaces with several types of stairs and plastic polyvinyl chloride pipes that the rats could move into or climb over. Novel objects made of hardly chewable plastic including balls, rings and a block of plate with predrilled holes as well as easily chewable objects such as ropes and paper nestles were provided and changed weekly. All enriched cages were receiving the same assortment of objects each time.

Tissue sampling and organ weights

At the end of the experiment, the rats (eight per group) were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.) between 8 am and 2 pm. The body weight of the offspring was measured. They were promptly decapitated. Trunk blood were immediately collected into anticoagulant-free sampling tubes. Brains were extracted excluding the olfactory bulb, and excluding parts of the brainstem posterior to the cerebellum. Brain wet weights was measured. The right whole amygdala were dissected free, using rat brain atlas of Paxinos and Watson, on an ice-cold petri dish within 3 min. The amygdala samples were stored at −70°C until used. The blood in sampling tubes were kept at room temperature to coagulate for 1 h[17] before centrifugation at 3000 rpm during 10 min at 4°C. The extracted serum was, then, divided into aliquots and stored at −70°C until the time of BDNF measurements.

Fig. 1. Big cage with a variety of toys were used to enrich the environment.

BDNF measurement

BDNF levels were determined with a commercial sandwich ELISA kit (ChemiKine, Catalogue No. CYT306; MERCK MILLIPORE, Germany). ChemiKine BDNF kit, according to the manufacture, is designed to measure the amount of BDNF in cell culture supernatants, tissue homogenates and biological fluid (serum, plasma and serum-free) samples from human and rat. The amygdala samples were homogenized in ice-cold homogenate buffer solution suggested by the manufacturer. Then, the amygdala and serum samples were separately diluted (1/10 v/v) with the ice-cold homogenate buffer solution. The homogenate buffer consisted of 100 mmol/l Tris/HCl, pH 7, 1 mol/l NaCl, 2% bovine serum albumin, 4 mmol/l EDTA-Na^2, 0.1% NaN_3, 2% Triton X-100 and the protease inhibitors (Sigma) 0.157 mg/ml benzamidine, 5 mg/ml aprotinin, 0.5 mg/ml antipain, 0.1 mg/ml pepstatin in 10% acetic acid in methanol and 17 mg/ml phenylmethyl-sulphonyl fluoride in 100% ethanol. Samples were centrifuged (14,000 g for 30 min). The diluted amygdala homogenate and serum samples were again diluted (1/10 and 1/2 v/v, respectively) directly on the plate with sample diluent solution provided by the kit. A standard curve (0–500 pg/ml) was prepared with standard BDNF and sample diluent solution. The rest of the procedure was performed according to the manufacturer's claim instructions. Optical density in each plate was measured at 450 nm using an ELISA plate reader (Stat fax 2100, Awareness, USA). According to the manufacturer's claim, the sensitivity of the assay was 7.8 pg/ml of BDNF, and the cross-reactivity with other related neurotrophic factors (nerve growth factor, neurotrophin-4/5 or neurotrophin-3) was nil. All samples were measured in the same assay, and were run in duplicate form.

Data analysis and statistics

Results are expressed as means ± S.E.M. Between group differences were analyzed using one-way analysis of variance (ANOVA). For statistically significant comparisons, *post-hoc* analyses were performed using least significance difference test, only where explained. A linear regression model was also used to test for any predictive effects of the amygdala BDNF levels on the serum BDNF levels. Body weight was investigated as a covariate in brain weight with a univariate analysis of the effect of environmental condition with one-way model analysis of covariance (ANCOVA), using rearing condition as random factor, and body mass as a covariate. In this case, the Bonferoni *post-hoc* test was used. Statistical significance is reported at the α level of 0.05.

Results

BDNF levels

Post-weaning rearing condition influenced BDNF levels of the amygdala [$F(2, 19) = 3.56$, $P = 0.04$] and serum [$F(2, 19) = 10.02$, $P = 0.001$] in adult (Fig. 2). Enriched animals showed significantly less BDNF levels in the amygdala (Fig. 2a) compared with SC rats ($P = 0.02$). Moreover, enriched animals showed marginally less BDNF levels in the amygdala than IC rats ($P = 0.05$). Parallel with these results were the smaller serum BDNF levels in EC rats (Fig. 2b) compared than those of both SC ($P = 0.002$) and IC ($P = 0.001$) animals. The linear regression analysis showed that the amygdala BDNF level predicted 24% variance in serum BDNF level (adjusted $R^2 = 0.24$, $P = 0.01$).

Body and brain weights

The animals were achieved differential final body weights in different environmental conditions [$F(2, 19) = 16.87$, $P = 0.001$].

https://doi.org/10.1017/S2040174418000144 Published online by Cambridge University Press

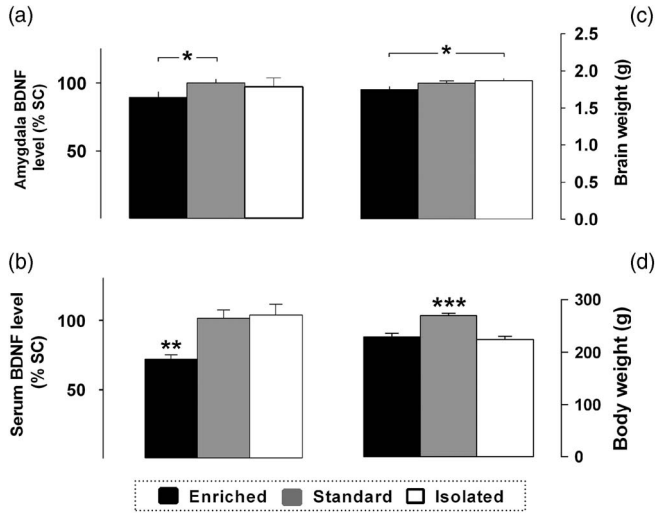

Fig. 2. Effects of post-weaning rearing condition on amygdala (*a*) and serum (*b*) brain-derived neurotrophic factor (BDNF) levels as well as brain (*c*) and body (*d*) weights in young adulthood. Results are expressed as means ± S.E.M. The BDNF data are represented as relative to the standard condition group (%SC). The lines upon the bars indicate significant differences among groups, which were omitted when one group differed from the both other groups at the same significance level: $^{*}P \leqslant 0.02$, $^{**}P \leqslant 0.002$, $^{***}P = 0.001$.

Animals in standard rearing condition showed higher body weights (Fig. 2d) compared with those of both EC ($P = 0.001$) and IC ($P = 0.001$) rats. Environmental enrichment also led to decreased brain weights [$F(2, 19) = 3.34$, $P = 0.05$; Fig. 2c] compared with the isolated rearing condition ($P = 0.02$). Since brain weight depends partly on body weight, the effect of the latter was removed by ANCOVA. ANCOVA also revealed a significant main effect of rearing condition [$F(2, 18) = 5.28$, $P = 0.01$]. However, this did significantly alter the primary effects indicated by the ANOVA, making the brain weight of EC animals significantly lower than SC rats ($P = 0.04$), but only marginally lower than IC animals ($P = 0.08$).

Discussion

Post-weaning environmental enrichment led to lower amygdala and serum BDNF levels in early adulthood. Moreover, enriched animals showed less brain weights compared to animals reared in impoverished conditions.

Enriched rats showed less amygdala BDNF levels compared with the SC animals. Consistently, we have shown previously that post-weaning EC reduces amygdala BDNF responses to foot shocks compared with the SC animals, 14 weeks after cessation of the enriched period.[9] However, in a recent study, post-weaning EC increased BDNF levels in central amygdala of trait anxiety rats.[10] These apparently contradictory results merit comment. The discrepancy can be easily explained if one keeps in mind that the amygdala consists of several nuclei,[18] while the current study have measured the BDNF levels in the whole amygdala. Chronic restraint stress paradigm leads to a lasting increase in the amygdala BDNF levels, which is associated with hypertrophy of this brain region and increased anxiety-like behavior.[19] The environmental enrichment might reduce amygdala BDNF levels by providing the animals with high-quality and/or less stressful condition.[9] Overall, current data suggest the existence of a critical age window during post-weaning period for tuning adult amygdala BDNF levels.

The current study, for the first time, demonstrated less serum BDNF levels in enriched rats compared with both SC and IC animals. The regulation of the BDNF levels in the peripheral blood is not yet fully understood.[20] The brain is considered as a major source of circulating BDNF.[21] Accordingly, the amygdala BDNF levels predicted 24% variance of the serum BDNF levels in current study. Indeed, stress impairs BDNF signaling in limbic areas.[22] It is likely that the stressful conditions in IC and SC rearing[23,24] have led to enhanced neuronal activity in some limbic areas, such as the amygdala, and then resulted in higher serum BDNF levels. In consistent, elevated serum BDNF levels in some animal models of depression have been reported in other studies.[25,26] It is noteworthy that there are some discrepancies between serum and brain levels of BDNF, and serum BDNF may not accurately reflect BDNF concentrations in the brain.[27] However, current findings, similar to the amygdala BDNF levels, suggest the existence of a critical age window during post-weaning period for tuning adult serum BDNF levels. As the implication of BDNF signaling in stress responses is gender-specific,[28] further research to investigate the effect of rearing condition on female rats would be productive.

SC animals showed higher body weight compared with both EC and IC rats. There is some controversy regarding the effect of environmental condition on body weight.[29] We suggest three possible explanations. First, crowding in standard laboratory cages might imply more competition between individuals, resulting in innate hoarding, which would result in enhanced body weight. Second, isolation in IC cages might has contributed to the decreased body weights of IC animals compared with SC rats.[30] Third, the endogenous rhythms, such as circatrigintan, could be adjusted by environmental factors.[31] Then, the circatrigintan rhythm might has differentially influenced the body weights in different environmental conditions in present study.

EC rats presented lower absolute brain weights compared with the isolation reared animals; when body mass is controlled for, the EC animals showed less brain weight compared with SC reared animals. Some studies have demonstrated higher brain weights in enriched rats compared with isolated animals later in life.[16,32] The effect of experiences on brain weight is plastic and depends on the duration of manipulation period.[32] Moreover, cage sizes, number of cage mates, shape of the objects in the enriched cage and other factors are usually different among studies.[33]

Normal brain development comprises the creation, growth and fine tuning of neural networks, and pruning is a necessary process in nervous system development.[34] For an example, the establishing and remodeling of neural networks in the cerebral cortex depends partly on sensory input via synaptic activity.[35] On the other hand, greater spine density have been reported in fragile X knockout mice, which has been suggested to reflect impaired synapse stabilization and pruning.[36] It is well established that the brain weight increases through increased synaptic area and supportive tissues.[37] As the single housing condition in male Wistar rats and the standard housing conditions of laboratory rodents are considered as impoverished rearing conditions, and cause stress reaction in these animals,[23,24] we suggest that the higher brain weights in IC and SC rats might be due to delayed pruning process in these groups. Of course, further extensive investigations are required to elucidate the fine anatomical consequences of rearing conditions on areas in the brain. Altogether, enriched rats might have developed well to encounter future stressful life events, as we have recently shown an antidepressant effect of EC in behavioral test with a study design similar to the present study work.[9,16]

https://doi.org/10.1017/S2040174418000144 Published online by Cambridge University Press

In conclusion, post-weaning environmental enrichment attenuates amygdala – and serum – BDNF levels, probably by providing high-quality/less stressful environment. The enriched animals also showed lower brain weight in early adulthood. Current results showed that the enriched rats might have gotten proper developmental trajectories. Our findings might offer insight into possible molecular mechanisms contributing to the life-long effects of early life experiences.

Acknowledgments. The authors thank Gh. Faridaalaee and A. Shiri.

Financial Support. Financial support for this study was provided by the Neuroscience Research Center (NSRC) of Tabriz University of Medical Sciences (grant number 90-61-5); Drug Applied Research Center of Tabriz University of Medical Sciences (grant number 44/91); and Maragheh University of Medical Sciences.

Conflicts of Interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (NIH; Publication No. 85-23, revised 1985) and has been approved by the institutional committee (Regional Ethics Committee of Tabriz University of Medical Sciences: No. 91/2-2/3).

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