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Behavioral alterations induced by post-weaning isolation rearing of rats are accompanied by reduced VGF/BDNF/TrkB signaling in the hippocampus

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A B S T R A C T

Post-weaning social isolation has been shown to be a relevant animal model for studying the mechanisms underlying psychopathological states induced by early-life stressful experiences. Besides extensively studied brain-derived neurotrophic factor (BDNF) and tyrosine receptor kinase B (TrkB) receptor, increasing attention is being given to a neuropeptide precursor VGF (non-acronymic). Several lines of evidence indicate an interplay between the neurotrophins and nitric oxide signaling. This study investigated the long-term consequences of post-weaning social isolation on behavior, VGF/BDNF/TrkB pathway and two isoforms of nitric oxide synthase (NOS) in the hippocampus and examined whether these effects were sex-specific. Male and female Sprague-Dawley rats were reared either in social isolation or social groups from postnatal day 21 for 9 weeks (n = 12–15/group and sex). Post-weaning social isolation induced impairments in sensorimotor gating and increased anxiety-like behavior in rats of both sexes. These behavioral alterations were accompanied by attenuated gene expression of VGF and TrkB receptor in the hippocampus. Isolation-induced reduction in VGF gene expression was more evident in male isolates. Similar changes were found in neuronal NOS (nNOS) gene expression with reduced mRNA levels in male isolates. Gene expression of BDNF and inducible NOS was not influenced by isolation rearing or sex. In addition, sex-specific patterns of VGF and nNOS gene expression in the hippocampus with higher mRNA levels in males than in females were revealed. The present study demonstrates a relationship between nNOS, VGF, BDNF, and TrkB confirming a link between nitric oxide and neurotrophins signaling pathways. Our findings indicate that long-term post-weaning social isolation alters signaling via VGF/BDNF/TrkB and nNOS that could interfere with neurodevelopmental processes which may contribute to pathological behavioral symptoms in adulthood. Future studies are needed to support this suggestion since the direct mechanistic link has not been approached in this study.

1. Introduction

There is no doubt that stressful events experienced early in life increase the susceptibility to psychopathology in adulthood. Long-term consequences of adverse early-life conditions on mental health are caused by disrupting the normal development of neural systems involved in the stress response, behavior and emotional states (Lukkes et al., 2009). It is of great importance to understand the mechanisms underlying psychopathological states induced by adverse early-life experiences. Animal models are fundamental to gain insights into the neurobiological and behavioral mechanisms underlying the short- and long-term effects of early stressful events (Cirulli et al., 2010). A large body of evidence suggests that post-weaning isolation rearing of rodents is an animal model that mimics some of the behavioral consequences of early-life stressful experiences in humans. Post-weaning social isolation involves rearing rats in isolation to prevent social

Abbreviations: BDNF, brain-derived neurotrophic factor; TrkB, tyrosine receptor kinase B; VGF, non-acronymic; NO, nitric oxide; NOS, nitric oxide synthase; nNOS, neuronal NOS; iNOS, inducible NOS; PPI, prepulse inhibition; PND, postnatal day

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contact with conspecifics, starting from the day of weaning (in a range from postnatal day 21–28 across studies) for several weeks. Isolation rearing of rats from weaning produces a range of persistent behavioral changes in the young adult, including an increase in anxiety-like behavior, a deficit in prepulse inhibition (PPI) of acoustic startle or hyperactivity in response to novelty (Fone and Porkess, 2008). Despite the fact that sex differences in neurobiological vulnerability to early life stress do exist (Gobinath et al., 2015; Goodwill et al., 2019), the majority of studies employing the model of long-term post-weaning isolation utilize male rats. Less attention has been given to females.

Current research postulates that stressful events during the brain development lead to defective neural connectivity and altered brain neurochemistry. The developmental and homeostatic neural processes are controlled by neurotrophic factors, signaling peptides which act on specific receptors to regulate the physiology of neurons and glial cells (Williams and Umemori, 2014; Marsh and Blurton-Jones, 2017). In addition to extensively studied brain-derived neurotrophic factor (BDNF), increasing attention is being given to a neuropeptide precursor VGF (non-acronymic). VGF, or better *vgf*, is a neurotrophin-inducible gene widely expressed in neuronal and neurosecretory cells. Numerous biologically active peptides of low molecular weight are derived from VGF precursor protein. VGF is involved in the processes of synaptic plasticity, neuronal growth and neurogenesis (Thakker-Varia et al., 2014; Bozdagi et al., 2008). The VGF expression is under the control of BDNF/ tyrosine receptor kinase B (TrkB) signaling pathway (Alder et al., 2003). BDNF/TrkB signaling induces expression of VGF and its C-terminal peptide TLQP-62 reinforces rapid BDNF secretion and/or TrkB signaling in the hippocampus. Thus, VGF has been proposed as a critical component of a positive BDNF/TrkB regulatory loop (Lin et al., 2015). Changes in VGF levels appear to be related to psychopathological states including bipolar disorder and schizophrenia (Busse et al., 2012). Recently, Jiang et al. (2018) have proposed a role of VGF/BDNF/TrkB feedback loop in rapid-acting antidepressant efficacy. We have reviewed in a systematic way the evidence for altered expression of BDNF in the brain of rats exposed to long-term social isolation (Murinova et al., 2017). The identified studies were rather consistent in reporting a decreased expression of BDNF in the hippocampus in isolated animals. To our knowledge, there is only one study available on the social isolation-induced changes in TrkB receptor (Djouma et al., 2006) reporting a decrease in TrkB expression in the cingulate cortex and the piriform cortex but an increase in the hippocampus and the retrosplenial cortex in rats. The influence of post-weaning isolation rearing of rats on VGF expression in the hippocampus has not been investigated yet. Moreover, scarce data are available on possible sexual dimorphism in the expression of these peptides in the hippocampus.

Another system which is linked to several neuronal and behavioral processes is the nitrergic system (Calabrese et al., 2007). Nitric oxide (NO) is a neurotrasmitter that is found throughout the central nervous system to participate in numerous different brain functions (Garthwaite, 2019). NO is produced by the enzyme NO synthase (NOS) with three isoforms that differ in their structure, distribution and regulation, namely neuronal (nNOS), inducible (iNOS) and endothelial (eNOS). nNOS is primarily expressed in neurons, while iNOS is mainly found in microglia and astrocytes and eNOS in endothelial cells of blood vessels (Förstermann and Sessa, 2012). In this study, we have focused on nNOS and iNOS as both of these NOS isoforms have been shown to be implicated in stress-related psychopathological states including schizophrenia (Nasyrova et al., 2015) and affective disorders (Zhou et al., 2018). Interestingly, several lines of evidence indicate an interplay between NO and BDNF/TrkB signaling since BDNF and NOS modulate each other in different cell types and experimental conditions (Canossa et al., 2002; Kolarow et al., 2014; Biojone et al., 2015).

The aim of the present study was to investigate the long-term consequences of post-weaning social isolation on behavior, VGF/BDNF/TrkB pathway, nNOS and iNOS gene expression in the rat hippocampus. Furthermore, as only a few studies focused on sexual dimorphism in behavioral and neurochemical consequences of isolation rearing, the present study was performed in both male and female rats to examine whether the effects of social isolation are sex-specific.

2. Material and methods

2.1. Animals

A total of eight adult timed-pregnant Sprague-Dawley rats (AnLab, Prague, Czech Republic) arrived at the animal facility on gestational day 16. Approximately a week later, the litter was born, and the day of birth was designated as postnatal day 0 (PND0). Litters were culled to eight pups, four males and four females per dam on PND7. This helped to avoid excessive food competition among the offspring during the first weeks of their life in which they depend on breastfeeding for nutrition. Rats were kept under standard housing conditions with a constant 12:12 h light/dark cycle (lights on at 06.00 h), temperature (22 °C ± 2 °C) and humidity (55 ± 10%). Food and water were available *ad libitum*. All experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic and conformed to the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2. Social isolation and experimental procedures

The pups were weaned on PND21. Males (n = 27) and females (n = 27) derived from eight litters were separated from their mothers and were randomly assigned to the isolation-reared (one rat per cage) and socially-reared (three rats per cage) groups. Animals were reared under these conditions for 9 weeks. According to the rearing conditions and sex, rats were divided into four groups: isolation/males (n = 12), social/males (n = 15), isolation/females (n = 12), social/females (n = 15). They were housed in plastic cages (55.5 x 34.5 × 19.5 cm for socially-reared groups and 43.5 x 28 × 23 cm for isolation-reared groups) containing sawdust bedding with metal grid lids, and had visual, auditory and olfactory contact with conspecifics in the same holding room. Rats were disturbed only for cleaning purposes (changing the cage once a week), the weekly body weight measurement and for behavioral testing throughout the experiment.

The duration of the isolation period was set according to previously published data showing that a minimum of 8 consecutive weeks following weaning is used to achieve the robust and significant isolation-induced behavioral disturbances (Varty et al., 1999; Fone and Porkess, 2008; Walker et al., 2019). Thus, behavioral testing was performed during the 8th-9th week of isolation and thereafter samples of blood and the hippocampus were collected for neurochemical measurements. Each rat was tested in a battery of paradigms designed to evaluate behavioral changes (Fig. 1). All behavioral tests were conducted during the light period of the light/dark cycle. Animals were transported in their home cages from the animal room to the testing room and left undisturbed for 1 h before testing. The experimenter was not in the room during the tests. The testing apparatus was thoroughly cleaned with 20% ethanol and dried prior the next animal was introduced. At the end of the test procedure, each animal was returned to its home cage. The open-field and elevated plus-maze tests were recorded by a video camera positioned above the testing apparatus. Tapes were analyzed using a video observation system (Ethovision XT 10, Noldus Information Technology, Wageningen, The Netherlands).

2.3. Behavioral testing

2.3.1. Prepulse inhibition of startle (PPI) paradigm

To assess changes in sensorimotor gating, rats were tested in the acoustic startle/PPI paradigm (Geyer and Swerdlow, 2001). Briefly, the test was performed in a single test station consisted of sound

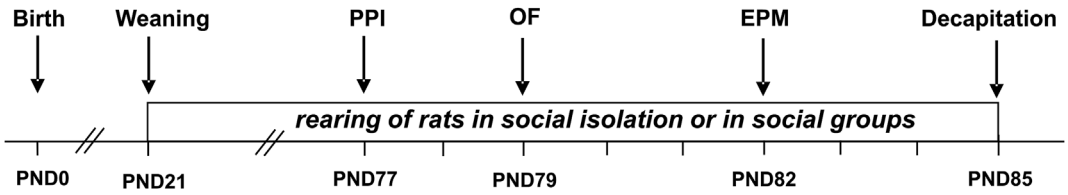

Fig. 1. Study design. Male (n = 27) and female (n = 27) Sprague-Dawley rats were reared in social isolation or in social groups from weaning (PND21) for 9 weeks. During the last week of the respective rearing conditions, each rat was behaviorally tested in the prepulse inhibition (PPI) of acoustic startle reflex paradigm on PND77, the open-field (OF) test on PND79 and the elevated plus-maze test on PND82. Animals were decapitated on PND85.

attenuating chamber (interior 50.8 x 33 × 30.5 cm; walls 1.9 cm thick) with a plexiglas cylinder situated on the top of a platform with a sensor that detects changes in strength made by the movements of the rat in each trial. Vibrations created by rat body movements were transduced and converted into a signal proportional to response amplitude. Auditory stimuli were delivered by two speakers situated on the sides of the cylinder (Med Associates, UK). The experimental session consisted of a 5-min acclimatization period to a 65-dB background noise, followed by three blocks of acoustic trials consisted of startle trials (pulse alone) and prepulse trial (prepulse + pulse). The first and third block tested acoustic startle response only and included six 120 dB pulse-alone trials (40 ms in duration). The second block assessed PPI and contained 10 pulse-alone trials (120 dB, 40 ms) and 15 prepulse + pulse trials (the 120 dB pulse preceded by a 76, 80 or 84 dB prepulse, 20 ms) presented in a randomized order. In the prepulse + pulse trials the pulse was administered 100 ms after prepulse stimulus. The following measures were calculated: *Startle reactivity* was defined as the mean startle response to the first block of acoustic trials (6 × 120 dB pulses). *Startle habituation* (% habituation) was calculated using the formula 100*(1-[mean startle for block3/mean startle for block1]). The *level of PPI (% PPI*) was determined according to the formula 100*(1-[startle magnitude on prepulse-pulse trials in block2/startle magnitude on pulse trials in block2]), such that 0% value indicated no difference between the responses to prepulse-pulse trials and pulse alone trials (i.e., no PPI). % PPI was calculated separately for each prepulse intensity along with average %PPI across all prepulse intensities.

2.3.2. Open-field test

Forty-eight hours following the acoustic startle/PPI paradigm (PND79), the open-field test was conducted to assess general locomotor activity and anxiety-like behavior (Hlavacova and Jezova, 2008). The apparatus consisted of a 90 cm × 90 cm square-shaped black arena with the 37 cm high black walls. The apparatus was illuminated by dim light with the intensity of 30 lx in the central area, and 25 lx in the peripheral area. Each animal was gently placed in the corner of the open field arena and allowed to explore the arena over a 15 min period. Locomotor activity (velocity and distance moved) and anxiety-like behavior (frequency and time spent in the central area) in the open field were evaluated.

2.3.3. Elevated plus-maze test

On the PND82, animals were subjected to the elevated plus-maze test to evaluate changes in anxiety-like behavior associated with isolation rearing. The black plastic apparatus was consisted of two opposite open (50 × 10 cm) and two enclosed (50 x 10 × 40 cm) arms that radiated from a central platform (10 × 10 cm) to form a plus sign. The maze was elevated to a height of 50 cm above the floor. The apparatus was illuminated by dim light with the intensity of 12 lx in the closed arms, and 45 lx in the open arms. Each rat was placed on the central platform of the maze facing an enclosed arm. Behavior scored, comprised spatiotemporal measures (number of open and closed arm entries, total arm entries, and the amount of time spent in each section of the maze (including the central platform), expressed as a percentage of the total test duration (Hlavacova and Jezova, 2008). The number of entries and time spent in the open arms were used as measures of the anxiety level. An arm entry was defined as all four paws entering the arm.

2.4. Tissue and blood collection

On the PND85, animals from all experimental groups were quickly decapitated with a guillotine. Blood was collected in polyethylene tubes containing EDTA and centrifuged immediately at 4 °C to separate plasma, which was stored at – 20 °C until analyzed. The brain was quickly removed from the skull. The hippocampus was quickly removed, frozen in liquid nitrogen and stored at – 70 °C until analyzed.

2.5. RNA isolation and real-time PCR analysis

Real-time qPCR was used for quantitative evaluation of gene expression of VGF, BDNF, TrkB receptor, nNOS and iNOS in the hippocampus. Total RNA from the hippocampus was isolated and purificated by TRIzol® Reagent (Life Technologies, California, USA). Isolated RNA (1 µg) was reverse-transcribed by oligo (dT) nucleotides using ProtoScript® First Strand cDNA Synthesis Kit (NEB, England). Real-time qPCR analysis was performed on a Fast Real-Time PCR System 7900 HT (Applied Biosystems, USA) using GoTaq qPCR Master Mix (Promega, USA). Specific primers (Table 1) for VGF, BDNF, TrkB receptor, nNOS and iNOS were designed by Primer BLAST NCBI software. Analysis was performed in a reaction volume of 20 µl by GoTaq qPCR Sybr Green Master Mix (Promega, USA) as described previously (Pokusa et al., 2016; Graban et al., 2017). Reaction buffer consisted of: 10 µl 2x GoTaq qPCR Master Mix (Promega, USA), 0.2 µl reference dye ROX (Promega, USA), 1 µl 5′-3′ and 3′-5′ complementary oligonucleotide from 5 µM

Table 1
Nucleotide sequence of primers used in gene expression evaluation of VGF (non-acronymic), brain-derived neurotrophic factor (BDNF), inducible NOS (iNOS), neuronal NOS (nNOS), peptidylprolyl isomerase A (PPIA) and TATA box binding protein (TBP).

Target gene	Forward primer 5′- 3′	Reverse primer 5′- 3′
VGF	GGCGCTCCGATGTTTATCCT	TGGGACGCTGTCATCCTTTG
BDNF	ACCATAAGGACGCGGACTTG	AGCAGAGGAGGCTCCAAAGG
TrkB	GCAGAAAACCTCGTCGGAGA	TGGCTCCGTTGTAGAACCAC
nNOS	CGCTACGCGGGCTACAAGCA	GCACGTCGAAGCGGCCTCTT
iNOS	TGGAGGTGCTGGAAGAGTT	GGAGGAGCTGATGGAGTAGT
PPIA	AAGCATACAGGTCCTGGCATCT	CATTCAGTCTTGGCAGTGCAG
TBP	TTCGTGCCAGAAATGCTGAA	GTTCGTGGCTCTCTTATTCTCATG

stock solution, 5–50 ng cDNA depending on the strength of expression and water added to final volume 20 µl. Specific oligonucleotids were used at a concentration of 0.25 pmol/µl. Initial denaturation at 95 °C for 10 min was followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s respectively. Melting curve analysis was performed and did not show any unspecific products of PCR. All data obtained by qPCR analysis were evaluated as an ng of mRNA (cDNA) according to a standard curve and was normalized to gene expression of peptidylprolyl isomerase A (PPIA) and TATA box binding protein (TBP) as reference genes (Table 1).

2.6. Plasma corticosterone concentrations

Plasma corticosterone concentrations were analyzed by a radioimmunoassay after dichloromethane extraction of the steroid from 10 µl aliquots of plasma as described previously (Jezova et al., 1994). The assay had a sensitivity of 0.1 µg/100 ml. The intra- and inter-assay coefficients of variances were 6 and 8%, respectively.

2.7. Statistical analysis

Data were processed and analyzed using SPSS 25 and R. All data were inspected for distributional properties and subsequently winsorized using a 15% two-tailed quantile trimming to treat the identified outlying observations (1.5 x interquartile range rule). A repeated measures ANOVA with the prepulse intensity (76, 80, and 84 dB) as a within-subject effect and rearing and sex as between-subject effects was used for analyzing PPI measures. All other data were analyzed by two-way analysis of variance (ANOVA) with main factors of rearing (social vs. isolation) and sex (male vs. female) followed by Tukey post-hoc test when appropriate. Partial η^2 was used as the measure of effect size. Relationships among selected parameters were assessed using Pearson's correlation analysis. Results are expressed as means ± SEM. The overall level of statistical significance was set as $p < 0.05$.

3. Results

3.1. Behavioral data

Since PPI was measured at different PPI intensities, the effect of this within-subjects variable was first assessed. Repeated measures ANOVA indicated no significant main effect of prepulse intensity ($F_{(2, 98)} = 1.79$, $p > 0.05$, $\eta^2 = 0.03$) and we therefore averaged PPI values across all PPI intervals for subsequent analyzes. Two-way ANOVA revealed a significant main effect of rearing on average PPI percentage ($F_{(1, 49)} = 7.02$, $p < 0.05$, $\eta^2 = 0.13$). As shown in Fig. 2, PPI was significantly decreased in animals reared in social isolation. There was also a significant interaction between sex and rearing ($F_{(1, 49)} = 5.7$, $p < 0.05$, $\eta^2 = 0.11$). Post-hoc comparisons indicated significantly reduced percentage of PPI in isolation-compared to socially-reared females ($p < 0.01$). Startle reactivity and startle habituation were not significantly affected by rearing, sex or by rearing x sex interaction (data not shown).

General locomotor activity in the open-field was not significantly affected by isolation rearing. A two-way ANOVA revealed a significant main effect of sex on the total distance travelled during the open-field test ($F_{(1, 49)} = 4.60$, $p < 0.05$, $\eta^2 = 0.09$) as well as on the percentage of time spent in the central area of the open-field ($F_{(1, 49)} = 5.4$, $p < 0.05$, $\eta^2 = 0.10$) showing that female rats were significantly more active during the test and spent more time in the central area than did male rats. There was no significant main effect of rearing or significant interaction between the factors (Table 2).

Isolation rearing affected anxiety-like behavior in the elevated plus-maze test regardless of sex (Table 2). There was a significant main effect of rearing on the frequency of entries ($F_{(1, 49)} = 5.49$, $p < 0.05$, $\eta^2 = 0.09$) and the percentage of time spent in the open arms ($F_{(1, 49)} = 6.33$, $p < 0.05$, $\eta^2 = 0.11$). Isolation-reared animals entered less often and spent less time in the open arms of the maze (Table 2). Neither the number of closed arm entries nor percentages of time spent in the closed arms or the central platform were significantly influenced by sex and rearing or an interaction between the factors.

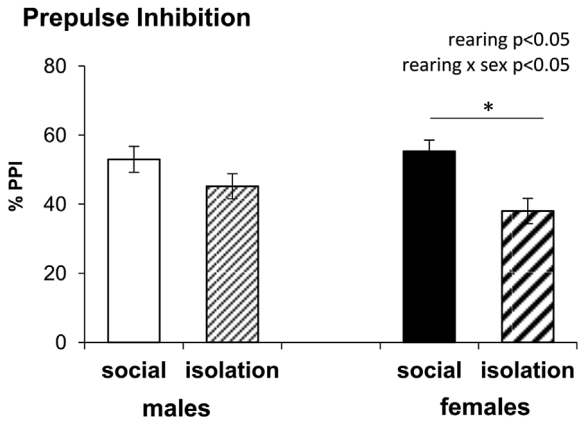

Fig. 2. Prepulse inhibition (PPI) of the acoustic startle response at the prepulse intensities 76, 80 and 84 (left side) and average %PPI across all prepulse intensities (right side) in male and female rats reared in social isolation or in social groups from weaning for 9 weeks. Each value represents mean ± SEM (n = 12–15 rats/group). Statistical significance as revealed by repeated measures ANOVA or by two-way ANOVA with subsequent Tukey post-hoc test when appropriate: **$p < 0.01$.

3.2. Neurochemical data

A two-way ANOVA showed significant main effects of rearing ($F_{(1, 39)} = 9.35$, $p < 0.01$, $\eta^2 = 0.16$) and sex ($F_{(1, 49)} = 13.61$, $p < 0.001$, $\eta^2 = 0.22$) on plasma corticosterone concentrations. Corticosterone levels were significantly lower in isolated compared to socially reared animals and were significantly higher in females than in males (Table 2). The interaction between rearing and sex was not significant.

Gene expression of VGF was significantly affected by rearing conditions ($F_{(1, 39)} = 14.95$, $p < 0.001$, $\eta^2 = 0.28$) and sex ($F_{(1, 39)} = 6.99$, $p < 0.05$, $\eta^2 = 0.15$). There was also a significant interaction between the two factors ($F_{(1, 39)} = 12.72$, $p < 0.001$, $\eta^2 = 0.25$). Post hoc comparisons showed that isolation significantly decreased VGF mRNA levels in males ($p < 0.001$) (Fig. 3A). In socially reared animals, the levels of VGF mRNA were higher in males compared to females ($p < 0.001$) (Fig. 3A).

There were no significant main effects of rearing and or a significant interaction between the factors on the gene expression of BDNF in the hippocampus (Fig. 3B). On the other hand, there was a significant main effect of rearing conditions on gene expression of TrkB receptor ($F_{(1, 40)} = 5.52$, $p < 0.05$, $\eta^2 = 0.12$), indicating that isolation rearing resulted in significantly decreased mRNA levels for TrkB receptor. There was no significant main effect of sex or an interaction between the factors (Fig. 3C).

A two-way ANOVA of data on gene expression of nNOS in the hippocampus revealed a significant main effect of sex ($F_{(1, 39)} = 5.59$, $p < 0.05$, $\eta^2 = 0.13$) showing significantly higher levels in males than in females (Fig. 4A). There was also a significant interaction between the rearing and sex ($F_{(1, 39)} = 5.37$, $p < 0.05$, $\eta^2 = 0.12$). Post hoc testing revealed significantly lower mRNA levels coding for nNOS in the group of isolation-reared males compared to socially-reared males ($p < 0.05$). Gene expression of nNOS was higher in socially-reared males than in socially-reared females ($p < 0.01$). Although there was a tendency toward lower iNOS mRNA levels in isolation-reared animals, the difference did not reach statistical significance (Fig. 4B). Gene expression of iNOS was not significantly influenced by sex or interaction

Table 2
Behavioral data obtained in the open-field and elevated plus maze tests as well as plasma corticosterone concentrations of male and female rats reared in social isolation or in social groups from weaning for 9 weeks. Each value represents mean ± SEM (n = 12–15 rats/group). Statistical significance as revealed by two-way ANOVA for main factors rearing conditions and sex.

	Males		Females		Statistics
	Social	Isolation	Social	Isolation	
Open-field test					
Total distance travelled (m)	52.7 ± 1.1	53.1 ± 1.9	54.9 ± 1	54.8 ± 1.7	sex $p < 0.05$
Time spent in the central area (%)	2.9 ± 0.3	2.7 ± 0.2	3.1 ± 0.3	3.3 ± 0.3	sex $p < 0.05$
Elevated plus-maze test					
Open arm entries (frequency)	6.7 ± 1.8	5.1 ± 0.9	12.1 ± 2.0	4.7 ± 0.8	rearing $p < 0.05$
Time spent in the open arms (%)	2.6 ± 0.6	1.9 ± 0.4	4.5 ± 0.7	1.8 ± 0.4	rearing $p < 0.05$
Plasma corticosterone (μg/100 ml)	2.7 ± 0.8	0.8 ± 0.2	8.4 ± 1.7	3.4 ± 0.6	rearing $p < 0.01$ sex $p < 0.001$

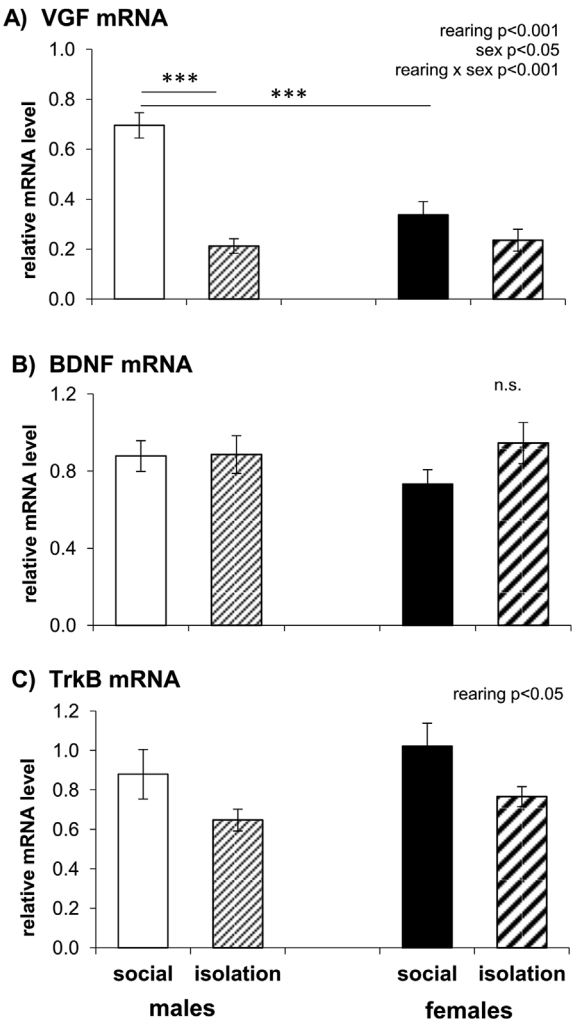

Fig. 3. Gene expression of VGF (A), BDNF (B) and TrkB receptor (C) in the hippocampus of male and female rats reared in social isolation or in social groups from weaning for 9 weeks. Each value represents mean ± SEM (n = 12–15 rats/group). Statistical significance as revealed by two-way ANOVA with subsequent Tukey post-hoc test when appropriate: ***$p < 0.001$.

between rearing and sex.

Pearson's correlation analysis revealed significant positive correlations between mRNA levels coding for VGF and TrkB receptor as well as VGF mRNA levels and nNOS mRNA levels. The correlation between the hippocampal mRNA levels for BDNF and TrkB receptor was negative. TrkB receptor mRNA levels positively correlated with nNOS mRNA levels. There were also significant negative correlations between the

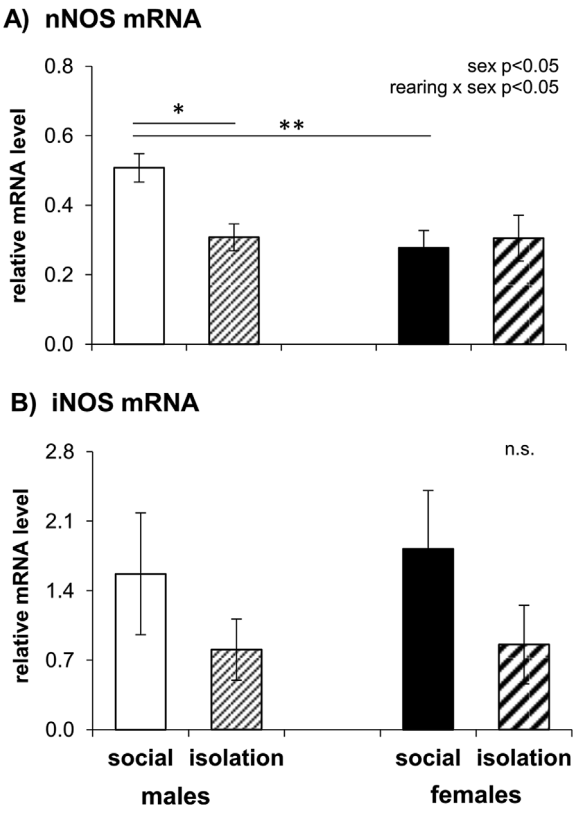

Fig. 4. Gene expression of nNOS (A) and iNOS (B) in the hippocampus of male and female rats reared in social isolation or in social groups from weaning for 9 weeks. Each value represents mean ± SEM (n = 12–15 rats/group). Statistical significance as revealed by two-way ANOVA with subsequent Tukey post-hoc test when appropriate: *$p < 0.05$, **$p < 0.01$.

Table 3
Pearson correlation coefficients and p-values between the levels of mRNAs coding for VGF, BDNF, TrkB, nNOS and iNOS in the rat hippocampus.

mRNA levels	VGF	BDNF	TrkB	nNOS	iNOS
VGF	-				
BDNF	r = −0.231 p = 0.147	-			
TrkB	**r = 0.463** **p = 0.002**	**r = -0.491** **p = 0.004**	-		
nNOS	**r = 0.607** **p = 0.000**	**r = -0.383** **p = 0.027**	**r = 0.416** **p = 0.007**	-	
iNOS	r = 0.165 p = 0.302	**r = -0.351** **P = 0.024**	r = 0.129 p = 0.422	r = −0.070 p = 0.662	-

mRNA levels for BDNF and nNOS and for BDNF and iNOS as well. The correlation coefficients and p-values are shown in Table 3.

4. Discussion

The results of the present study demonstrate that behavioral alterations induced by post-weaning social isolation are accompanied by attenuated gene expression of VGF and TrkB receptor in the hippocampus. Isolation-induced reduction in VGF gene expression was more evident in male isolates. Similar changes were found in nNOS gene expression with reduced mRNA levels in male isolates. Expression of BDNF and iNOS were not influenced by isolation rearing or sex. Sex-specific patterns of VGF and nNOS gene expression in the hippocampus with higher mRNA levels in males compared to females were revealed. As expected, post-weaning social isolation induces impairments in sensorimotor gating and increased anxiety-like behavior in rats of both sexes. Notably, the reduction in prepulse inhibition occurred to a greater extent in female isolates.

The main finding of this study is a marked reduction of VGF and TrkB receptor gene expression in isolated rats indicating attenuated VGF/BDNF/TrkB signaling in animals reared in social isolation. Interestingly, the reduction of mRNA levels coding for VGF was more pronounced in male isolates, while the mRNA levels of TrkB receptor were significantly reduced in isolated animals regardless of sex. Surprisingly, we did not find any effect of isolation rearing on BDNF mRNA expression following 9 weeks of isolation. Although the majority of studies are rather consistent in reporting a decreased expression of BDNF mRNA or protein in the hippocampus in isolated animals (Murinova et al., 2017), there are few studies showing no effect of social isolation on BDNF at the protein level (Parks et al., 2008; Simpson et al., 2012). Since the measurements of BDNF and TrkB receptor were performed only at the nucleic acid level, we do not know what happened at the protein level. We may speculate that a reduction in BDNF (both mRNA and protein) occurs at earlier stages of isolation rearing as suggested by a decrease in gene expression of TrkB receptor and VGF observed in our study. It has been shown that the VGF expression is strongly dependent on the BDNF/TrkB signaling pathway (Alder et al., 2003) and VGF regulates hippocampal synaptic plasticity through a BDNF-dependent mechanism (Bozgadi et al., 2008). The decrease in VGF expression could be caused by a reduced stimulatory effect of BDNF due to decreased TrkB receptor expression. In addition, gene expression of TrkB receptor in the present study positively correlated with VGF gene expression, being consistent with the assumption that the VGF expression is controlled by the BDNF/TrkB signaling pathway (Alder et al., 2003).

A few clinical and several pre-clinical studies strongly suggest the involvement of the NO signaling pathway in stress-related disorders (Wegener and Volke, 2010). In the present study, post-weaning social isolation induced reduction in hippocampal nNOS mRNA levels in males, but not in females. Although there was a tendency toward decline in iNOS gene expression in isolation-reared animals, the difference did not reach statistical significance. This is in contrast with previous reports showing increased nNOS and iNOS expression in the hippocampus and the prefrontal cortex of male rats exposed to chronic stress of isolation conducted in adulthood (Zlatkovic et al., 2013, 2014). Rats in the present study were reared in social isolation from weaning whereas in studies by Zlatkovic et al. (2013, 2014) isolation housing was initiated in adulthood when the rats were mature. It has been shown that the isolation effect depends upon the developmental timing of the manipulation since the social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences (Hall, 1998). Thus, the effects of social isolation on nNOS and iNOS appear to be specific to the developmental stage during which the isolation is experienced.

There is a growing body of evidence supporting the relationship between NO and neurotrophins signaling (Biojone et al., 2015; Chmelova et al. 2019). Present results obtained by correlation analysis support this idea. Hippocampal mRNA levels coding for nNOS positively correlated with both BDNF and TrkB receptor mRNA levels. Interestingly, we revealed positive correlation between nNOS and VGF mRNA levels. As no information on the direct relationship between VGF and nNOS has been reported so far, this is the first study to report such association. In this study, we put attention to sex differences in both neurotrophins and NOS gene expressions.

An intriguing finding of this study is the sexual dimorphism in VGF and nNOS gene expression with significantly higher mRNA levels in males than females. Notably, this finding applies to the group of socially-reared animals, nut not the isolation-reared ones. We could propose that rearing of rats in social groups allowing social interactions leads to increase in VGF and nNOS gene expression in males, but not females. Higher hippocampal nNOS mRNA levels in males compared to females is consistent with the study by Chen et al. (2014). According to Hu et al. (2012), substantially lower levels of 17β-estradiol in the female compared to the male hippocampus account for lower local NO production via estrogen receptor β–mediated nNOS expression. To our knowledge, this is the first study demonstrating sex-specific pattern of VGF gene expression in the hippocampus which biological implications remain to be elucidated in future research.

Consistent with numerous previous studies reporting isolation-induced behavioral abnormalities (Fone and Porkess, 2008; Murinova and Riecansky, 2016), isolation-reared animals exhibited significant deficits in PPI and increased anxiety-like behavior compared to socially-reared animals when tested in adulthood. Present finding of reduced PPI in isolation-reared rats confirms previous postulate that social isolation paradigm produces strong deficits in PPI magnitude (Bakshi and Geyer, 1999). Although isolation rearing induced impairment in PPI in both sexes, the deficit was more evident in female isolates. Isolation rearing-induced deficit in PPI was not associated with an increased locomotor activity, supporting previous suggestion that isolation-induced PPI deficits and locomotor hyperactivity are dissociable (Varty et al., 2000). The fact that we did not observe any effect of rearing conditions on the total distance traveled in the open-field test indicates that locomotor activity in isolated animals was not impaired. On the other hand, we noticed sex differences in the total distance traveled as well as the time spent in the central area of the open-field test. Females were more active during the test and showed less anxious behavior than did males. This is in agreement with generally accepted view that in comparison with males, female rats exhibit less fear-related behaviors manifested by higher horizontal and vertical locomotor activity (Donner and Lowry, 2013). In this study, both males and females reared in social isolation consistently exhibited a robust increase in anxiety-like behavior measured in the elevated plus maze test. Increased anxiety-like behavior induced by social isolation has been repeatedly reported in male rats (Weiss et al., 2004). Our study along with few other studies in female rats suggest that isolation rearing and/or social deprivation appears to increase anxiety-like behavior of female rats regardless of onset of isolation (Leussis and Andersen, 2008; Regenass et al., 2018; Harvey et al., 2019). The lack of any sex differences observed in the elevated plus-maze test may be caused by the fact that we compared male and female rats without considering the phase of the estrous cycle in females. It has been demonstrated that the estrous cycle phase and gonadal hormones influence conditioned fear extinction (Milad et al., 2009). This should be taken into account in future studies.

We found that isolation rearing was associated with decreased plasma corticosterone concentrations in both sexes indicating attenuated activity of the hypothalamic-pituitary-adrenocortical axis in rats reared in social isolation. Findings of studies describing the impact of post-weaning social isolation on plasma corticosterone levels are very controversial. Some authors reported increased (Gamallo et al., 1986), unchanged (Ravenelle et al., 2014; Zlatkovic et al., 2014) but also decreased (Pisu et al., 2016; Regenass et al., 2018) concentrations of corticosterone when measured in adulthood. These inconsistencies may

be due to differences in the timing, methods of social isolation, strain and sex of rats used. It is likely that corticosterone concentrations were only increased during an early phase of isolation rearing and later, adaptive changes occurred. Moreover, we and others showed that increased anxiety is associated with attenuated responsiveness of the hypothalamic-pituitary-adrenocortical axis in humans (Jezova et al., 2004, 2010; Petrowski et al., 2013; Hlavacova et al., 2017) and rodents (Lisieski et al., 2018; Harvey et al., 2019).

The limitation of this study is that the measurements were performed only at the level of gene expression. The findings obtained should be verified at the protein level in future studies. Another limitation is that we did not check the phase of the estrous cycle in females during which circulating levels of gonadal hormones fluctuate and may influence a variety of biological processes including behavior.

In conclusion, the present study demonstrates a relationship between nNOS, VGF, BDNF and TrkB confirming a link between NO and neurotrophins signaling pathways. Our findings indicate that long-term post-weaning social isolation alters signaling via VGF/BDNF/TrkB and nNOS that could interfere with neurodevelopmental processes which may contribute to pathological behavioral symptoms in adulthood. Future studies are needed to support this suggestion since the direct mechanistic link has not been approached in this study.

Declarations of interest

None.

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Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104473.

References

Alder, J., Thakker-Varia, S., Bangasser, D.A., Kuroiwa, M., Plummer, M.R., Shors, T.J., Black, I.B., 2003. Brain-derived neurotrophic factor-induced gene expression reveals novel actions of VGF in hippocampal synaptic plasticity. J. Neurosci. 23, 10800–10808.

Bakshi, V.P., Geyer, M.A., 1999. Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiol. Behav. 67, 385–392.

Biojone, C., Casarotto, P.C., Joca, S.R., Castren, E., 2015. Interplay between nitric oxide and brain-derived neurotrophic factor in neuronal plasticity. CNS Neurol. Disord. - Drug Targets 14, 979–987.

Bozdagi, O., Rich, E., Tronel, S., Sadahiro, M., Patterson, K., Shapiro, M.L., Alberini, C.M., Huntley, G.W., Salton, S.R., 2008. The neurotrophin-inducible gene Vgf regulates hippocampal function and behavior through a brain-derived neurotrophic factor-dependent mechanism. J. Neurosci. 28, 9857–9869. https://doi.org/10.1523/JNEUROSCI.3145-08.2008.

Busse, S., Bernstein, H.G., Busse, M., Bielau, H., Brisch, R., Mawrin, C., Müller, S., Sarnyai, Z., Gos, T., Bogerts, B., Steiner, J., 2012. Reduced density of hypothalamic VGF-immunoreactive neurons in schizophrenia: a potential link to impaired growth factor signaling and energy homeostasis. Eur. Arch. Psychiatry Clin. Neurosci. 262, 365–374. https://doi.org/10.1007/s00406-011-0282-7.

Calabrese, V., Mancuso, C., Calvani, M., Rizzarelli, E., Butterfield, D.A., Stella, A.M., 2007. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nat. Rev. Neurosci. 8, 766–775. https://doi.org/10.1038/nrn2214.

Canossa, M., Giordano, E., Cappello, S., Guarnieri, C., Ferri, S., 2002. Nitric oxide down-regulates brain-derived neurotrophic factor secretion in cultured hippocampal neurons. Proc. Natl. Acad. Sci. U. S. A 99, 3282–3287. https://doi.org/10.1073/pnas.042504299.

Chen, F., Zhou, L., Bai, Y., Zhou, R., Chen, L., 2014. Sex differences in the adult HPA axis and affective behaviors are altered by perinatal exposure to a low dose of bisphenol A. Brain Res. 1571, 12–24. https://doi.org/10.1016/j.brainres.2014.05.010.

Chmelova, M., Jezova, D., Riecansky, I., Hlavacova, N., 2019. Post-weaning social isolation of rats induces reduction in the gene expression of vascular endothelial growth factor (VEGF) in the hippocampus. Gen Physiol Biophys 5 (in press).

Cirulli, F., Berry, A., Bonsignore, L.T., Capone, F., D'Andrea, I., Aloe, L., Branchi, I., Alleva, E., 2010. Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels. Neurosci. Biobehav. Rev. 34, 808–820. https://doi.org/10.1016/j.neubiorev.2010.02.008.

Djouma, E., Card, K., Lodge, D.J., Lawrence, A.J., 2006. The CRF1 receptor antagonist, antalarmin, reverses isolation-induced up-regulation of dopamine D2 receptors in the amygdala and nucleus accumbens of fawn-hooded rats. Eur. J. Neurosci. 23, 3319–3327. https://doi.org/10.1111/j.1460-9568.2006.04864.x.

Donner, N.C., Lowry, C.A., 2013. Sex differences in anxiety and emotional behavior. Pflügers Archiv 465, 601–626. https://doi.org/10.1007/s00424-013-1271-7.

Fone, K.C., Porkess, M.V., 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci. Biobehav. Rev. 32, 1087–1102. https://doi.org/10.1016/j.neubiorev.2008.03.003.

Förstermann, U., Sessa, W.C., 2012. Nitric oxide synthases: regulation and function. Eur. Heart J. 33, 829–837. https://doi.org/10.1093/eurheartj/ehr304.

Gamallo, A., Villanua, A., Trancho, G., Fraile, A., 1986. Stress adaptation and adrenal activity in isolated and crowded rats. Physiol. Behav. 36, 217–221.

Garthwaite, J., 2019. NO as a multimodal transmitter in the brain: discovery and current status. Br. J. Pharmacol. 176, 197–211. https://doi.org/10.1111/bph.14532.

Geyer, M.A., Swerdlow, N.R., 2001. Measurement of startle response, prepulse inhibition, and habituation. Curr. Protoc. Neurosci (Chapter 8), Unit 8.7. https://doi.org/10.1002/0471142301.ns0807s03.

Gobinath, A.R., Mahmoud, R., Galea, L.A., 2015. Influence of sex and stress exposure across the lifespan on endophenotypes of depression: focus on behavior, glucocorticoids, and hippocampus. Front. Neurosci. 8, 420. https://doi.org/10.3389/fnins.2014.00420.

Goodwill, H.L., Manzano-Nieves, G., Gallo, M., Lee, H.I., Oyerinde, E., Serre, T., Bath, K.G., 2019. Early life stress leads to sex differences in development of depressive-like outcomes in a mouse model. Neuropsychopharmacology 44, 711–720. https://doi.org/10.1038/s41386-018-0195-5.

Graban, J., Hlavacova, N., Jezova, D., 2017. Increased gene expression of selected vesicular and glial glutamate transporters in the frontal cortex in rats exposed to voluntary wheel running. J. Physiol. Pharmacol. 68, 709–714.

Hall, F.S., 1998. Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. Crit. Rev. Neurobiol. 12, 129–162.

Harvey, B.H., Regenass, W., Dreyer, W., Möller, M., 2019. Social isolation rearing-induced anxiety and response to agomelatine in male and female rats: role of corticosterone, oxytocin, and vasopressin. J. Psychopharmacol. 33 (5), 640–646. https://doi.org/10.1177/0269881119826783.

Hlavacova, N., Jezova, D., 2008. Chronic treatment with the mineralocorticoid hormone aldosterone results in increased anxiety-like behavior. Horm. Behav. 54, 90–97. https://doi.org/10.1016/j.yhbeh.2008.02.004.

Hlavacova, N., Solarikova, P., Marko, M., Brezina, I., Jezova, D., 2017. Blunted cortisol response to psychosocial stress in atopic patients is associated with decrease in salivary alpha-amylase and aldosterone: focus on sex and menstrual cycle phase. Psychoneuroendocrinology 78, 31–38. https://doi.org/10.1016/j.psyneuen.2017.01.007.

Hu, Y., Wu, D.L., Luo, C.X., Zhu, L.J., Zhang, J., Wu, H.Y., Zhu, D.Y., 2012. Hippocampal nitric oxide contributes to sex difference in affective behaviors. Proc. Natl. Acad. Sci. U. S. A 109, 14224–14229. https://doi.org/10.1073/pnas.1207461109.

Jezová, D., Guillaume, V., Juránková, E., Carayon, P., Oliver, C., 1994. Studies on the physiological role of ANF in ACTH regulation. Endocr. Regul. 28, 163–169.

Jezova, D., Makatsori, A., Duncko, R., Moncek, F., Jakubek, M., 2004. High trait anxiety in healthy subjects is associated with low neuroendocrine activity during psychosocial stress. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 28, 1331–1336. https://doi.org/10.1016/j.pnpbp.2004.08.005.

Jezova, D., Vigas, M., Hlavacova, N., Kukumberg, P., 2010. Attenuated neuroendocrine response to hypoglycemic stress in patients with panic disorder. Neuroendocrinology 92, 112–119. https://doi.org/10.1159/000283560.

Jiang, C., Lin, W.J., Sadahiro, M., Labonté, B., Menard, C., Pfau, M.L., Tamminga, C.A., Turecki, G., Nestler, E.J., Russo, S.J., Salton, S.R., 2018. VGF function in depression and antidepressant efficacy. Mol. Psychiatry 23, 1632–1642. https://doi.org/10.1038/mp.2017.233.

Kolarow, R., Kuhlmann, C.R., Munsch, T., Zehendner, C., Brigadski, T., Luhmann, H.J., Lessmann, V., 2014. BDNF-induced nitric oxide signals in cultured rat hippocampal neurons: time course, mechanism of generation, and effect on neurotrophin secretion. Front. Cell. Neurosci. 8, 323. https://doi.org/10.3389/fncel.2014.00323.

Leussis, M.P., Andersen, S.L., 2008. Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. Synapse 62, 22–30. https://doi.org/10.1002/syn.20462.

Lin, W.J., Jiang, C., Sadahiro, M., Bozdagi, O., Vulchanova, L., Alberini, C.M., Salton,

S.R., 2015. VGF and its C-terminal peptide TLQP-62 regulate memory formation in Hippocampus via a BDNF-TrkB-dependent mechanism. J. Neurosci. 35, 10343–10356. https://doi.org/10.1523/JNEUROSCI.0584-15.2015.
Lisieski, M.J., Eagle, A.L., Conti, A.C., Liberzon, I., Perrine, S.A., 2018. Single-prolonged stress: a review of two decades of progress in a rodent model of post-traumatic stress disorder. Front. Psychiatry 9, 196. https://doi.org/10.3389/fpsyt.2018.00196.
Lukkes, J.L., Mokin, M.V., Scholl, J.L., Forster, G.L., 2009. Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. Horm. Behav. 55, 248–256. https://doi.org/10.1016/j.yhbeh.2008.10.014.
Marsh, S.E., Blurton-Jones, M., 2017. Neural stem cell therapy for neurodegenerative disorders: the role of neurotrophic support. Neurochem. Int. 106, 94–100. https://doi:10.1016/j.neuint.2017.02.006.
Milad, M.R., Igoe, S.A., Lebron-Milad, K., Novales, J.E., 2009. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience 164, 887–895. https://doi:10.1016/j.neuroscience.2009.09.011.
Murinova, J., Riecansky, I., 2016. Neurodevelopmental rat models of schizophrenia. Activitas Nervosa Superior Rediviva 58, 43–46.
Murínová, J., Hlaváčová, N., Chmelová, M., Riečanský, I., 2017. The evidence for altered BDNF expression in the brain of rats reared or housed in social isolation: a systematic review. Front. Behav. Neurosci. 11, 101. https://doi.org/10.3389/fnbeh.2017.00101.
Nasyrova, R.F., Ivashchenko, D.V., Ivanov, M.V., Neznanov, N.G., 2015. Role of nitric oxide and related molecules in schizophrenia pathogenesis: biochemical, genetic and clinical aspects. Front. Physiol. 11, 6–139. https://doi.org/10.3389/fphys.2015.00139.
Parks, E.A., McMechan, A.P., Hannigan, J.H., Berman, R.F., 2008. Environmental enrichment alters neurotrophin levels after fetal alcohol exposure in rats. Alcohol Clin. Exp. Res. 32, 1741–1751. https://doi.org/10.1111/j.1530-0277.2008.00759.x.
Petrowski, K., Wintermann, G.B., Schaarschmidt, M., Bornstein, S.R., Kirschbaum, C., 2013. Blunted salivary and plasma cortisol response in patients with panic disorder under psychosocial stress. Int. J. Psychophysiol. 88, 35–39. https://doi.org/10.1016/j.ijpsycho.2013.01.002.
Pisu, M.G., Garau, A., Boero, G., Biggio, F., Pibiri, V., Dore, R., Locci, V., Paci, E., Porcu, P., Serra, M., 2016. Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. Neuroscience 320, 172–182. https://doi.org/10.1016/j.neuroscience.2016.02.009.
Pokusa, M., Hlavacova, N., Csanova, A., Franklin, M., Zorad, S., Jezova, D., 2016. Adipogenesis and aldosterone: a study in lean tryptophan-depleted rats. Gen. Physiol. Biophys. 35, 379–386. https://doi.org/10.4149/gpb_2016020.
Ravenelle, R., Santolucito, H.B., Byrnes, E.M., Byrnes, J.J., Donaldson, S.T., 2014. Housing environment modulates physiological and behavioral responses to anxiogenic stimuli in trait anxiety male rats. Neuroscience 270, 76–87. https://doi.org/10.1016/j.neuroscience.2014.03.060.
Regenass, W., Möller, M., Harvey, B.H., 2018. Studies into the anxiolytic actions of agomelatine in social isolation reared rats: role of corticosterone and sex. J. Psychopharmacol. 32, 134–145. https://doi.org/10.1177/0269881117735769.
Simpson, J., Bree, D., Kelly, J.P., 2012. Effect of early life housing manipulation on baseline and drug-induced behavioural responses on neurochemistry in the male rat. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 37, 252–263. https://doi.org/10.1016/j.pnpbp.2012.02.008.
Thakker-Varia, S., Behnke, J., Doobin, D., Dalal, V., Thakkar, K., Khadim, F., 2014. VGF (TLQP-62)-induced neurogenesis targets early phase neural progenitor cells in the adult hippocampus and requires glutamate and BDNF signaling. Stem Cell Res. 12, 762–777. https://doi.org/10.1016/j.scr.2014.03.005.
Varty, G.B., Braff, D.L., Geyer, M.A., 1999. Is there a critical developmental 'window' for isolation rearing-induced changes in prepulse inhibition of the acoustic startle response? Behav. Brain Res. 100, 177–183. https://doi.org/10.1016/S0166-4328(98)00129-6.
Varty, G.B., Paulus, M.P., Braff, D.L., Geyer, M.A., 2000. Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biol. Psychiatry 47, 864–873. https://doi.org/10.1016/S0006-3223(99)00269-3.
Walker, D.M., Cunningham, A.M., Gregory, J.K., Nestler, E.J., 2019. Long-term behavioral effects of post-weaning social isolation in males and females. Front. Behav. Neurosci. 13, 66. https://doi.org/10.3389/fnbeh.2019.00066.
Wegener, G., Volke, V., 2010. Nitric oxide synthase inhibitors as antidepressants. Pharmaceuticals 3, 273–299. https://doi.org/10.3390/ph3010273.
Weiss, I.C., Pryce, C.R., Jongen-Rêlo, A.L., Nanz-Bahr, N.I., Feldon, J., 2004. Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav. Brain Res. 152, 279–295. https://doi.org/10.1016/j.bbr.2003.10.015.
Williams, A.J., Umemori, H., 2014. The best-laid plans go oft awry: synaptogenic growth factor signaling in neuropsychiatric disease. Front. Synaptic Neurosci. 6, 4. https://doi.org/10.3389/fnsyn.2014.00004.
Zhou, Q.G., Zhu, X.H., Nemes, A.D., Zhu, D.Y., 2018. Neuronal nitric oxide synthase and affective disorders. IBRO Rep 5, 116–132. https://doi.org/10.1016/j.ibror.2018.11.004.
Zlatković, J., Filipović, D., 2013. Chronic social isolation induces NF-κB activation and upregulation of iNOS protein expression in rat prefrontal cortex. Neurochem. Int. 63, 172–179. https://doi.org/10.1016/j.neuint.2013.06.002.
Zlatković, J., Bernardi, R.E., Filipović, D., 2014. Protective effect of Hsp70i against chronic social isolation stress in the rat hippocampus. J. Neural Transm. 121, 3–14. https://doi.org/10.1007/s00702-013-1066-1.