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Research report

Dual influences of early life stress induced by limited bedding on walking adaptability and *Bdnf/TrkB* and *Drd1/Drd2* gene expression in different mouse brain regions

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ABSTRACT

Introduction Evidence suggests early life stress impairs development, quality of life and increases vulnerability to disease. One important aspect of the stress experience is its impact on cognitive-motor performance, which includes the ability to adapt walking according to the environmental conditions. This study aimed to investigate how early-life stress affects walking adaptability of mice, while investigating BDNF/TrkB and Drd1/Drd2 expression in different brain regions.

Methods Briefly, we exposed male C56BL/6 to the limited bedding protocol (LB) from post-natal day (PND) 2 to PND9 and then tested animals in the ladder walking task at PND60. RT-qPCR was used to investigate gene expression in the mPFC, hippocampus, motor cortex and cerebellum 2 h after the task

Results LB induced a wide range of variability and therefore two distinct subgroups of animals within the LB group were established: a) superior performance (LB-SP); and b) inferior performance (LB-IP), compared to controls. Additionally, *Drd1* gene expression was increased in the mPFC of LB-IP animals and in the cerebellum of LB-SP animals, while *Drd2* expression was reduced in the hippocampus of the LB-IP group. *BDNF exon IV* gene expression in the mPFC and motor cortex was increased in both the LB-IP and LB-SP subgroups. *TrkB* gene expression in the hippocampus was reduced in the LB-IP group. A strong negative correlation was found between walking adaptability performance and *BDNF exon IV* gene expression in the motor cortex. Conversely, a positive correlation was found between walking adaptability performance and TrkB expression in the mPFC and a negative correlation in the hippocampus. Both *Drd1* and *Drd2* gene expression were negatively correlated with the ability to adapt walking.

Conclusions Overall, our findings suggest exposure to early life stress leads to distinct walking adaptability phenotypes, which may be related to *Drd1*, *Drd2*, *Bdnf exon IV* and *TrkB* gene expression in brain regions that influence walking adaptability.

1. Introduction

The impact of stress and social vulnerability on neural development has been studied for many years, since they decrease quality of life and increase vulnerability to a wide range of diseases [1,2]. Deprivation of basic resources such as food and shelter has critical consequences during childhood. Premature birth and being exposed to stressful environment in the first three years of life increases the likelihood of growth, intelligence and behavior-related problems [3]. Also, children submitted to constant stressors tend to experience inadequate neurodevelopment [4], which seems to change their performance in skilled motor tasks [5] and physical activity engagement [6,7].

Whilst vulnerability is linked with stress exposure, not all individuals that experience stressful life events, trauma or chronic

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adversity will present this vulnerability-like phenotype [8]. An early study led by Bradley in 1994 found children living in very stressful conditions showed early signs of resilience, functioning in the normal range for cognitive, health and growth parameters at age three [9]. However, the resilience-related mechanisms that explain these findings remain unclear.

Although clinical studies show stress may influence skilled tasks performance in children, few experiments have attempted to shed light on the neurobiological mechanisms involved. For instance, chronic pharmacological activation of glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) led to impairments in a skilled-reaching motor task in rats [10]. More recently, Kokubo et al. showed early-life stress exposure resulted in motor coordination dysfunction in adult mice [11]. Moreover, the dopaminergic connection between the cerebellum and basal ganglia constitutes an important pathway within the motor control system [12]. Additionally, dopamine activity in the motor cortex has been shown to play a major role in regulating motor learning and motor-cortex plasticity [13]. Using antagonists to block dopamine in the motor cortex induced a decrease in long-term potentiation, which is an important mechanism in learning [13,14].

Synergistically with dopaminergic neurotransmission, BDNF plays a role in memory and learning, as well as in synaptic plasticity and neurogenesis [15]. For instance, Fritsch and cols. have shown activity-induced BDNF and TrkB activation in the primary motor cortex are required for the acquisition of new motor skills [16]. Indeed, the loss of BDNF has been linked to neurodegenerative diseases such as Parkinson's Disease, Huntington's Disease and Amyotrophic Lateral Sclerosis [17]. BDNF levels and its receptor TrkB are crucial for motor development [14,17] and may provide insights into the relationship between early life stress and walking adaptability. Thus, a better understanding of the mechanisms behind early life stress exposure could contribute towards reducing the negative effects such stress has on motor control.

However, to the best of our knowledge, no previous studies have investigated the impact of early life stress on walking adaptability in the adulthood. In the same way, whether dopaminergic and BDNF gene expression in the medial pre-frontal cortex, motor cortex, cerebellum and hippocampus influence walking adaptability is still unknown. Therefore, the present exploratory study was designed to address these issues.

2. Material and methods

The present study was conducted in accordance with the guidelines of the Brazilian Association for Laboratory Animal Sciences and all the procedures described above were approved by the local Ethics Committee for Animal Research under the #15/00475. In this study, adult female C57BL/6 acquired from CEMBE/PUCRS were mated with a male for 48 h. The day of birth of the resulting litters was considered post-natal day (PND) 1. From PND2 to PND9, the pups were exposed to either early life stress induced by limited bedding or control condition (described in detail below) and weaned at PND21, when they were placed with same-sex littermates in 2–3 per cage. At P60, animals were assessed using the ladder walking task (described in detail below). A total of 20 litters were used (10 × 10) and to avoid any potential litter effects, no more than 2 animals per litter were used. Animals from the same litter were assigned to additional ongoing research projects. The animals were maintained in automatically controlled room (temperature 23 °C + -1 °C and 12 h light/12 h dark cycle) with mouse chow and water *ad libitum* throughout the entire study.

The early life stress induced by limited bedding was performed based on a previously described protocol [18,19]. The cage floor was covered with bedding to absorb feces and urine and a stainless steel wire floor was placed 2 cm above the floor. The 0.6 mm gauge wire floor has 10 mm squared openings. Dams were unable to retrieve bedding from the floor to build nests, however 1 g of autoclaved cotton was provided for this purpose. In the control group cages, there was no wire floor and the normal amount of bedding (~150 g) was provided in addition to 4 g of cotton for nest building. On PND2, dams were assigned to either limited bedding protocol or control conditions. Briefly, dams were firstly removed from the home cage. Then, pups were removed, one-by-one by hand from the home cage, weighed and placed in a new cage. The dam and the litter were left undisturbed until PND9, when they were removed from the limited bedding condition and returned to a cage similar to that of controls. In addition, the cages were cleaned on a PND16 when the bedding was replaced. Dams from control conditions were left undisturbed, except for regular cage cleaning on PND2, PND9 and PND16.

To assess the ability to adapt walking of the mice, we used the adapted version of the ladder walking task, as previously described [20,21]. Briefly, mice were encouraged to cross a 1 m long horizontal ladder with variably spaced rungs situated 30 cm above the floor. During the task session, mice were recorded crossing the ladder three times using a GoPro Hero 4 with 12mp and an acquisition rate of 240 FPS. The first trial was considered habituation and was discounted from the analysis. Thus, the performance score was calculated using the average number of total errors (forelimb errors + hindlimb errors) committed on trials 2 and 3. The numbers of errors were normalized to the control group using the following formula: (Number of total errors/ Mean of total errors from control group) * 100. Hence, a percentage (%) lower than 100% means that animals committed fewer errors than controls, thus performing better. A percentage higher than 100% means that animals committed more errors than controls, thus performing worse.

Locomotor activity and measures of anxiety-like behaviors were evaluated with the open field (OF) and elevated plus maze (EPM) tests. In the OF, the animals were placed in the center of a squared Plexiglas box (33 × 33 cm) and allowed to explore for 5 min. Video was recorded with a professional video camera and then analyzed using AnyMaze Software (Stoelting CO, Wood Dale, IL, USA). The box was divided in the software into 16 squares, where 4 center squares were defined as the central zone and the other 12 squares as the peripheral zone. Outcomes measures included the total distance travelled (in meters), time spent in the center zone, time in the peripheral zone and number of rearings. The EPM apparatus was constructed with black Plexiglas and consisted of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) connected via a central platform (5 cm × 5 cm). The apparatus was raised to a height of 50 cm above the ground. Mice were transported to the experimental room and left undisturbed for 30 min prior to testing. They were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The number of entries in each arm, the time spent in the open or closed arms, as well as in the center of the maze was recorded. In addition, the frequency of the following RA behaviors was recorded: 1) when mice dipped their heads below the level of the maze floor (head dipping); 2) when stretching the head/shoulders from the center of the maze towards open arms (peeping out); and 3) when the animal stretches to its full length with the forepaws keeping the hind paws in the same place and turns back to the anterior position while exploring the center of the maze (stretched-attend posture). The sum of these behaviors were computed as RA behaviors in the EPM. All EPM data were video recorded and the time spent in each arm, as well as the number of entries were analyzed automatically using the Anymaze Software (Stoelting CO, Wood Dale, IL, USA). Moreover, one independent observer blinded to the rearing conditions recorded the RA behaviors manually.

Mice brains were collected 2 h after the last trial in the ladder walking task and rapidly hand-free dissected on ice to access the medial Pre-Frontal Cortex (mPFC), Motor Cortex and Cerebellum. After dissection, brain regions were snap frozen in dry ice and maintained at -80 °C until analysis. Total RNA were isolated from tissue using Trizol® (Qiagen - Hilden, Germany) according to the manufacturer's protocol and reconstituted in 20 uL of RNase-free water. The concentration of RNA was measured using NanoDrop (Thermo Fisher – Waltham, USA)

Table 1
Primers used in RT-qPCR analysis.

Gene	Forward	Reverse	Size (bp)
GAPDH	5'-TCATATTTCTCGTGGTTCACACC-3'	5'-CTGAGTATGTCGTGGAGTCTACTGG-3'	149
Bdnf exon IV	5'-GCAGCTGCCTTGATGTTTAC-3'	5'-GCATGGCATAGTAGTTGTAGTGG-3'	147
TrkB	5'-CTCGGTAGCTGGAAGCACAT-3'	5'-GGACTCTTTGGGTCGCAGAA-3'	155
Drd1	5'-ATGGCTCCTAACACTTCTACCA-3'	5'-GGGTATTCCCTAAGAGAGTGGAC-3'	124
Drd2	5'-ACCTGTCCTGGTACGATGATG-3'	5'-GCATGGCATAGTAGTTGTAGTGG-3'	105

Legends. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; Bdnf: Brain derived neurotrophic factor gene, exon IV; TrkB: tyrosine receptor kinase B gene; Drd1: Dopamine receptor D1 gene; Drd2: Dopamine receptor D2 gene; bp: base pair.

and a total of 500 ng of RNA from each sample was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems - Foster City, USA). The cDNA used in each real-time quantitative PCR (RT-qPCR) reaction in the RotorGene (Qiagen - Hilden, Germany) machine was processed with the miScript SYBR green kit (Qiagen - Hilden, Germany).

The primers used for RT-qPCR analysis are described in Table 1. Gradient PCR and DNA gels were checked for specificity analysis, as well as melting curve analysis. Each RT-qPCR was run in duplicate for each sample and repeated once. The fold-change relative expression was calculated using the ΔΔCt method (with the control group as reference) and the GAPDH Ct values as endogenous controls for mRNA analysis.

All statistical analyses were performed using the SPSS 20.0 (IBM – New York, USA) and the graphs were constructed using the Prism GraphPad 6.0 (La Jolla, USA). Data normality distribution was analyzed using the Shapiro-Wilk's test. In order to analyze the effects of early life stress on walking adaptability and gene expression, one-way ANOVA was performed followed by Tukey's multiple comparison adjustments. To investigate whether the behavior correlated with gene expression, Pearson's correlation was used. In all the analyses, data are expressed as mean ± SEM and the level of statistical significance was set at 5%.

3. Results

In the ladder walking task, the average percentage of errors in the control group was 100.1 ± 13.13 (n = 13). The animals exposed to early life stress showed distinct patterns of error in this task, and were therefore divided into two subgroups: a) superior performance (LB-SP) - mice showing at least one standard deviation below the mean of the control group (n = 6, showed fewer errors during the task) and b) inferior performance (LB-IP) - mice exhibiting at least one standard deviation above mean of the control group (n = 7, showing more errors during the task). Another six animals exposed to early life stress performed similarly to the controls (performance score between 86.97-113.23) and therefore were not included in the analysis. These selection criteria were adapted from a previous study led by Albuquerque Filho et. al. [22].

One-way ANOVA showed a significant difference among groups when comparing the performance in the ladder walking task [Fig. 1; $F(2,23) = 28.99$, $p < 0.0001$), as expected. Subsequent analyses using Tukey's post hoc test confirmed that animals in the LB-SP group had fewer errors relative to the control group ($p < 0{,}001$). Animals in the LB-IP group committed more errors relative to controls ($p < 0{,}01$). No between-group effects were found when analyzing the open field and elevated plus maze tests ($p \geq 0.09$) (Table 2). Together, the results suggest animals did not exhibit gross motor/locomotor deficits, reinforcing the argument that early-life stress probably changes the mechanisms involved in the ability to adapt walking, but not of the overall motor function.

Results related to gene expression in the mPFC, hippocampus, motor cortex and cerebellum are shown in Fig. 2. Unfortunately, samples from some animals were not approved in the standardization and quality control and therefore were excluded from the assessment. To

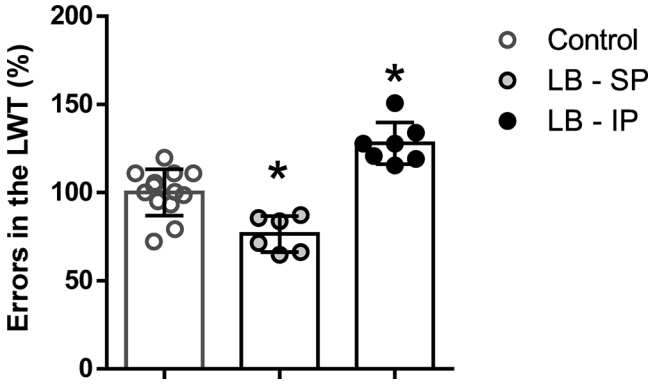

Fig. 1. Walking adaptability of mice exposed to early life stress induced by limited bedding. Performance in the task is presented as percentage of errors compared to the control group. The average (%) of errors from the control group was 100,1 ± 13.13 (n = 13); Superior Performance n = 6; Inferior Performance n = 7. Data are expressed as mean ± SEM; * $p < 0.05$ compared to the control group. LB – IP: limited bedding group, inferior performance; LB – SP: limited bedding group, superior performance. LWT: ladder walking task.

investigate the connection between the expression of dopaminergic receptors and walking adaptability, we first assessed Drd1 gene expression. We observed Drd1 expression was increased in the mPFC of the LB-IP animals [$F(2,16) = 9.89$, $p = 0.0016$)] (Fig. 2A). No differences were found in the hippocampus and motor cortex (Fig. 2B and C, respectively). We also showed Drd1 increased in the cerebellum of the LB-SP group compared to controls [$F(2,15) = 6.97$, $p = 0.007$)] (Fig. 2D). Secondly, regarding the Drd2 expression there were no between-group differences in the mPFC, motor cortex and cerebellum [$F(2,17) = 0.03$, $p = 0.96$; $F(2,15) = 0.02$, $p = 0.97$ and $F(2,16) = 1.35$, $p = 0.28$, respectively] (Fig. 2D,F and G). Moreover, Drd2 gene expression in the hippocampus was reduced in the LB-IP group when compared to the controls [$F(2,16) = 7.03$ $p = 0.006$] (Fig. 2E). Interestingly, we found a negative correlation between the expression of both Drd1 and Drd2 in the cerebellum and errors in the ladder walking task, which suggests lower cerebellar Drd1 and Drd2 gene expression may be associated with poor performance in adaptive walking (Table 3).

One-way ANOVA showed a significant difference in the mPFC for BDNF exon IV gene expression [$F(2,16) = 7.18$, $p = 0.005$)]. Tukey's post-hoc analyses revealed both the LB-SP and LB-IP groups increased the expression within the mPFC when compared to controls ($p < 0.05$ and $p < 0{,}01$, respectively) (Fig. 2H). A trend for increased BDNF exon IV gene expression in the hippocampus was observed in this study [$F(2,16) = 2.95$, $p = 0.08$)] (Fig. 2I). BDNF exon IV expression in the motor cortex was significantly different between the LB-SP and LB-IP groups [$F(2,15) = 7.86$, $p = 0.004$); Tukey's post-hoc $p < 0.01$] (Fig. 2J). No between-group differences for this gene were observed in the cerebellum [$F(2,15) = 1.68$, $p = 0.21$)] (Fig. 2K). A strong negative correlation was found between BDNF exon IV expression in the motor cortex and walking adaptability (Table 3). This finding suggests lower expression of this transcript in the motor cortex is also associated

Table 2
Between-group performance (mean ± SEM) in the open field and elevated plus maze tests. LB: Limited bedding protocol; LB-SP: limited bedding group showing superior performance in the ladder walk test; LB-IP: limited bedding group showing inferior performance in the ladder walk test; Sig: significance level. SEM: standard error. NS: Not statistically significant in the one-way ANOVA.

Variable	Control group	LB-SP group	LB-IP group	Sig.
Open field				
Travelled distance (meters)	110.97 ± 8.89	112.73 ± 10.62	118.90 ± 7.85	0.80 (NS)
Time - central zone (sec)	38.43 ± 5.81	31.98 ± 6.86	21.84 ± 3.25	0.09 (NS)
Time - peripheral zone (sec)	261.57 ± 5.81	268.02 ± 6.86	278.14 ± 3.25	0.09 (NS)
Rearing (number of events)	24.00 ± 2.96	23.83 ± 1.74	23.71 ± 1.25	0.99 (NS)
Elevated Plus Maze				
Number of entries (open arms)	1.43 ± 0.43	0.83 ± 0.16	1.57 ± 0.43	0.38 (NS)
Number of entries (closed arms)	10.14 ± 1.10	10.33 ± 1.23	11.57 ± 1.11	0.28 (NS)
Time in the closed arms (sec)	267.71 ± 6.43	275.17 ± 7.21	258.50 ± 7.35	0.11 (NS)
Time in the opened arms (sec)	13.71 ± 4.53	4.33 ± 1.60	18.50 ± 5.60	0.93 (NS)
Time in the central zone (sec)	18.57 ± 3.48	20.50 ± 6.76	21.00 ± 4.50	0.63 (NS)
Risk assessment (number of events)	19.14 ± 2.70	21.00 ± 6.51	18.00 ± 2.08	0.72 (NS)

with poor performance in adaptive walking.

Finally, we measured the expression of TrkB, a BDNF receptor, in the same brain regions. A one-way ANOVA revealed decreased TrkB gene expression in the hippocampus of LB-IP animals when compared to the controls [$F(2,16) = 7.03$, $p < 0.05$)] (Fig. 2M). No additional between-group differences were found ($p < 0.40$) (Fig. 2L,N,O). Furthermore, a positive correlation between TrkB expression in the mPFC and the ladder walking task performance was found while a negative correlation was found in the hippocampus, which suggests TrkB gene expression modulation in different brain regions may play a role to adapt walking (Table 3).

4. Discussion

In this study, we have used a mouse model to investigate: a) the impact of stress early in life on walking adaptability in adulthood; and b) whether the early life stress-related changes in dopaminergic receptors (*Drd1 and Drd2*) and *Bdnf* gene expression in the medial prefrontal cortex, motor cortex, cerebellum and hippocampus influence walking adaptability. As expected, animals responded differently to the effects of early life stress induced by the limited bedding protocol - some animals showed a superior performance in the ladder walking task, while other animals showed an inferior performance. This finding suggests that some individuals exposed to stressful conditions are more resilient and maintain normal physiological and behavioral responses. Whilst several reports have described the effects of early life stress on animal physiology and behavior, little is known about the stress response of individuals [23]. Moreover, the biological mechanisms underlying the different responses after suffering threats are still unclear. Our study has started to fill this gap in the literature by subdividing the animals based on their performance in the ladder walking task and trying to establish some neurobiological correlations to guide this research field. Indeed, the open filed and elevated plus maze tests suggested animals did not exhibit gross motor/locomotor deficits, reinforcing the early-life stress probably change the mechanisms involved in the ability to adapt walking, but not of the overall motor function.

The mPFC establishes stress-related behaviors [24] and plays a role in adaptive walking [25]. We observed the LB-IP group increases Drd1 expression in mPFC, whereas early life stress increased BDNF exon IV expression in both the LB-IP and LB-SP groups. No differences were found for Drd2 expression. On the one hand, dopamine has both positive and negative effects on cognition, exhibiting an inverted U-shaped dose-response curve [25]. Gene expression changes in the dopaminergic signaling pathway are linked with the resilient/vulnerability profile, which is in agreement with our data [26]. In addition, we also found a negative correlation between cerebellar Drd1 gene expression and walking adaptability performance in mice. The literature shows dysfunction in dopaminergic signaling impairs gait automatism by modifying cerebellar processing [27], which is aligned with the current results. On the other hand, stress triggers an activity-dependent BDNF upregulation, as seen in the mPFC results, probably acting as a compensatory mechanism to recover and reestablish neuronal function and prevent neuronal loss [28–30]. However, the performance in the ladder walking task was uncorrelated with the expression of both Drd1 and BDNF exon IV genes in the mPFC. Hence, studies assessing other genes are needed to clarify how the mPFC acts to control walking adaptability.

To the best of our knowledge, this is the first study to show a positive correlation between BDNF exon IV expression in the motor cortex and performance in adaptive walking. In other words, higher levels of BDNF exon IV in the motor cortex may induce more precise walking. This hypothesis is supported by previous studies, since BDNF mediates important downstream signaling pathways involved in learning and memory, such as the long-term potentiation [31] and TrkB activation [32]. Thus, walking adaptability might involve BDNF exon IV as a signaling mediator in the motor cortex, which is indeed a matter for further investigation.

Whilst the TrkB expression remained the same for all studied groups, we found a positive correlation between TrkB mRNA expression in the mPFC and errors in adaptive walking. This finding suggests TrkB signaling in the mPFC may play a role in linking higher brain functions (such as working memory and attention) to the process of adjusting interlimb coordination to the environmental context. BDNF and other neurotrophic factors such as NT-3 and NT-4 (involved in learning and brain plasticity) bind with high affinity to TrkB receptors [33]. Additionally, Choi et al 2012 has shown inhibiting both BDNF and TrkB in the mPFC impairs the formation of memory [34], which may be required to adjust and adapt walking. Notwithstanding, further studies blocking TrkB receptors in the mPFC (using techniques such as lentiviral-mediated knockdown or antagonist administration directly in the mPFC) are needed to establish a causal relationship between TrkB activation and adaptive walking.

Timing of early life stress experience may influence neurogenesis, gliogenesis, synaptogenesis, oligodendrocyte maturation and some behavioral patterns that coincide with developmental-related changes. While mouse brain volume is stable by approximately post-natal week three, myelination increases until post-natal month six [35]. Thus, in the first post-natal week (when the LB protocol was used) all the studied brain regions (mPFC, motor cortex, cerebellum and hippocampus) are undergoing plastic activity and are not fully matured. Since walking adaptability is influenced by prefrontal cortex, cerebral cortex, cerebellum and hippocampal activity [36–38], we can speculate early life stress might change the maturation/functional connectivity of these structures in vulnerable subjects and therefore influence their ability to adapt walking later in life. However, the relative weight of each neural mechanism/structure on the ability to adapt walking after stressful

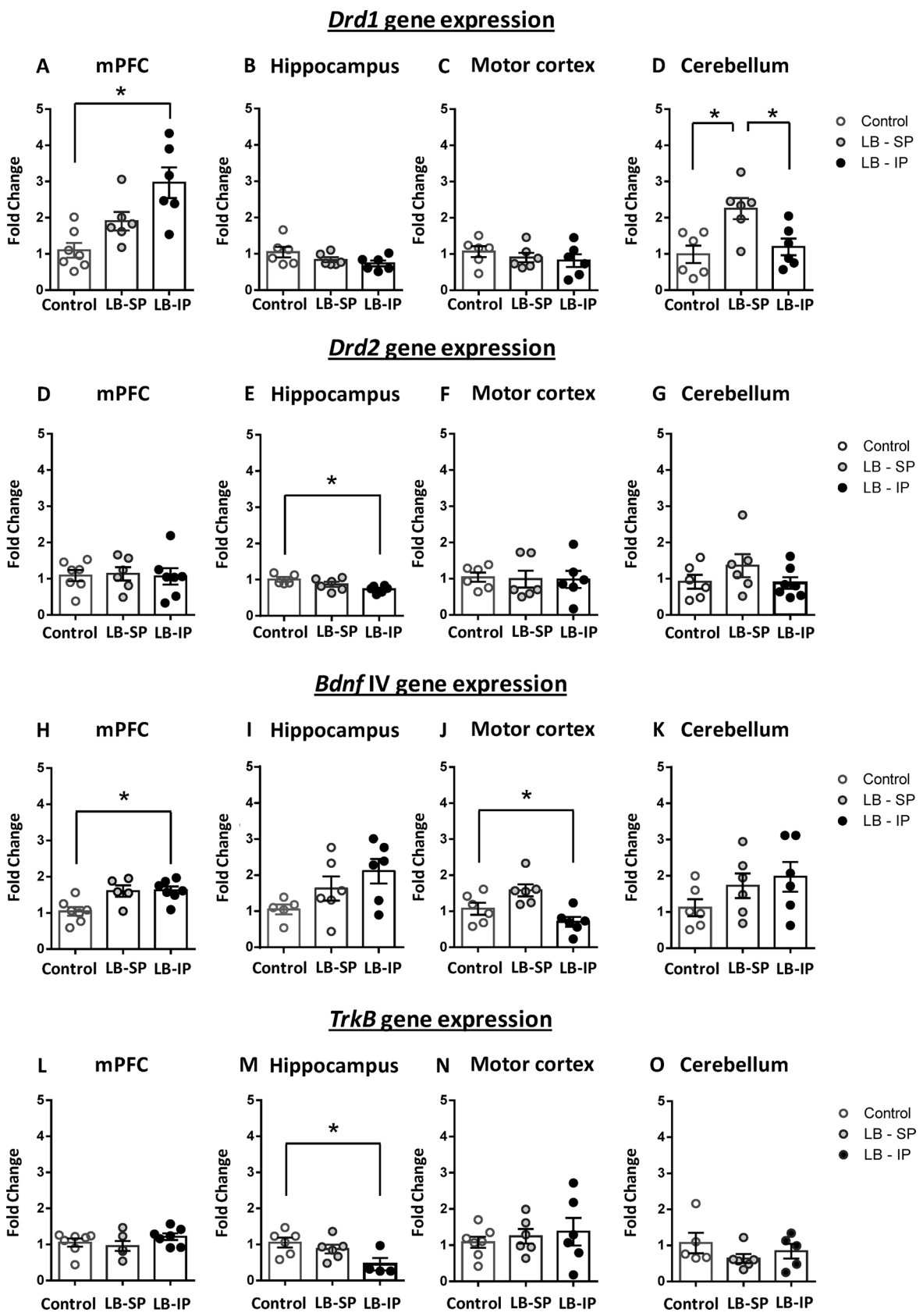

Fig. 2. Drd1, Drd2, BDNF exon IV and TrkB gene expression in the mPFC, hippocampus, motor cortex and cerebellum 2 h after the ladder walking task. A-D) Expression of Drd1 mRNA gene levels. E-H) Expression of Drd2 mRNA gene levels; I-L) Expression of BDNF exon IV gene levels; M-O) Expression of TrkB mRNA gene levels. The fold-change relative expression calculation with ΔΔCt method was used with the control group as reference and the Pgk Ct values as endogenous controls for mRNA analysis; Control group n = 7; Superior Performance n = 6; Inferior Performance n = 7 Data are expressed as mean ± SEM; *significant between-group difference at $p < 0.05$.

Table 3
Pearson's correlation index between the percentage of errors in the ladder walking task and gene expression (*Drd1, Drd2, Bdnf IV, TrkB*) in the studied brain regions. r: Pearson's correlation index; LWT: ladder walking task; Sig.: significance level. * statistically significant difference.

Structure / Gene expression	% Errors (LWT)	Sig.
mPFC – *Drd1*	r = 0.35	0.14
mPFC – *Drd2*	r = - 0.16	0.51
mPFC – *Bdnf IV*	r = 0.07	0.766
mPFC – *TrkB*	r = 0.52	0.021*
Hippocampus – *Drd1*	r = - 0.30	0.22
Hippocampus – *Drd2*	r = - 0.40	0.11
Hippocampus – *Bdnf IV*	r = 0.29	0.25
Hippocampus – *TrkB*	r = - 0.51	0.04*
Motor Cortex – *Drd1*	r = - 0.12	0.63
Motor Cortex – *Drd2*	r = - 0.29	0.24
Motor Cortex – *Bdnf IV*	r = - 0.71	0.001*
Motor Cortex – *TrkB*	r = 0.02	0.95
Cerebellum – *Drd1*	r = - 0.63	0.005*
Cerebellum – *Drd2*	r = - 0.52	0.02*
Cerebellum – *Bdnf IV*	r = 0.26	0.29
Cerebellum – *TrkB*	r = 0.25	0.35

experiences is still a matter for further investigation.

We observed up-regulation of BDNF exon IV in the mPFC and a trend of significance in the hippocampus in response to limited bedding experience, regardless of walking adaptability performance. The expression of this specific transcript is dynamic and activity-dependent [39]. Multiple cellular mechanisms can engage and regulate BDNF exon IV expression in an experience-dependent manner, which results in increased or decreased expression [40–42]. On one hand, the pioneer studies looking at the effects of early life stress on BDNF regulation showed stress reduces BDNF and leads to impaired neurogenesis and plasticity [43]. One the other hand, more recently, experiments have shown the patterns of BDNF expression following early life stress are complex and can be influenced by a number of factors such as developmental stage, duration and severity of stress, stress paradigm, studied brain areas, sex and genetic background of the animals [44]. Contrasting with the idea that early life stress decreases BDNF, other studies have shown rats exposed to an early in life maternal separation model exhibit increased BDNF expression in the hippocampus but decreased BDNF expression in the mPFC [45]. Suri et al. also found BDNF is increased in the hippocampus of early life stress-exposed animals [46]. Clearly, early life stress has an impact on the neurotrophic signaling pathway.

However, the behavioral consequences of these differential expression patterns require further investigation. It may be the case that reduced BDNF exon IV gene expression in the motor cortex of the subjects vulnerable to stress indicates they had limited plasticity in this structure, which might explain the greater difficulty they had to adapt walking. Several studies in humans have suggested motor difficulties increase the demand on attentional and cognitive systems to overcome their problems to adapt walking [47,48]. In addition, limb coordination while walking is influenced by cognitive load [47]. In our mice, BDNF exon IV gene expression may have increased in the mPFC and hippocampus as a compensatory mechanism in an attempt to maintain motor performance. However, further studies are needed to confirm this preliminary hypothesis.

This study has some limitations. Firstly, only male mice were studied, which prevents the generalization of the current results for female animals. Further studies comparing the influence of sexual dimorphism on the ability to adapt walking should be performed to address this issue. Secondly, we investigated molecular targets looking at the gene expression layer. Although useful as an exploratory screening, further studies using Western blotting and microdialysis techniques may be useful to address the proteins at cell-type level and the levels of dopamine while the rodents are walking, respectively, to better determine the function of the studied brain areas. Furthermore, using specific receptor antagonists or agonists could help to establish a cause-effect relationship. Finally, the free-hand technique of brain dissection prevents sub region analyses, which should be considered when generalizing the molecular results. Additional studies assessing mid-performers and dividing controls into SP and IP groups might also help to elucidate whether the behavioral variability found in this study is an individual survival strategy or a subgroup behavior pattern.

5. Conclusion

Together, our findings suggest an early in life stressful condition can lead to distinct phenotypes (superior or inferior) of walking adaptability in adolescent mice. In addition, motor cortex *Bdnf* exon IV, cerebellar Drd1 and Drd2, *mPFC TrkB and* hippocampus *TrKB* gene expressions were correlated with walking adaptability performance and may play a role in its neural control. Further studies following the findings raised here should provide more conclusive evidence relating early life stress and walking adaptability.

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References

[1] E. Mendenhall, B.A. Kohrt, S.A. Norris, D. Ndetei, D. Prabhakaran, Non-communicable disease syndemics: poverty, depression, and diabetes among low-income populations, Lancet 389 (10072) (2017) 951–963.
[2] N. Spencer, L. Strazdins, Socioeconomic disadvantage and onset of childhood chronic disabling conditions: a cohort study, Arch. Dis. Child. 100 (4) (2015) 317–322.
[3] R.H. Bradley, R.F. Corwyn, Socioeconomic status and child development, Annu. Rev. Psychol. 53 (2002) 371–399.
[4] D.A. Hackman, M.J. Farah, M.J. Meaney, Socioeconomic status and the brain: mechanistic insights from human and animal research, Nat. Rev. Neurosci. 11 (9) (2010) 651–659.
[5] D. Morley, K. Till, P. Ogilvie, G. Turner, Influences of gender and socioeconomic status on the motor proficiency of children in the UK, Hum. Mov. Sci. 44 (2015) 150–156.
[6] K.S. Iivonen, A.K. Saakslahti, A. Mehtala, J.J. Villberg, T.H. Tammelin, J.S. Kulmala, et al., Relationship between fundamental motor skills and physical activity in 4-year-old preschool children, Percept. Mot. Skills 117 (2) (2013) 627–646.
[7] L.E. Robinson, D.D. Wadsworth, C.M. Peoples, Correlates of school-day physical activity in preschool students, Res. Q. Exerc. Sport 83 (1) (2012) 20–26.
[8] G. Wu, A. Feder, H. Cohen, J.J. Kim, S. Calderon, D.S. Charney, et al., Understanding resilience, Front. Behav. Neurosci. 7 (2013) 10.
[9] R.H. Bradley, L. Whiteside, D.J. Mundfrom, P.H. Casey, K.J. Kelleher, S.K. Pope, Early indications of resilience and their relation to experiences in the home environments of low birthweight, premature children living in poverty, Child Dev. 65 (2 Spec No) (1994) 346–360.
[10] N.M. Jadavji, R.D. Supina, G.A. Metz, Blockade of mineralocorticoid and glucocorticoid receptors reverses stress-induced motor impairments, Neuroendocrinology 94 (4) (2011) 278–290.
[11] M. Kokubo, S. Toya, I. Amano, Y. Takatsuru, Early-life stress induces motor coordination dysfunction in adult mice, J. Physiol. Sci. (2017).
[12] P. Giompres, F. Delis, Dopamine transporters in the cerebellum of mutant mice, Cerebellum 4 (2) (2005) 105–111.
[13] K. Molina-Luna, A. Pekanovic, S. Röhrich, B. Hertler, M. Schubring-Giese, M.S. Rioult-Pedotti, et al., Dopamine in motor cortex is necessary for skill learning and synaptic plasticity, PLoS One 4 (9) (2009) e7082.
[14] M.S. Rioult-Pedotti, A. Pekanovic, C.O. Atiemo, J. Marshall, A.R. Luft, Dopamine promotes motor cortex plasticity and motor skill learning via PLC activation, PLoS One 10 (5) (2015) e0124986.
[15] C. Cunha, R. Brambilla, K.L. Thomas, A simple role for BDNF in learning and memory? Front. Mol. Neurosci. 3 (1) (2010).
[16] B. Fritsch, J. Reis, K. Martinowich, H.M. Schambra, Y. Ji, L.G. Cohen, B. Lu, Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning, Neuron 66 (2) (2010) 198–204.
[17] Y.Y. He, X.Y. Zhang, W.H. Yung, J.N. Zhu, J.J. Wang, Role of BDNF in central motor structures and motor diseases, Mol. Neurobiol. 48 (3) (2013) 783–793.
[18] C.J. Rice, C.A. Sandman, M.R. Lenjavi, T.Z. Baram, A novel mouse model for acute and long-lasting consequences of early life stress, Endocrinology 149 (10) (2008)

4892–4900.
[19] H. Heun-Johnson, P. Levitt, Early-life stress paradigm transiently alters maternal behavior, dam-pup interactions, and offspring vocalizations in mice, Front. Behav. Neurosci. 10 (2016) 142.
[20] B.J. Cummings, C. Engesser-Cesar, G. Cadena, A.J. Anderson, Adaptation of a ladder beam walking task to assess locomotor recovery in mice following spinal cord injury, Behav. Brain Res. 177 (2) (2007) 232–241.
[21] G.A. Metz, I.Q. Whishaw, The ladder rung walking task: a scoring system and its practical application, J. Vis. Exp. 28 (2009).
[22] M.O. Albuquerque Filho, B.S. de Freitas, R.C. Garcia, P.C. Crivelaro, N. Schroder, M.N. de Lima, Dual influences of early-life maternal deprivation on histone deacetylase activity and recognition memory in rats, Neuroscience 344 (2017) 360–370.
[23] R. Yehuda, J.D. Flory, S. Southwick, D.S. Charney, Developing an agenda for translational studies of resilience and vulnerability following trauma exposure, Ann. N. Y. Acad. Sci. 1071 (2006) 379–396.
[24] H. Shimada, K. Ishii, K. Ishiwata, K. Oda, M. Suzukawa, H. Makizako, et al., Gait adaptability and brain activity during unaccustomed treadmill walking in healthy elderly females, Gait Posture 38 (2) (2013) 203–208.
[25] J. Savitz, M. Solms, R. Ramesar, The molecular genetics of cognition: dopamine, COMT and BDNF, Genes Brain Behav. 5 (4) (2006) 311–328.
[26] E. Azadmarzabadi, A. Haghighatfard, A. Mohammadi, Low resilience to stress is associated with candidate gene expression alterations in the dopaminergic signalling pathway, Psychogeriatrics 18 (May (3)) (2018) 190–201.
[27] M. Gilat, P.T. Bell, K.A. Ehgoetz Martens, M.J. Georgiades, J.M. Hall, C.C. Walton, S.J.G. Lewis, J.M. Shine, Dopamine depletion impairs gait automaticity by altering cortico-striatal and cerebellar processing in Parkinson's disease, Neuroimage 15 (May (152)) (2017) 207–220.
[28] F. Zheng, X. Zhou, Y. Luo, H. Xiao, G. Wayman, H. Wang, Regulation of brain-derived neurotrophic factor exon IV transcription through calcium responsive elements in cortical neurons, PLoS One 6 (12) (2011) e28441.
[29] S.S. Shi, S.H. Shao, B.P. Yuan, F. Pan, Z.L. Li, Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus, Yonsei Med. J. 51 (5) (2010) 661–671.
[30] M.A. Smith, S. Makino, S.Y. Kim, R. Kvetnansky, Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary, Endocrinology 136 (9) (1995) 3743–3750.
[31] S.W. Ying, M. Futter, K. Rosenblum, M.J. Webber, S.P. Hunt, T.V. Bliss, et al., Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis, J. Neurosci. 22 (5) (2002) 1532–1540.
[32] A. Yoshii, M. Constantine-Paton, Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease, Dev. Neurobiol. 70 (5) (2010) 304–322.
[33] A.M. Davies, L. Minichiello, R. Klein, Developmental changes in NT3 signalling via TrkA and TrkB in embryonic neurons, EMBO J. 14 (18) (1995) 4482–4489.
[34] D.C. Choi, S.L. Gourley, K.J. Ressler, Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning, Transl. Psychiatry 2 (2012) e205.
[35] Semple B.D, Blomgren K, Gimlin K, Ferriero D.M, Noble-Haeusslein L.J, Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species, Prog. Neurobiol. (106-107) (2013) 1–16.
[36] D. Meester, E. Al-Yahya, H. Dawes, P. Martin-Fagg, C. Piñon, Associations between prefrontal cortex activation and H-reflex modulation during dual task gait, Front. Hum. Neurosci. 8 (2014).
[37] H. Shimada, K. Ishii, K. Ishiwata, K. Oda, M. Suzukawa, H. Makizako, T. Doi, T. Suzuki, Gait adaptability and brain activity during unaccustomed treadmill walking in healthy elderly females, Gait Posture 38 (2) (2013) 203–208.
[38] T. Mitchell, F. Starrs, J.P. Soucy, A. Thiel, C. Paquette, Impaired sensorimotor processing during complex gait precedes behavioral changes in middle-aged adults, J. Gerontol. Ser. A Biol. Sci. Med. Sci. (Sep 21) (2018), https://doi.org/10.1093/gerona/gly210.
[39] F. Zheng, H. Wang, NMDA-mediated and self-induced bdnf exon IV transcriptions are differentially regulated in cultured cortical neurons, Neurochem. Int. 54 (5-6) (2009) 385–392.
[40] Kuzumaki N, Ikegami D, Tamura R, Hareyama N, Imai S, Narita M, Torigoe K, Niikura K, Takeshima H, Ando T, Igarashi K, Kanno J, Ushijima T, Suzuki T, Narita M, Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment, Hippocampus. 21 (February (2)) (2011) 127–132, https://doi.org/10.1002/hipo.20775.
[41] Roth TL, Lubin FD, Funk AJ, Sweatt JD, Lasting epigenetic influence of early-life adversity on the BDNF gene, Biol. Psychiatry. 65 (9) (2009) 760–769.
[42] A. Katz, N. Meiri, Brain-derived neurotrophic factor is critically involved in thermal-experience-dependent developmental plasticity, J. Neurosci. 26 (15) (2006) 3899–3907.
[43] M.A. Smith, S. Makino, R. Kvetnansky, R.M. Post, Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus, J. Neurosci. 15 (3 Pt 1) (1995) 1768–1777.
[44] Bondar NP, Merkulova TI, Brain-derived neurotrophic factor and early-life stress: multifaceted interplay, J. Biosci. 41 (4) (2016) 751–758.
[45] Q. Wang, F. Shao, W. Wang, Maternal separation produces alterations of forebrain brain-derived neurotrophic factor expression in differently aged rats, Front. Mol. Neurosci. 8 (2015) 49.
[46] D. Suri, V. Veenit, A. Sarkar, D. Thiagarajan, A. Kumar, E.J. Nestler, S. Galande, V.A. Vaidya, Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, BDNF expression, and cognition, Biol. Psychiatry. 73 (7) (2013) 658–666.
[47] T. Ghanavati, M. Salavati, N. Karimi, H. Negahban, I. Ebrahimi Takamjani, M. Mehravar, M. Hessam, Intra-limb coordination while walking is affected by cognitive load and walking speed, J. Biomech. 47 (10) (2014) 2300–2305.
[48] T.J. Ellmers, W.R. Young, Conscious motor control impairs attentional processing efficiency during precision stepping, Gait Posture 63 (2018) 58–62.