European Archives of Psychiatry and Clinical Neuroscience (2019) 269:439–447
https://doi.org/10.1007/s00406-018-0882-6

ORIGINAL PAPER

Altered prepulse inhibition of the acoustic startle response in BDNF-deficient mice in a model of early postnatal hypoxia: implications for schizophrenia

Juan M. Lima-Ojeda[1,2] · Anne S. Mallien[1] · Christiane Brandwein[1] · Undine E. Lang[3] · Dimitri Hefter[1] · Dragos Inta[1,3]

Received: 5 September 2017 / Accepted: 12 February 2018 / Published online: 16 February 2018

Abstract

The brain-derived neurotrophic factor (BDNF) is a major proliferative agent in the nervous system. Both BDNF-deficiency and perinatal hypoxia represent genetic/environmental risk factors for schizophrenia. Moreover, a decreased BDNF response to birth hypoxia was associated with the disease. BDNF expression is influenced by neuronal activity and environmental conditions such as hypoxia. Thus, it may partake in neuroprotective and reparative mechanisms in acute or chronic neuronal insults. However, the interaction of hypoxia and BDNF is insufficiently understood and the behavioral outcome unknown. Therefore, we conducted a battery of behavioral tests in a classical model of chronic early postnatal mild hypoxia (10% O_2), known to significantly impair brain development, in BDNF-deficient mice. We found selective deficits in measures associated with sensorimotor gating, namely enhanced acoustic startle response (ASR) and reduced prepulse inhibition (PPI) of ASR in BDNF-deficient mice. Unexpectedly, the alterations of sensorimotor gating were caused only by BDNF-deficiency alone, whereas hypoxia failed to evoke severe deficits and even leads to a milder phenotype in BDNF-deficient mice. As deficits in sensorimotor gating are present in schizophrenia and animal models of the disease, our results are of relevance regarding the involvement of BDNF in its pathogenesis. On the other hand, they suggest that the effect of perinatal hypoxia on long-term brain abnormalities is complex, ranging from protective to deleterious actions, and may critically depend on the degree of hypoxia. Therefore, future studies may refine existing hypoxia protocols to better understand neurodevelopmental consequences associated with schizophrenia.

Keywords BDNF · Schizophrenia · Neurodevelopment · Perinatal hypoxia · Sensorimotor gating

Introduction

BDNF regulates neuronal plasticity and survival during all stages of development and aging at multiple levels. It promotes synaptic formation, dendritic and axonic sprouting, neuronal genesis and migration and modulates multiple transmitter systems including glutamatergic, serotonergic and GABAergic systems [1]. BDNF expression is regulated by neuronal activity, especially by NMDA-receptor (NMDAR)-mediated calcium influx and may be increased in response to hypoxia–ischemia [2]. Unsurprisingly, multiple lines of evidence suggest its involvement in the pathophysiology of various neuropsychiatric diseases such as stroke, Huntington´s disease, Alzheimer´s disease, Parkinson disease, depression, and schizophrenia [3].

Schizophrenia is a chronic mental illness characterized by positive symptoms such as hallucinations and delusions and negative symptoms such as impaired cognition,

Juan M. Lima-Ojeda, Anne S. Mallien, Undine E. Lang, Dimitri Hefter, and Dragos Inta have contributed equally to this work.

✉ Dragos Inta
dragos.inta@upkbs.ch

[1] RG Animal Models in Psychiatry, Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany

[2] Department of Psychiatry and Psychotherapy, University of Regensburg, Regensburg, Germany

[3] Department of Psychiatry (UPK), University of Basel, Wilhelm Klein-Strasse 27, 4012 Basel, Switzerland

social withdrawal and emotional flattening [4]. The neurodevelopmental hypothesis of schizophrenia suggests that a combination ("Two-Hit") of early-life environmental influences and genetic risk factors may lead to subtle perturbations of CNS physiology that manifest as severe functional deficits of large brain networks as found in adult patients [5, 6]. Disturbances in network function lead to deficient information processing by the brain; thus, sensory information cannot be sorted efficiently and may not result in appropriate behavioral response. Sensorimotor deficits are a common prominent feature in patients and animal models [7, 8].

BDNF represents one main genetic risk factor that was linked to schizophrenia, manifested as low levels of BDNF or low-functioning BNDF polymorphisms. Indeed, low plasma and serum levels of BDNF have been reported in schizophrenia patients [9]. Lack of this trophic agent might contribute to many of the morphological and functional deficits found in patients and animal models of schizophrenia such as reduced dendritic arborization, aberrant synaptic connectivity and plasticity, neuronal loss due to decreased proliferation and increased susceptibility to insult [10].

Perinatal hypoxia, e.g., resulting from obstetric complications, represents an important environmental risk factor for schizophrenia [11–13]. While severe hypoxic insults result in permanent neurological deficits and hypoxic encephalopathy, short or mild periods of low oxygen supply may be fully compensated and even protects against subsequent hypoxic or ischemic insults [14]. However, a relatively mild insult which may be compensated in a person without genetic predisposition, may lead to subtle deficits in an individual with BDNF-deficiency, resulting in chronic pathophysiological changes as described above. Indeed, BDNF has been previously shown to exert protective effects in animal models of hypoxic insult to the brain [15]. Moreover, one large retrospective clinical study in which BDNF probes were taken at birth and stored for 45–50 years, reported a significant correlation between perinatal hypoxia and low BDNF levels at birth in the later development of schizophrenia [14].

In this study, we examined the relationship between BDNF-deficiency and perinatal hypoxia in mice. For this purpose, we exposed genetically modified heterozygous BDNF-deficient (BDNF+/-) mice to mild continuous hypoxia at the age of Postnatal Day 3 (P3)– P7, which corresponds to the third trimester of pregnancy in humans. We report selective deficits in the acoustic startle response (ASR), a fast twitch of facial and body muscles due to a sudden and intense stimulus, and its prepulse inhibition (PPI), an operational measure of sensorimotor gating, in BDNF+/− mice, independently of hypoxia, and only mild effects of hypoxia.

Materials and methods

Experimental design

The subjects were exposed to either hypoxic or normoxic conditions on P3–P7. For that purpose the home cages were either introduced into the hypoxia chamber or remained in conventional racks within the same colony room. The room temperature was maintained at 23 ± 3 °C and 50 ± 5% relative humidity. Pups were not separated from their mother until weaning (P28). The behavioral experiments started at P92 with the Nest test and subsequently they were tested in the Open Field test on P99, the rotarod test on P102 and the acoustic startle response and prepulse inhibition test on P142. Hence, there was an interval of at least 72 h between the experiments to avoid distress. All mice were tested in the behavioral tests and all experiments were conducted in the active phase of the animals. Experimenters were blind to genotype and treatment throughout the testing. The orders of the animals in the tests were randomly assigned for each experiment. All experiments were approved by the local German animal welfare authorities (Regierungspräsidium Karlsruhe, 35-9185.81/6189/09).

Animals

All experiments were conducted in BDNF-heterozygous male mice ($n = 12$) and wild-type littermate controls on a C57Bl/6N background ($n = 19$), which were bred and maintained in the animal facility of the Central Institute of Mental Health, Mannheim. Mice were weaned after the hypoxia treatment at the age of 4 weeks, and were then single-housed for 8 weeks in standard type II macrolon cages. The behavioral testing was performed at an age of 12–16 weeks. Additionally to the bedding material, nesting material (unbleached tissue) was provided. Mice were kept on a 12:12-h reversed dark–light cycle (lights on at 6.00 p.m.). Water and food pellets were available ad libitum. The body weight was assessed weekly under red-light conditions inside the housing room, when the cages were changed.

Hypoxia procedure

BDNF+/− males were mated with 6 week old C57Bl6/N female mice from Charles River, Sulzfeld, Germany and received a daily plug-check. As previously described in rodents [16, 17], mice were placed in a Plexiglas chamber (BioSpherix Ltd., Lacona, NY) with a nitrogen / compressed air gas delivery system that mixes the nitrogen with room air using a compact oxygen controller (BioSpherix Ltd.,Pro:OX). The animals (6 BDNF+/- and 12 BDNF+/+)

were continuously subjected to hypoxia (90% N2, 10% O2) at postnatal day 4 (P3) for 5 consecutive days until P7. The temperature within the chamber was identical to the conditions of normoxic mice. A separate group of control mice (6 BDNF+/− and 7 BDNF+/+) was kept under normoxic conditions. No animals died during or shortly following the hypoxia exposure.

Behavioral testing

Openfield

The openfield test was performed as described previously [18]. Briefly, to evaluate locomotor and exploratory behavior, mice were individually placed into a white, open arena measuring 50×50 cm^2 and illuminated from above by 25 lx. Their locomotion was monitored for 10 min with a video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision XT8.0 (Noldus Information Technology, Wageningen, Netherlands). For each sample, the system recorded position and the occurrence of defined events. Parameters assessed in the present study were total distance moved, velocity, and thigmotaxis (i.e., the percentage of time spent in a corridor with a maximal distance of 10 cm to the walls).

Rotarod

Deficits in locomotion were tested in the accelerating rotarod test, as described previously [19]. Briefly, mice were placed on a rotating rod with initially 2.5 rpm which was subsequently accelerated up to 25 rpm within 4.5 min (TSE, Bad Homburg Type 337,650). The scored parameter was the latency to fall within a maximum 5 min.

Nest test

The test was performed as described in [20]. The nest building was assessed by two independent raters as reported earlier [21]. A cotton nestlet was introduced into the home cages 1 h before the dark phase. Nest building was evaluated 5 and 24 h after introduction of the nesting material according to a rating scale based on cohesion and shape.

PPI

We assessed acoustic startle response and PPI as described in [22, 23] in a startle chamber (SR-LAB; San Diego Instruments), a motion-sensitive setup. Briefly, after habituation to white noise in a relatively low intensity, a pseudorandomized sequence of startle stimuli with or without prepulse presentation was executed. A white noise pulse of 110 dB and 40 ms was used as startle stimulus and less intense white noise (72, 76, 80, and 84 db) with shorter duration 20 ms served as prepulse displayed 100 ms before startle. Inhibition was calculated as percentage of motion response after the prepulse compared to the mean acoustic startle response (ASR) without prepulse.

Statistical analysis

Statistical analysis was performed using SPSS 20 for Windows. Inter-group comparisons were calculated using two factorial ANOVA design with genotype (BDNF+/+ and BDNF+/-) and condition (hypoxia and normoxia) as factors. Repeated measures ANOVA was used to explore the dependence of treatment effects on time (bodyweight). Bonferroni's post hoc tests were performed. The significance threshold was set at $p < 0.05$. All data are means ± SEM. The figures were created using Microsoft Excel and edited in Corel Draw X6.

Results

Effects on ASR and PPI of the ASR

To analyze the effects of BDNF-deficiency and hypoxia, four groups were built: wild-type mice raised under normoxic conditions (BDNF+/+*normoxia), wild-type mice subjected to hypoxia at P3–P7 (BDNF+/+*hypoxia), heterozygous BDNF-deficient mice raised under normoxic conditions (BDNF+/−*normoxia) and BDNF-deficient mice subjected to hypoxia (BDNF+/−*hypoxia). BDNF+/+*normoxia mice exhibited the smallest ASR with 94 ± 19 units, while BDNF-deficiency and hypoxia increased the ASR to 196 ± 27 and 174 ± 15 units, respectively (Fig. 1a). Pairwise comparisons revealed significant differences between BDNF+/+*normoxia and BDNF+/−*normoxia ($p < 0.05$), BDNF+/+*hypoxia and BDNF+/−* hypoxia ($p < 0.05$) and between BDNF+/+*normoxia and BDNF+/−*hypoxia ($p = 0.001$) groups. Effects of both, BDNF and hypoxia, were significant in two factorial ANOVA ($p < 0.001$, $F(1,27) = 16.53$ and $p < 0.05$, $F(1,27) = 6.94$, respectively). However, no interactions between the genotype and treatment were observed.

To measure PPI of the ASR, the startling stimulus was preceded by a weaker stimulus between 72 and 84 dB. As expected, the magnitude of PPI positively correlated with the strength of the prepulse, ranging from approximately 20% at 72 dB to 80% at 84 dB (Fig. 1b). However, there were some significant differences between the four groups. BDNF-deficient mice exhibited consistently reduced PPI over all prepulse intentions; this effect reached statistical significance at 76 and 80 dB ($p < 0.05$, $F(1,27) = 5.51$, $p = 0.05$, $F(1,27) = 4.17$, respectively). In contrast, exposure

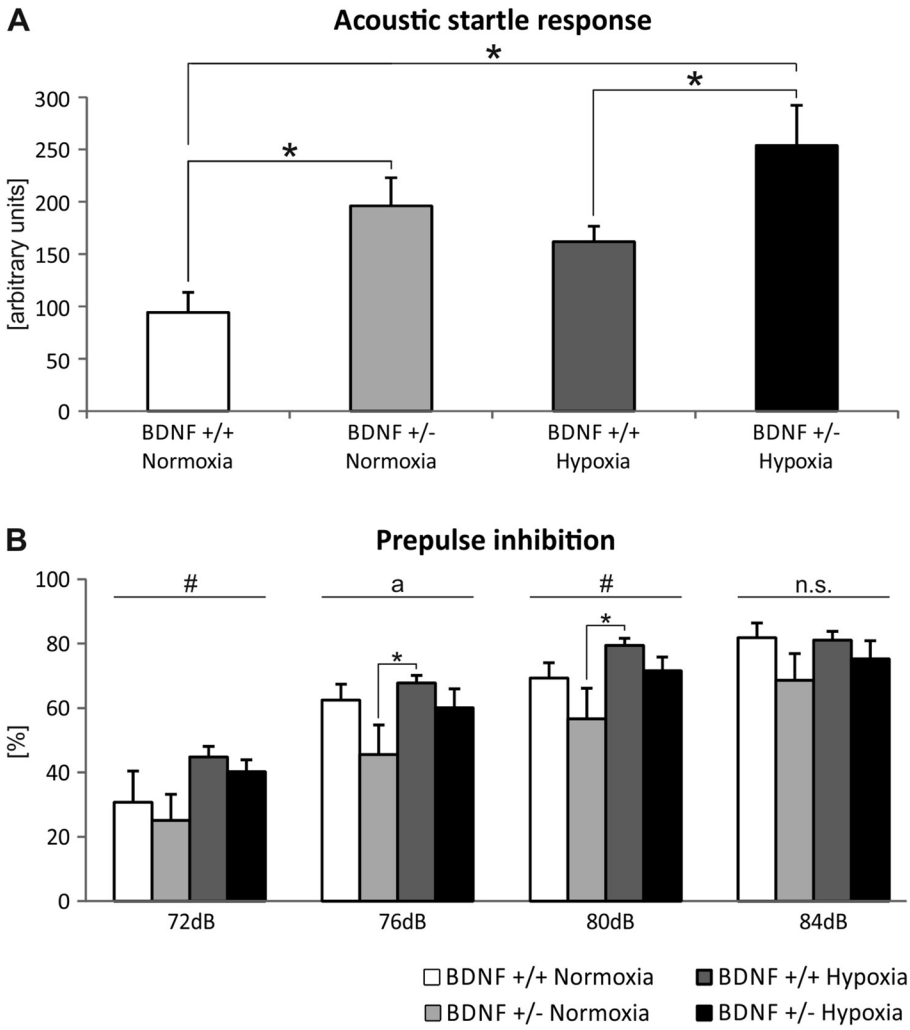

Fig. 1 Sensorimotor gating deficits in BDNF-deficient mice. **a** Acoustic startle response. Effect of BDNF: $p < 0.001$, $F = 16.53$; effect of hypoxia: $p < 0.05$, $F = 6.94$, two factorial ANOVA. **b** Prepulse inhibition of the acoustic startle response in dependence of the prepulse strength. Effects of BDNF at 72 and 76 dB: $p < 0.05$, $F = 5.51$, $p = 0.05$, $F = 4.17$, respectively. Effects of hypoxia at 72 and 80 dB: $p < 0.05$, $F = 5.26$, $p < 0.05$, $F = 6.16$, respectively, two factorial ANOVA. Data are presented as mean ± SEM. White bars: BDNF+/+*normoxia; light grey bars: BDNF+/−*normoxia; dark grey bars: BDNF+/+*hypoxia; black bars: BDNF+/−*hypoxia. *, $p < 0.05$ in post hoc pairwise comparisons between groups; #, treatment (hypoxia) effect; a, genotype (BDNF) effect

to hypoxia lead to increased PPI of the ASR with significant effects at 72 and 80 dB ($p < 0.05$, $F(1,27) = 5.26$, $p < 0.05$, $F(1,27) = 6.16$, respectively). No interactions between the genotype and treatment were observed. The differences between the genotypes were less pronounced in the hypoxia condition compared to the normoxia treated groups. Post hoc analysis detected significant effects between the reduced response of BDNF+/−*normoxia and the increased response of BDNF+/+*hypoxia at 76 dB ($p = 0.022$) and 80 dB ($p = 0.013$).

Increased body weight and impaired performance on the rotarod in BDNF-deficient mice

BDNF+/-*normoxia mice gained significantly more weight as compared to other groups, reaching 40.4 ± 1.2 g (vs. 30–32 g in BDNF+/+*normoxia and BDNF+/−*hypoxia, $p < 0.001$ in both) at the age of 17 weeks, (Fig. 2a). In contrast, BDNF+/+*hypoxia mice grew significantly lighter (26.9 ± 0.9 g), also as compared to BDNF-deficient mice raised under hypoxic conditions ($p < 0.001$ vs BDNF+/−*hypoxia). Interestingly, BDNF+/−*hypoxia mice exhibited an intermediary phenotype closely to wild-type in normoxia. Both hypoxia and BDNF significantly influenced the body weight (hypoxia: $p < 0.001$, $F(1,27) = 23.7$;BDNF: $p < 0.01$, $F(1,27) = 8.72$, two factorial ANOVA). Genotype and treatment interactions were not observed.

The performance on the accelerating rotarod was significantly impaired in BDNF+/−*normoxia mice (Fig. 2B). They lasted only for 8.7 ± 3.7 s, while BDNF+/+*normoxia mice stayed for 111 ± 29 s on the wheel (effect of BDNF: $p < 0.05$, $F(1,27) = 5.23$, two factorial ANOVA). Although BDNF+/+*hypoxia and BDNF+/−*hypoxia groups tended to perform between the normoxic wild-type and BDNF-deficient mice, the effect of hypoxia on rotarod performance was not significant. No interactions between the genotype and treatment were observed. The observed differences in performance on the rotarod test cannot be explained by merely a correlation to the bodyweight because

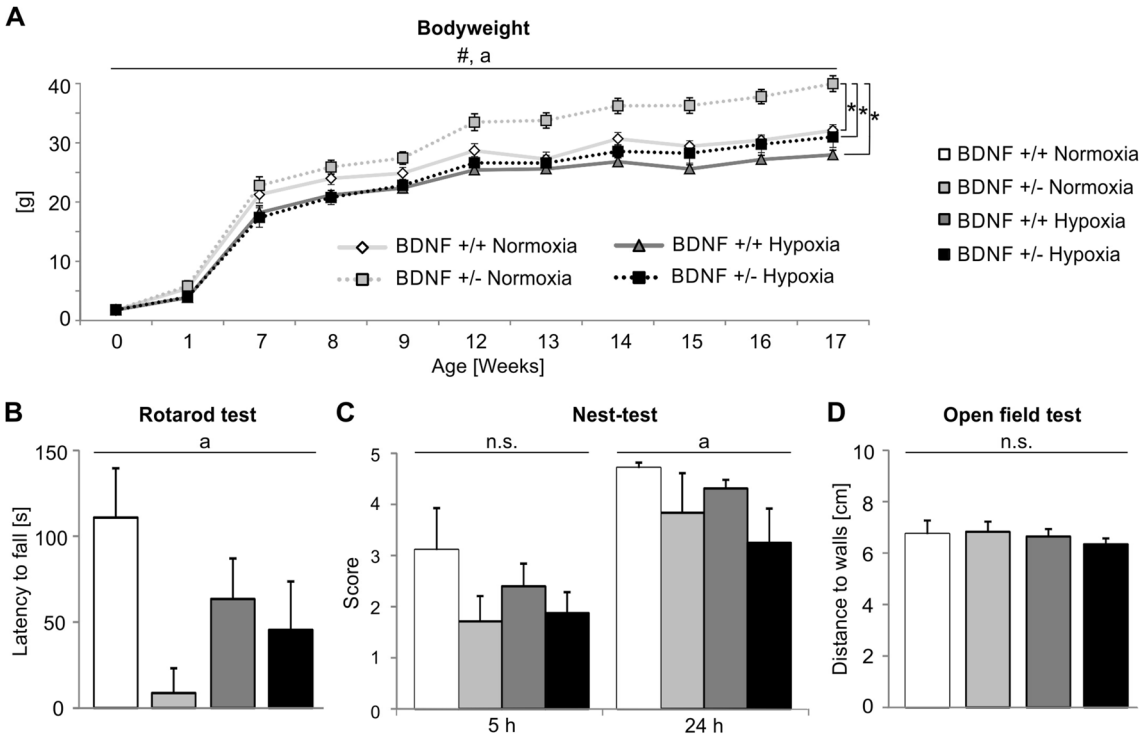

Fig. 2 **a** Development of body weight between birth and postnatal week 17. Effect of BDNF: $p<0.01$, $F=8.72$; effect of hypoxia: $p<0.001$, $F=23.7$, two factorial ANOVA. **b** Latency to fall on the accelerating rotarod test. Effect of BDNF: $p<0.05$, $F=5.23$, two factorial ANOVA. **c** Nest test. Effect of BDNF after 24 h: $p<0.05$, $F=5.13$, two factorial ANOVA. **d** Distance to walls in the open field test. Data are presented as mean ± SEM. White bars: BDNF+/+*normoxia; light grey bars: BDNF+/−*normoxia; dark grey bars: BDNF+/+*hypoxia; black bars: BDNF+/−*hypoxia. *, $p<0.05$ in post hoc pairwise comparisons between groups; #, treatment (hypoxia) effect; a, genotype (BDNF) effect

we did not find a correlation between these parameters over all groups (Pearson´s $R=0$, t-value = 0). Within the BDNF+/−*normoxia group there was a small, but not significant negative correlation ($R=-0.37$, t-value = −0.81), while within the BDNF+/−*hypoxia group bodyweight and rotarod performance correlated positively ($R=0.72$, t-value = 2.51); however, the data highly vary between 0 s and over 3 min latency to fall. Other factors besides bodyweight need to be taken into account.

Deficits in nesting behavior of BDNF-deficient mice

Nest building behavior is an essential element of the behavioral repertoire of male and female mice, as it in preserves body heat and lowers the risk of predation; therefore, it is not limited to maternal behavior [20]. BDNF-deficient mice showed deficits in nesting behavior, reaching lower scores at the nesting test as compared to wild-type mice in normoxia (1.71 ± 0.50 vs 3.11 ± 0.82 after 5 h and 3.83 ± 0.77 vs 4.71 ± 0.10 after 24 h, respectively) (Fig. 2c). The effect of BDNF was significant after 24 h ($p<0.05$, $F(1,27)=5.13$). Hypoxia alone or in combination with BDNF-deficiency had no significant effects on nesting behavior.

Open field test

There were no significant differences in the behavior in the open field between the four groups or due to the genotype (Fig. 2d). Mice from all groups moved in the field with a similar distance to the walls of 6–7 cm and traveled comparable distances with similar velocity (data not shown).

Discussion

In the current study, we examined the combined effects of heterozygous BDNF-deficiency and mild chronic early postnatal hypoxia in an established model [16] on the behavioral phenotype of mice, using tests that were shown to reveal abnormalities that correlate both with positive symptoms (Openfield, PPI) [24] and negative symptoms of schizophrenia (Nest Test) [25]. We report selective deficits caused by BDNF-deficiency alone whereas hypoxia failed to evoke severe deficits and even lead to a milder phenotype in BDNF+/− mice.

Most prominently, in our study BDNF+/− mice exhibited a more than doubled ASR amplitude as compared to

wild-type. Hypoxia also significantly increased ASR, albeit not as strongly as BDNF. The effects of hypoxia and BDNF-deficiency did not cumulate. The ASR is a basic protective response conserved across all mammals studied [26]. It is mediated by specific pontine nuclei which receive input from central auditory pathways and project to the reticular formation [26]. The ASR is sensitized by averse stimuli and fear conditioning, which is mediated by the amygdala [27]. In humans it is enhanced in anxiety and posttraumatic stress disorders [27]. Thus, BDNF-deficiency as well as perinatal hypoxia may lead to increased anxiety. However, unaltered behavior in the open field test contradicts the hypothesis of generally increased anxiety levels in these mice. The deficits seem to be rather specific and may be constrained to the auditory system.

If two auditory stimuli follow within a time frame of 30–500 ms, the ASR in response to the second stimulus is suppressed in dependence of the first stimulus´ strength. This phenomenon, called PPI, is a fundamental measure of sensorimotor gating which is the brain´s ability to distinguish relevant sensory input from contemporaneous noise and sequentially organize a proper behavioral response [28]. PPI does not require learning and occurs already at the first trial. Reduced PPI of the ASR is not only a prominent feature of schizophrenic patients [29, 30], but is also reliably reproduced in various animal models of the disease, e.g., following application of NMDA antagonists and dopaminergic agonists [31] or lesions of hippocampus and amygdala [32]. Thus, it constitutes an inherent hallmark of dysfunctional information processing in schizophrenia and may be used as a diagnostic or prognostic marker [7]. Strikingly, in our model BNDF deficiency alone lead to mildly, but significantly reduced PPI of the ASR at medium prepulse intensities. These results indicate that genetically reduced levels of BDNF may be enough to lead to a schizophrenia-like phenotype. In contrast, mice exposed to mild chronic hypoxia partially exhibited even slightly increased PPI. The effect of BDNF on PPI appears complex with various different effects reported by other studies. BDNF administration in mice was shown to increase the startle response and restore PPI [33]. Mice with a Val66Met mutation causing reduced BDNF release showed significantly reduced PPI upon exposure to dopamine antagonist apomorphine or NMDAR antagonist MK-801 [34]. In contrast to these results, a study on BDNF+/- rats showed no alterations in PPI [35]. Moreover, previous data show decreased ASR and PPI in mice overexpressing BDNF [36]. Thus, our findings extend earlier reports that support the role of BDNF in the regulation of sensorimotor gating. Regarding the effect of hypoxia, while in our study mild hypoxia did not inhibit PPI, in an other study rats exposed to mild hypoxia with 11% O_2 between P4–P8 developed prominent PPI deficits in adulthood [8], indicating possible species-specific effects. BDNF+/− mice in normoxia gained significantly more body weight as compared to wild-type, in agreement to data shown before. These results are in line with previous findings as BDNF was shown to be an important regulator of appetite and BDNF mutations are consistently associated with obesity in humans and mice [37, 38]. Therefore increased body weight can be considered as an indirect validation of BDNF-deficiency in our model. In contrast, mice exposed to hypoxia had a reduced body weight, which indicates that exposure to this mild insult did have some negative impact on overall development and nutrition. Once again, no cumulative effects of hypoxia plus BDNF-deficiency were detected; the phenotype of these mice was rather indistinguishable from wild-type in normoxia. Furthermore, BDNF+/− mice performed significantly worse at the accelerating rotarod test, while exposure to hypoxia did not significantly impair performance. The rotarod tests cerebellum-dependent sensorimotor coordination [39], but may be affected by reduced neuromuscular strength or in obesity. Individuals with schizophrenia show reduced gyrification of the cerebellum, hinting towards neurodevelopmental disturbances as simulated by our model [40]. These disturbances might lead to subtle motor dysfunction as found in these patients [41]. Unaltered performance in mice exposed to hypoxia indicates that this mild-level insult is insufficient to cause long-lasting neuromuscular or cerebellar deficits.

BDNF-deficient mice had a slightly reduced performance in the nesting test, whereas no effects of hypoxia were detected. Nesting is an important social behavior in rodents which is impaired in response to pain or reduced well-being as well as in various models of neuropsychiatric diseases [42]. BDNF was shown to reduce anxiety and facilitate social behavior in rodents [43].

As BDNF is one of the central neurotrophic factors in the CNS and affects neuronal morphology and function on multiple levels, there are myriads possibilities how its deficiency may contribute to neurodevelopmental deficits which lead to dysfunctional networks in schizophrenia. In this section we highlight some prominent mechanisms how BDNF-deficiency may lead to sensorimotor gating impairments, as found in our study. BDNF strengthens glutamatergic synapses and weakens GABAergic synapses [1]. Thus, BDNF-deficiency may lead to impaired glutamatergic transmission and reduced NMDAR signaling. Multiple lines of evidence converge towards a pivotal role of NMDAR hypofunction in schizophrenia pathophysiology [44, 45]. Furthermore, NMDAR hypofunction is indeed associated with reduced PPI of the ASR [46]. An interplay between glutamate and dopamine in mesolimbic structures regulates PPI, with low glutamate leading to hyperdopaminergia and reduced PPI [47]. In contrast, one study reported unaltered ASR and

PPI in forebrain-restricted brain-derived neurotrophic factor mutant mice [48]. Thus, forebrain structures may play a minor role in the circuitry of these reflexes.

The neurodevelopmental hypothesis of schizophrenia suggests that genetic predisposition in combination with environmental stressors in early-life contributes to the development of this disease [6]. As BDNF levels are reduced in schizophrenic patients and individuals who suffered perinatal hypoxia are more likely to develop the disease, it is tempting to suggest cumulative effects of BDNF-deficiency and hypoxia in schizophrenia [9, 11]. BDNF´s anti-apoptotic, anti-oxidative and trophic effects may protect in acute early-life hypoxic insult as well as against neurodegeneration during chronic time course of the disease [49].

One of the most prominent neurotoxic mechanisms in hypoxia–ischemia is excessive release of glutamate and subsequent over-activation of NMDAR. NMDAR-mediated excitotoxicity disrupts BNDF signaling in various ways, e.g., leading to its degradation or deactivation of its downstream targets [2]. However, calcium influx following moderate NMDAR activation potently activates BDNF expression [50]. In line with these findings, BNDF levels are reduced within the ischemic core in animal models of stroke, but elevated in the ischemic penumbra [51]. Various studies show protective acute role of BDNF in hypoxia–ischemia and excitotoxicity which is able to compensate insults of moderate severity [15, 52, 53].

Strikingly, a large longitudinal study showed that birth hypoxia was associated with a significant increase in BDNF in cord samples of healthy subjects, while BDNF levels were significantly decreased following birth hypoxia in individuals diagnosed with psychotic disorders later on in their life [12]. These data strongly support a role of BDNF-deficiency and hypoxia in schizophrenia.

Despite the convincing evidence on the cumulative effects of BDNF-deficiency and hypoxia, in our study BDNF+/- mice exposed to hypoxia partially had an even milder phenotype as compared to BDNF+/− mice in normoxia. We suppose that this model of continuous mild hypoxia is insufficient to suppress BDNF and lead to neuropsychiatric deficits. Indeed, it was shown that severe hypobaric hypoxia of 180 Torr, corresponding to 5% oxygen, down-regulates levels of BDNF, whereas mild intermittent hypoxia, corresponding to 10% oxygen (levels used in our study), enhances BDNF expression [50]. Furthermore compensatory mechanisms need to be taken into account since BDNF-deficiency may be compensated by overexpression of the receptor TrkB or transport and release of BDNF [49].

Although the model of hypoxia in this study has been successfully employed to induce severe temporary neuronal loss, ventriculomegaly and lasting deficits of GABAergic interneurons and sensorimotor gating [8, 54], reassembling findings in schizophrenic patients, the deficits can be mostly compensated by neurogenesis [16]. We found in our previous work no lasting behavioral or morphological abnormalities in chronic or intermittent mild postnatal hypoxia [17]. Furthermore, mild early postnatal hypoxia may even act neuroprotective through preconditioning mechanisms [14]. These effects may explain the seemingly contraintuitive effects of hypoxia in the present study. Additionally, strain and species differences may play an important role. Thus, in contrast to mice, rats exposed to postnatal hypoxia exhibit PPI deficits [8, 55].To successfully mimic human pathological conditions in rodents, it is necessary to titrate a hypoxic insult severe enough to elicit lasting deficits and circumvent the positive effects of hypoxic preconditioning. On the other hand, in order to reproduce a schizophrenia-like phenotype, the insult should not lead to focal necrosis and pronounced neurological deficits. Models with oxygen concentrations below 8% may be employed, but animal welfare has to be considered. Possibly the Vannucci model of hypoxia–ischemia may be a more elegant way to accomplish this task. In this model of unilateral carotid artery ligation plus subsequent exposure to hypoxia, the insult severity can be tuned resulting in subtle functional deficits or severe focal brain damage [56]. Additionally, models of acute obstetrical injury were developed, like the model of acute birth anoxia in rats, in which pups extracted by Caesarean section are submersed in water for 15 min [57]. Even in this model, Caesarean section, but not anoxia is responsible for dopaminergic impairment that was associated with schizophrenia [58]. However, this model is not possible to be performed in mice (hampering gene-environment interaction studies using genetic modified mouse lines), due to very high mortality of mouse pups compared to rat pups in this paradigm (own unpublished data).

In conclusion, our work shows that BDNF-deficiency leads to schizophrenia-typical sensorimotor gating deficits, underlying that BDNF-deficiency alone might already constitute a risk factor for schizophrenia. Further research should focus on the underlying mechanisms. However, hypoxia models currently employed in psychiatric animal research need to be refined.

Acknowledgements This work was supported by grants from the Deutsche Forschungsgemeinschaft (IN168/3-1), the Ingeborg Ständer Foundation and the Research Fund of the UPK Basel to D.I.

Author Contributions DI conceived and designed the experiments and contributed reagents/materials/analysis tools. UL contributed reagents/materials/analysis tools. JL and CB performed the experiments. JL, CB, AM and DH analyzed the data. DH, DI, AM and CB wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval The manuscript does not contain clinical studies or patient data.

References

1. Binder DK, Scharfman HE (2004) Brain-derived neurotrophic factor. Growth Factors 22:123–131. https://doi.org/10.1016/j.bbi.2008.05.010
2. Tejeda GS, Diaz-Guerra M (2017) Integral characterization of defective BDNF/TrkB signalling in neurological and psychiatric disorders leads the way to new therapies. Int J Mol Sci 18:1–24. https://doi.org/10.3390/ijms18020268
3. Chourbaji S, Brandwein C, Gass P (2011) Altering BDNF expression by genetics and/or environment: Impact for emotional and depression-like behaviour in laboratory mice. Neurosci Biobehav Rev 35:599–611. https://doi.org/10.1016/j.neubiorev.2010.07.003
4. van Os J, Kapur S (2009) Schizophrenia Lancet 374:635–645. https://doi.org/10.1016/S0140-6736(09)60995-8
5. Brown AS (2011) The environment and susceptibility to schizophrenia. Prog Neurobiol 93:23–58. https://doi.org/10.1016/j.pneurobio.2010.09.003
6. Maynard TM, Sikich L, Lieberman JA, LaMantia A-S (2001) Neural Development, Cell-Cell Signaling, and the "Two-Hit" Hypothesis of Schizophrenia. Schizophr Bull 27:457–476
7. Quednow BB, Frommann I, Berning J et al (2008) Impaired Sensorimotor Gating of the Acoustic Startle Response in the Prodrome of Schizophrenia. Biol Psychiatry 64:766–773. https://doi.org/10.1016/j.biopsych.2008.04.019
8. Fendt M, Lex A, Falkai P et al (2008) Behavioural alterations in rats following neonatal hypoxia and effects of clozapine: Implications for schizophrenia. Pharmacopsychiatry 41:138–145. https://doi.org/10.1055/s-2008-1058107
9. Fernandes BS, Steiner J, Berk M et al (2015) Peripheral brain-derived neurotrophic factor in schizophrenia and the role of antipsychotics: meta-analysis and implications. Mol Psychiatry 20:1108–1119. https://doi.org/10.1038/mp.2014.117
10. Favalli G, Li J, Belmonte-de-Abreu P et al (2012) The role of BDNF in the pathophysiology and treatment of schizophrenia. J Psychiatr Res 46:1–11. https://doi.org/10.1016/j.jpsychires.2011.09.022
11. Zornberg GL, Buka SL, Tsuang MT (2000) Hypoxic-ischemia-related fetal/neonatal complications and risk of schizophrenia and other nonaffective psychoses: a 19-year longitudinal study. Am J Psychiatry 157:196–202. https://doi.org/10.1176/appi.ajp.157.2.196
12. Cannon TD, Yolken R, Buka S, Torrey EF (2008) Decreased Neurotrophic Response to Birth Hypoxia in the Etiology of Schizophrenia. Biol Psychiatry 64:797–802. https://doi.org/10.1016/j.biopsych.2008.04.012
13. Schmitt A, Malchow B, Hasan A, Falkai P (2014) The impact of environmental factors in severe psychiatric disorders. Front Neurosci 8:1–10. https://doi.org/10.3389/fnins.2014.00019
14. Bouslama M, Adla-Biassette H, Ramanantsoa N et al (2015) Protective effects of intermittent hypoxia on brain and memory in a mouse model of apnea of prematurity. Front Physiol 6:1–11. https://doi.org/10.3389/fphys.2015.00313
15. Xie H, Leung KL, Chen L et al (2010) Brain-derived neurotrophic factor rescues and prevents chronic intermittent hypoxia-induced impairment of hippocampal long-term synaptic plasticity. Neurobiol Dis 40:155–162. https://doi.org/10.1016/j.nbd.2010.05.020
16. Fagel DM, Ganat Y, Silbereis J et al (2006) Cortical neurogenesis enhanced by chronic perinatal hypoxia. Exp Neurol 199:77–91. https://doi.org/10.1016/j.expneurol.2005.04.006
17. Lima-Ojeda JM, Vogt MA, Richter SH et al (2014) Lack of protracted behavioral abnormalities following intermittent or continuous chronic mild hypoxia in perinatal C57BL/6 mice. Neurosci Lett 577:77–82. https://doi.org/10.1016/j.neulet.2014.06.022
18. Richter SH, Garner JP, Zipser B et al (2011) Effect of population heterogenization on the reproducibility of mouse behavior: a multi-laboratory study. PLoS ONE. https://doi.org/10.1371/journal.pone.0016461
19. Domanskyi A, Geissler C, Vinnikov IA et al (2011) Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models. FASEB J Off Publ Fed Am Soc Exp Biol 25:2898–2910. https://doi.org/10.1096/fj.11-181958
20. Deacon RMJ (2006) Assessing nest building in mice. Nat Protoc 1:1117–1119
21. Biedermann SV, Fuss J, Steinle J et al (2016) The hippocampus and exercise: histological correlates of MR-detected volume changes. Brain Struct Funct 221:1353–1363. https://doi.org/10.1007/s00429-014-0976-5
22. Schneider M, Spanagel R (2008) Appetitive odor-cue conditioning attenuates the acoustic startle response in rats. Behav Brain Res 189:226–230. https://doi.org/10.1016/j.bbr.2007.12.017
23. Inta D, Vogt MA, Lima-Ojeda JM et al (2011) Lack of long-term behavioral alterations after early postnatal treatment with tropisetron: Implications for developmental psychobiology. Pharmacol Biochem Behav 99:35–41. https://doi.org/10.1016/j.pbb.2011.03.020
24. van den Buuse M (2010) Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr Bull 36:246–270. https://doi.org/10.1093/schbul/sbp132
25. Pedersen CS, Sorensen DB, Parachikova AI, Plath N (2014) PCP-induced deficits in murine nest building activity: employment of an ethological rodent behavior to mimic negative-like symptoms of schizophrenia. Behav Brain Res 273:63–72. https://doi.org/10.1016/j.bbr.2014.07.023
26. Yeomans JS, Frankland PW (1996) The acoustic startle reflex: neurons and connections. Brain Res Rev 21:301–314
27. Koch M (1999) The neurobiology of startle. Prog Neurobiol 59:107–128. https://doi.org/10.1016/S0301-0082(98)00098-7
28. Blumenthal TD, Schicatano EJ, Chapman JG et al (1996) Prepulse effects on magnitude estimation of startle-eliciting stimuli and startle responses. Percept Psychophys 58:73–80
29. Swerdlow NR, Geyer MA (1998) Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr Bull 24:285–301
30. Maier W, Mössner R, Quednow BB et al (2008) From genes to psychoses and back: The role of the 5HT2α-receptor and prepulse inhibition in schizophrenia. Eur Arch Psychiatry Clin Neurosci 258:40–43. https://doi.org/10.1007/s00406-008-5011-5
31. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 156:117–154
32. Daenen EWPM, Wolterink G, Van Der Heyden JA et al (2003) Neonatal lesions in the amygdala or ventral hippocampus disrupt prepulse inhibition of the acoustic startle response; implications for an animal model of neurodevelopmental disorders like schizophrenia. Eur Neuropsychopharmacol 13:187–197
33. Naumenko VS, Bazovkina DV, Morozova MV, Popova NK (2013) Effects of brain-derived and glial cell line-derived neurotrophic factors on startle response and disrupted prepulse inhibition in mice of DBA/2J inbred strain. Neurosci Lett 550:115–118. https://doi.org/10.1016/j.neulet.2013.06.056
34. van den Buuse M, Lee JJW, Jaehne EJ (2017) Interaction of brain-derived neurotrophic factor Val66Met genotype and history of

stress in regulation of prepulse inhibition in mice. Schizophr Res. https://doi.org/10.1016/j.schres.2017.08.019
35. van den Buuse M, Biel D, Radscheit K (2017) Does genetic BDNF deficiency in rats interact with neurotransmitter control of prepulse inhibition? Implications for schizophrenia. Prog Neuro 75:192–198. https://doi.org/10.1016/j.pnpbp.2017.02.009
36. Papaleo F, Silverman JL, Aney J et al (2011) Working memory deficits, increased anxiety-like traits, and seizure susceptibility in BDNF overexpressing mice. Learn Mem 18:534–544. https://doi.org/10.1101/lm.2213711
37. Vanevski F, Xu B (2013) Molecular and neural bases underlying roles of BDNF in the control of body weight. Front Neurosci 7:1–10. https://doi.org/10.3389/fnins.2013.00037
38. Chourbaji S, Hellweg R, Brandis D et al (2004) Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. Brain Res Mol Brain Res 121:28–36. https://doi.org/10.1016/j.molbrainres.2003.11.002
39. Curzon P, Zhang M, Radek RJ, Fox GB (2009) The behavioral assessment of sensorimotor processes in the mouse: acoustic startle, sensory gating, locomotor activity. CRC Press, Boca Raton
40. Schmitt A, Schulenberg W, Bernstein H-G et al (2011) Reduction of gyrification index in the cerebellar vermis in schizophrenia: a post-mortem study. World J Biol Psychiatry 12(Suppl 1):99–103. https://doi.org/10.3109/15622975.2011.598379
41. Hirjak D, Kubera KM, Thomann PA, Wolf RC (2017) Motor dysfunction as an intermediate phenotype across schizophrenia and other psychotic disorders: progress and perspectives. Schizophr Res. https://doi.org/10.1016/j.schres.2017.10.007
42. Jirkof P (2014) Burrowing and nest building behavior as indicators of well-being in mice. J Neurosci Methods 234:139–146. https://doi.org/10.1016/j.jneumeth.2014.02.001
43. Bahi A (2017) Hippocampal BDNF overexpression or microR124a silencing reduces anxiety- and autism-like behaviors in rats. Behav Brain Res 326:281–290. https://doi.org/10.1016/j.bbr.2017.03.010
44. Gonzalez-Burgos G, Lewis DA (2012) NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957. https://doi.org/10.1093/schbul/sbs010
45. Ju P, Cui D (2015) The involvement of N-methyl-d-aspartate receptor (NMDAR) subunit NR1 in the pathophysiology of schizophrenia. Acta Biochim Biophys Sin 48:209–219. https://doi.org/10.1093/abbs/gmv135 (Shanghai)
46. Wolf R, Dobrowolny H, Matzke K et al (2006) Prepulse inhibition is different in two inbred mouse strains (CPB-K and BALB/cJ) with different hippocampal NMDA receptor densities. Behav Brain Res 166:78–84. https://doi.org/10.1016/j.bbr.2005.07.027
47. Koch M, Schnitzler H-U (1997) The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. Behav Brain Res 89:35–49. https://doi.org/10.1016/S0166-4328(97)02296-1
48. Gorski JA, Balogh SA, Wehner JM, Jones KR (2003) Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. Neuroscience 121:341–354. https://doi.org/10.1016/S0306-4522(03)00426-3
49. Chen S, Wu CL, Hwang WC, Yang DI (2017) More insight into BDNF against neurodegeneration: anti-apoptosis, anti-oxidation, and suppression of autophagy. Int J Mol Sci 18:1–12. https://doi.org/10.3390/ijms18030545
50. Samoilov M, Churilova A, Gluschenko T, Rybnikova E (2014) Neocortical pCREB and BDNF expression under different modes of hypobaric hypoxia: role in brain hypoxic tolerance in rats. Acta Histochem 116:949–957. https://doi.org/10.1016/j.acthis.2014.03.009
51. Kokaia Z, Zhao Q, Kokaia M et al (1995) Regulation of brain-derived neurotrophic factor gene expression after transient middle cerebral artery occlusion with and without brain damage. Exp Neurol 136:73–88. https://doi.org/10.1006/exnr.1995.1085
52. Ferrer I, Krupinski J, Goutan E et al (2001) Brain-derived neurotrophic factor reduces cortical cell death by ischemia after middle cerebral artery occlusion in the rat. Acta Neuropathol 101:229–238
53. Mattson MP (2003) Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders. Neuromol Med 3:65–94. https://doi.org/10.1385/NMM:3:2:65
54. Ment LR, Schwartz M, Makuch RW, Stewart WB (1998) Association of chronic sublethal hypoxia with ventriculomegaly in the developing rat brain. Dev Brain Res 111:197–203. https://doi.org/10.1016/S0165-3806(98)00139-4
55. Griva M, Lagoudaki R, Touloumi O et al (2017) Long-term effects of enriched environment following neonatal hypoxia-ischemia on behavior, BDNF and synaptophysin levels in rat hippocampus: effect of combined treatment with G-CSF. Brain Res 1667:55–67. https://doi.org/10.1016/j.brainres.2017.05.004
56. Vannucci RC, Connor JR, Mauger DT, et al (1999) Rat model of perinatal hypoxic-ischemic brain damage. J Neurosci Res 55:158–163
57. El-Khodor BF, Boksa P (1997) Long-term reciprocal changes in dopamine levels in prefrontal cortex versus nucleus accumbens in rats born by Caesarean section compared to vaginal birth. Exp Neurol 145:118–129. https://doi.org/10.1006/exnr.1997.6437
58. El-Khodor B, Boksa P (2001) Caesarean section birth produces long term changes in dopamine D1 receptors and in stress-induced regulation of D3 and D4 receptors in the rat brain. Neuropsychopharmacology 25:423–439. https://doi.org/10.1016/S0893-133X(01)00228-7