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BDNF^{+/-} rats exhibit depressive phenotype and altered expression of genes relevant in mood disorders

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Abstract

Major depressive disorder (MDD) is a leading contributor to the global burden of disease. However, the causal relationship of risk factors, such as genetic predisposition or experience of augmented stress, remain unknown. Numerous studies in humans and rodents have implicated brain-derived neurotrophic factor (BDNF) in MDD pathology, as a genetic risk factor and a factor regulated by stress. Until now, the majority of preclinical studies have employed genetically modified mice as their model of choice. However, mice display a limited behavioural repertoire and lack expression of circulating BDNF, which is present in rats and humans. Therefore, heterozygous BDNF (BDNF^{+/-}) rats were tested for affective behaviours and accompanying expression of key genes associated with affective disorders in the brain. We found that BDNF^{+/-} rats, which have reduced BDNF levels in brain and plasma, displayed symptoms of anhedonia, a core symptom of MDD, and anxiety-like behaviour, but no behavioural despair or cognitive impairments. This was accompanied by changes in the expression of genes that are implicated in modulation of the stress response and affective disorders. Hence, glucocorticoid receptor, neuregulin 1 and disrupted-in-schizophrenia 1 gene expression were upregulated in the prefrontal cortex of BDNF^{+/-} rats, whereas FK506 binding protein 5 levels were decreased in the hippocampus. We conclude that a reduction in BDNF levels alters expression of genes associated with affective disorders, which may contribute to the development of depressive-like symptoms.

Introduction

Major depressive disorder (MDD) is the leading cause of disability worldwide affecting 300 million people and their socio-economic environment (World Health Organisation 2018). Stressful life events, such as sustained stress load at work or family disharmony, can trigger MDD development, in particular in predisposed individuals (Kessler 1997; Kendler *et al.* 1999; de Kloet *et al.* 2005; Heim & Binder 2012). By implication, MDD pathology emerges from a gene x environment interaction eliciting a heterogeneity of symptoms (Otte *et al.* 2016). Heritability of MDD is presumed to be 30–40% (Sullivan *et al.* 2000). However, many genes which exert small effects on their own may interact to contribute to the overall pathogenesis of MDD (Hyman 2014); confounding the identification of MDD-specific candidate genes. Furthermore, MDD patients display high comorbidities with other neuropsychiatric diseases, such as anxiety disorders, which additionally enhances the complexity of MDD (Kessler *et al.* 2003; De Carlo *et al.* 2016). Hence, the aetiology of MDD pathogenesis is still insufficiently understood, which precludes the tailoring of antidepressant treatment and reduces drug efficacy.

Brain-derived neurotrophic factor (BDNF), involved in neural circuit function and plasticity (Park & Poo 2013), was identified as a possible contributor to MDD pathogenesis and drug efficacy (Angelucci

1 *et al.* 2005; Sen *et al.* 2008). In humans, the BDNF polymorphism Val66Met, linked to reduced BDNF
2 activity (Egan *et al.* 2003; Hosang *et al.* 2014), was shown to have a strong interaction with stressful life
3 events in MDD pathogenesis (Hosang *et al.* 2014). Moreover, stress, which is known as a major
4 environmental risk factor for MDD development across species (Kessler 1997; Kendler *et al.* 1999;
5 Martis *et al.* 2018), reduces BDNF levels in the hippocampus (HPC) in preclinical studies (Smith *et al.*
6 1995; Murakami *et al.* 2005). The HPC is a brain region that also shows reduced plasticity in
7 environmentally-induced preclinical MDD models (Jayatissa *et al.* 2006) and atrophy in humans with
8 MDD (Bremner *et al.* 2000). Furthermore, reduced BDNF levels in prefrontal cortex (PFC) and HPC were
9 found in *post-mortem* tissue of MDD patients (Dwivedi *et al.* 2003); whereas MDD patients medicated
10 with antidepressants showed elevated BDNF levels in the HPC *post-mortem* (Chen *et al.* 2001). Similarly,
11 serum BDNF was decreased in depressed patients and elevated following medication (Shimizu *et al.*
12 2003; Karege *et al.* 2005; Sen *et al.* 2008). Moreover in preclinical studies, infusion of BDNF in the
13 midbrain induced an antidepressant-like effect in the learned helplessness paradigm (Siuciak *et al.*
14 1997) and antidepressant drug efficacy was shown to be BDNF level dependent (Castrén & Rantamäki
15 2010; Autry *et al.* 2011; Björkholm & Monteggia 2016). Hence, these findings promoted preclinical
16 research into MDD using mice with genetically reduced BDNF expression.

17 However, these preclinical studies resulted in inconsistent findings with genetically-reduced
18 levels of BDNF provoking depressive-like phenotypes in only a limited proportion of mouse studies
19 (Chourbaji *et al.* 2004; Castrén & Rantamäki 2010; Autry & Monteggia 2012). One possible explanation
20 may be that mice, unlike humans and rats, do not express peripheral BDNF (Klein *et al.* 2011). Peripheral
21 administration of BDNF in mice altered gene expression in the brain and produced an antidepressant-
22 like and anxiolytic behavioural response, although the precise mechanism of action remains to be
23 determined (Schmidt & Duman 2010). Furthermore, peripheral BDNF levels in humans are a potential
24 biomarker for MDD and antidepressant treatment (Karege *et al.* 2005; Lee *et al.* 2007; Sen *et al.* 2008).
25 Thus, peripheral BDNF levels might contribute to the pathogenesis and treatment of depression and
26 highlight that rats may be a more appropriate species to investigate the relationship between BDNF
27 and MDD. Furthermore, rats exhibit a more extensive behavioural repertoire than mice and are
28 considered translationally more relevant to humans (Hirst *et al.* 2003; Klein *et al.* 2011; Czéh *et al.* 2016;
29 Ellenbroek & Yoon 2016). Finally, most behavioural tests are designed to characterize rat behaviour
30 (Czéh *et al.* 2016), making the interpretation of BDNF mouse studies difficult. Rats heterozygous for the
31 BDNF gene (BDNF^{+/-}) express lower BDNF levels in the brain and periphery (Harris *et al.* 2016) and may
32 be a more relevant preclinical model, overcoming the inconsistent findings in mice and generating more
33 translational results. Indeed, BDNF^{+/-} rats display impaired fear related learning which correlates with

1 altered brain processing as demonstrated by functional magnetic resonance imaging (Harris *et al.*
2 2016).

3 Therefore, the present study aimed to determine the direct effect of reduced BDNF levels on
4 behavioural alterations and affective disease-related gene expression levels in the brain. Rats
5 heterozygous for the BDNF gene were behaviourally phenotyped for anxiety and depressive-like
6 behaviours as well as cognitive performance. Reduced plasticity and altered release of hypothalamic
7 neuropeptides in response to lower BDNF levels might impair the homeostasis of the hypothalamic-
8 pituitary-adrenal (HPA) axis, which is an important regulator of the stress response and often altered in
9 patients with affective disorders (Tapia-Arancibia *et al.* 2004; de Kloet *et al.* 2005; Alexander *et al.* 2010;
10 Bains *et al.* 2015). Thus, we measured the expression of genes involved in regulating the stress response
11 (the glucocorticoid receptor (GR), mineralocorticoid receptor (MR), corticotropin releasing hormone
12 (*Crh*), and FK506 binding protein 5 (*Fkbp5*)) and expression of genes relevant in neuropsychiatric
13 diseases (disrupted in schizophrenia-1 (*Disc1*), glycogen synthase kinase 3 beta (*Gsk3b*) and neuregulin
14 1 (*Nrg1*)). We hypothesized these genes would be differentially regulated in BDNF^{+/-} rats compared to
15 controls and be associated with depressive- and/or anxiety-like behaviours.

16 **Material and Methods**

17 ***Animals***

18 All animal experiments were approved by the University of Edinburgh Ethical Review Committee and
19 studies were carried out in strict accordance with the UK Home Office Animals (Scientific Procedures)
20 Act 1986 and the European Communities Council Directive of 22 September 2010 (Directive
21 2010/63/EU).

22 Animals were generated by crossing male Sprague-Dawley (Hsd:SD) rats that were
23 heterozygous for a BDNF knockdown mutation (HET, SD-BDNF^{tm1sage}; generated using zinc finger
24 nuclease technology, SAGE®Labs, St Louis, MO, USA) with control female SD rats (SAGE®Labs, St Louis,
25 MO, USA). Litters comprised of BDNF^{+/+} wild type rats (WT) and BDNF heterozygous rats (BDNF^{+/-}) and
26 were bred in-house. BDNF^{+/-} (*n* = 13) and WT rats (*n* = 14) were 11–12 weeks old and weighed an
27 average of 384 ± 49 g at the beginning of behavioural testing (except for Morris water maze (MWM)
28 test). For the MWM test, a separate cohort of 5 WT and 10 BDNF^{+/-} rats were employed (30 weeks of
29 age). Another group of 10 BDNF^{+/-} and 9 WT rats at 11–13 weeks of age were used for testing brain
30 gene expression levels. Only male animals were used and housed in mixed genotype groups of 3–4.
31 Rats had free access to food and water and were kept on a 12 h light/dark cycle (lights on at 7:00 am).

All of the following behavioural tests are listed in the order that they were conducted and took place in the first half of the light cycle (except for sucrose preference test (SPT) which was assessed over 48 h).

The behavioural test battery was conducted in the following order: sucrose preference test, elevated-plus-maze, novelty induced hypophagia, spontaneous alternation behaviour, open field and forced swim test. This order was chosen to minimize the interference of stressful tests with other tasks with more robust tests at the end of the test battery.

Sucrose preference test

The sucrose preference test (SPT) measures the hedonic state of each animal (Muscat & Willner 1992). Rats were habituated to drink a palatable sucrose solution (1.5%) for two days. A bottle of water and a bottle of the sucrose solution were made available in the animals' home cage. One and a half days after habituation, animals were single-housed and exposed to two bottles, one with water and one with the sucrose solution, for 48 h. Bottle position (left/right) was counterbalanced across cages and switched after 24 h during habituation and test phase. Water and sucrose solution consumption, body weight and food intake were measured after 48 h.

Elevated plus-maze

Anxiety-related behaviour was assessed in the elevated plus-maze (EPM) 3–5 days after the SPT. Rats were habituated in their home cage to the experimental room 1 h prior to testing. The EPM consisted of a maze shaped like a plus sign (arm size: 10 cm width, 45 cm length) and elevated 66 cm from the floor. Two opposing arms, the closed arms, were enclosed with high walls (50 cm height), the two other arms were open, leaving a central area (10 x 10 cm) in the middle of the EPM. Illumination in the closed arms was 2.5 lux and 45 lux in the open arms. In a randomized order, rats were positioned in the centre of the EPM facing a closed arm. Each rat was allowed to explore the maze for 5 min. EPM was cleaned with 70% ethanol between animals. Distance travelled and time spent in open or closed arms as well as head dips and rearing were recorded using ANY-maze automatic tracking software (ANY-maze, Stoelting Co., Wood Dale, IL, USA).

Novelty induced hypophagia

The novelty induced hypophagia (NIH) task tested anhedonic-like behaviour (decreased motivation to consume a reward) and anxiety-related behaviour (fear of eating in a novel and open environment) (Wyrwoll *et al.* 2015). Eight days after the EPM test, rats were habituated to eat a chocolate chip (280 mg; Milk chocolate chips, Wm Morrison Supermarket PLC, Bradford, UK) in their home cage on four consecutive days. On the following day, the animals were moved to the experimental room 30 min prior

to testing. The room illumination was adjusted to approximately 65–70 lux. After acclimation to the experimental room, a chocolate chip was positioned at the one end (35 lux) of an experimental box (66 cm length, 28 cm width, 40 cm height, non-transparent) and the rat was placed at the other end of the box (24–25 lux). Latency to consume the chocolate chip was manually scored from recorded videos. Rats which did not consume the chocolate chip within the time limit were listed with the full experimental duration of 15 min. Two animals were tested in parallel and boxes were cleaned with 70% ethanol between animals.

Spontaneous alternation behaviour test

The spontaneous alternation behaviour (SAB) task assesses working memory in rodents (Henningsson *et al.* 2009). The SAB test was carried out 5 days after the NIH task and according to K. Henningsen, Woldbye, & Wiborg (2013). Animals were acclimatized to the testing room 45 min prior to testing. Light intensity was 10–13 lux in the arms (49 cm length, 17 cm width, 32 cm height) and 17 lux in the triangular centre of the Y-shaped maze (120° angles). Each rat was placed at the end of the same arm facing the back wall and allowed to explore the Y-maze for 10 min. Arm entries (all four paws in arm) were recorded using ANY-maze automatic tracking software (ANY-maze, Stoelting Co., Wood Dale, IL, USA). The primary readout was alternation ratio, which was calculated by the number of alternations (visiting all three arms consecutively) divided by the maximum possible alternation score (number of arm entries minus two). A high alternation ratio shows that the rat is not re-entering an arm that was previously visited, indicating intact working memory. The apparatus was cleaned with 70% ethanol between animals.

Forced swim test and open field

The forced swim test (FST) is primarily used to investigate immobility behaviour (floating with minimum movements to keep the head above water) indicating a rat's propensity to surrender to a seemingly hopeless situation. On the contrary, swimming (horizontal movements throughout the cylinder) or struggling (vertical movements with the forepaws, usually against to cylinder wall) are counted as active escape attempts of the situation. Increased immobility is associated with a depressive-like phenotype (Slattery & Cryan 2012). The two-day FST protocol included OF testing (Abildgaard *et al.* 2017; Liebenberg *et al.* 2018) to assess locomotor activity, an important cofounder of the FST. On day one of the FST, 4 weeks after the SAB test, rats were acclimatized to the testing room (150 lux) 1 h prior to testing. In parallel, two rats were immersed in transparent cylinders (20 cm diameter, 50 cm height) filled with water (38 cm depth; 24 ± 1 °C) for 15 min. The water was renewed between rats. On the following day, locomotor activity was assessed in the open field (OF). Rats were acclimatized to an

adjacent room with dim illumination containing an OF (97 x 97 cm) for 1 h. The OF was divided into a centre area (31 cm from the edge, 30 lux), an outer area (within 12 cm from edge, 25 lux) and a middle area in between the centre and the outer area. Each rat was placed in the centre of the OF and tracked using ANY-maze automatic tracking software (ANY-maze, Stoelting Co., Wood Dale, IL, USA) for 10 min. Time spent and distance travelled in each zone as well as number of fecal boli were measured. After the OF test, the rat was transferred to the room of the FST and exposed to 7 min of forced swimming. Predominant behaviour (immobility, swimming or struggling) was scored manually from recorded videos of the second swim with time-sampling technique (5 sec) by an examiner blindfolded to group identity until score reliability was within 10%. Diving was scored as struggling.

Morris water maze

Spatial learning and memory, as well as reversal learning was examined in the MWM (2 m diameter, 0.5 m height) (Vorhees & Williams 2006) with a naïve cohort of rats (5 WT, 10 BDNF^{+/-}). Rats were trained to find a hidden Atlantis escape platform (12 cm diameter; Ugo Basile, Italy) for five days with two trials per day. Each rat was released from one of four release points in a pre-determined random order. Rats were allowed up to 120 s to find and mount the platform and were guided there if they failed to find it. Latency, swim speed and distance were recorded using Actimetrics Watermaze software (Actimetrics, IL, USA). On day six (24 h after the last test), each rat received a 60 s probe trial in which the escape platform was absent, but rose up after 60 s (to prevent extinction of the location). During the probe trial, % time spent in quadrant, % thigmotaxis (swimming within 15 cm of the pool edge), swim speed and number of platform crossings were analysed. On day seven and eight, perseverant behaviour was assessed using a reversal protocol in which the platform was moved to a randomly determined location (middle of one of the four quadrants). Rats received two swims on day seven and one swim on day eight.

To assess visual ability, a trial in which the platform was visible (i.e. the water level was lowered) was conducted two weeks after the probe trial with curtains pulled around the maze to hide spatial cues (both groups reached the platform in under 12 ± 2 s; data not shown).

Tissue collection and RT-qPCR

Quantitative PCR (qPCR) was used to investigate the cerebral expression of genes associated with neuropsychiatric disease and hypothalamic-pituitary-adrenal (HPA) axis regulation. Naïve WT ($n = 9$) and BDNF^{+/-} rats ($n = 10$) were sacrificed by decapitation. The HPC and PFC were quickly removed from the right hemisphere, immediately frozen on dry ice and stored at -80 °C. Approximately 70–80 mg (PFC) and 50–60 mg (HPC) of tissue was homogenised in Qiazol Lysis Reagent (Qiagen GmbH, North

Rhine-Westphalia, Germany) according to the manufacturer's instructions. RNA was then isolated from the supernatant using the RNase MiniKit (250) (Qiagen GmbH) with an on column DNase step to reduce possible sample contamination with DNA (Qiagen GmbH). RNA quantification and integrity (absorbance at 280/260 nm > 2.10, 260/230 nm > 1.45) was analysed by Nanodrop (Spectrophotometer ND-1000, Software ND-1000 V3.8.1; NanoDrop Technologies Inc., Delaware, USA). High-Capacity cDNA Reverse Transcription Kit (4368814, Applied Biosystems, ThermoFisher, Massachusetts, USA) was used for reverse transcription PCR, by adding 500 ng of RNA to 10 µl of reverse transcription reagent, resulting in a final volume of 20 µl. Samples were incubated for 10 min at 25 °C, 120 min at 37°C, 5 min at 85 °C, cooled down to 4 °C and stored at -20 °C. For the qPCR, cDNA was diluted 1:20 with diethyl pyrocarbonate treated water and triplicates of 2 µl of the sample and 8 µl of master mix (Roche, LightCycler 480 Probes Master, Baden-Württemberg, Germany) with TaqMan Gene expression Assays (Applied Biosystems, ThermoFisher) were used. Reference genes and target genes are listed in Table 1. The thermal conditions for the qPCR were 5 min at 95 °C, followed by 50 cycles of 10 s denaturation at 95 °C and 30 s annealing at 60 °C. Finally, qPCR was completed with 30 s extension at 40 °C.

A standard curve of eight two-fold dilutions was run for each target and reference gene on the same plate as the respective samples. PCR efficiency was calculated from the standard curve's slope and outliers within the triplicate were excluded if standard deviation (SD) of the triplicates was > 0.4. A combination of reference genes were chosen based on good correlation with other reference genes and low variance within the reference gene across groups and tissue. Hence, an average of the relative concentration of Actb and Hprt1 (Silver *et al.* 2008) were used to normalise target genes.

Table 1: Details of genes and primers used for qPCR.

<i>Used gene abbreviation</i>	<i>Gene name</i>	<i>Probe</i>	<i>Amplicon length</i>	<i>Application</i>
GR (<i>Nr3c1</i>)	Nuclear receptor subfamily 3, group C, member 1 coding for glucocorticoid receptor	Rn00561369_m1	73	Target gene
MR (<i>Nr3c2</i>)	Nuclear receptor subfamily 3, group C, member2 coding for mineralocorticoid receptor	Rn00565562_m1	79	Target gene
<i>Crh</i>	Corticotropin releasing hormone	Rn01462137_m1	112	Target gene
<i>Fkbp5</i>	FK506 binding protein 5	Rn01768371_m1	74	Target gene
<i>Disc1</i>	Disrupted in schizophrenia 1	Rn00598264_m1	73	Target gene
<i>Nrg1</i>	Neuregulin 1	Rn01482168_m1	86	Target gene
<i>Gsk3b</i>	Glycogen synthase kinase 3 beta	Rn01444108_m1	96	Target gene
<i>Actb</i>	Beta Actin	Rn00667869_m1	91	Reference gene
<i>Hprt1</i>	Hypoxanthine phosphoribosyltransferase 1	Rn01527840_m1	64	Reference gene
45S	45S pre-ribosomal RNA	Rn03928990_g1 RN45s	61	(Reference gene)

Statistical analysis

Behavioural and qPCR data (normalised to WT group mean) were analysed with Student's *t*-test or Welch's unequal variance *t*-test if the assumption of homogeneity of variance was violated. Normality was assessed with QQ-plots and Shapiro Wilk test, and if violated, data was log-transformed or non-parametric Mann-Whitney *U* test was applied. Two BDNF^{+/-} animals were excluded from the SPT because their total fluid intake was < 5 g within 48 h, which could be due to a measurement error. To reduce the type 1 error rate, qPCR results were corrected with the Benjamini-Hochberg procedure (*Q* = 25%). Repeated measurement data of the MWM was analysed with multivariate repeated measures ANOVA. Outliers were removed according to Grubb's ($\alpha = 0.05$, two-sided) or ROUT test (*Q* = 1%; GraphPad Prism 6, GraphPad Software Inc., California, USA). Data analysis was carried out in Stata (Stata 14.0, StataCorp, Texas, USA). Graphs were created with GraphPad Prism 5.

Results

Results are reported according to readout category and not in the testing order (SPT, EPM, NIH, SAB, OF, FST; MWM; qPCR) to provide a more transparent overview of the rats' phenotype.

BDNF^{+/-} rats exhibit anhedonic-like behaviour but not behavioural despair

Forced swim test reveals no difference in behavioural despair

The FST assesses behavioural despair indicated by a longer time spend passively coping, i.e. immobile, relative to actively coping behaviours, i.e. swimming or struggling, in an unescapable situation. No difference between genotypes was observed for time spent immobile, engaged in swimming or struggling behaviour. Salient is the bimodal distribution in the WT group for struggling behaviour and the low immobility scores of the WT group (nearly 40% of WT animals have a lower immobility score than the minimum score for BDNF^{+/-}; Fig. 1).

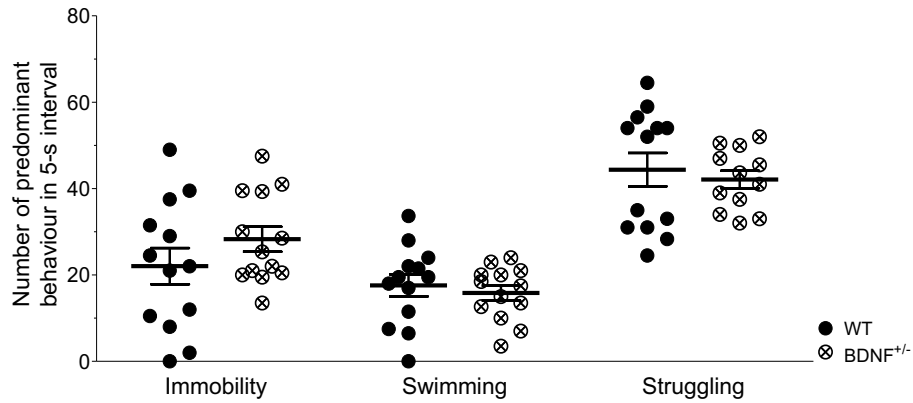


Figure 1: Behavioural parameters during the forced swim test. Time spent engaged in immobility, swimming and struggling during 5-s time intervals during 7 min FST. Group mean (\pm SEM) as well as individual scores are shown.

BDNF^{+/-} rats exhibit anhedonic behaviour in the sucrose preference test

The SPT assesses the hedonic state of the rats by measuring the preference for a sucrose solution over water intake. No significant difference was observed in total fluid intake between the WT and BDNF^{+/-} rats (Fig. 2a). Sucrose preference (i.e. sucrose solution consumption normalised to total fluid intake) was significantly different between groups ($t_{(14.61)} = 2.82$, $P = 0.013$) with BDNF^{+/-} animals exhibiting a lower sucrose preference than WT animals (Fig. 2b), thus indicating increased anhedonic-like behaviour of the BDNF^{+/-} rats compared to WT controls. Food intake and relative change in body weight (both normalised to body weight) were not significantly different between the genotypes during single-housing.

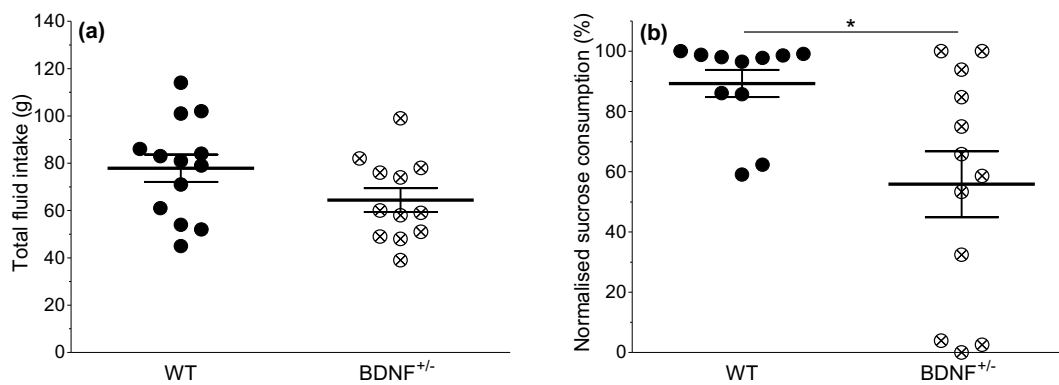


Figure 2: Sucrose preference test. (a) Total fluid consumption. (b) Percent normalised sucrose consumption to total fluid intake, i.e. sucrose preference. The individual results are plotted with mean (\pm SEM) for each group. Statistical significance between groups is indicated with $*P < 0.05$.

Novelty induced hypophagia is similar between genotypes

In the NIH test, the drive to consume a palatable chocolate reward competes with the fear of a novel environment and thus assesses anxiety- as well as anhedonic-like behaviour (Dulawa & Hen 2005). No significant difference was found for reward collection latency between WT (254.69 ± 183.40 s, *Median* = 220) and BDNF^{+/-} rats (344.00 ± 334.54 s, *Median* = 202). These data suggest there is no difference between WT and BDNF^{+/-} rats in a combined readout of anhedonic-like behaviour and anxiety-related behaviour. However, three BDNF^{+/-} rats did not consume the chocolate reward within the time limit (900 s) and hence the time limit was used as their collection latency although the true value could have been much higher.

BDNF^{+/-} rats display anxiety-like behaviour in open field test

BDNF^{+/-} rats display normal anxiety-like behaviour in the elevated plus-maze

Anxiety-like behaviour was evaluated in BDNF^{+/-} and WT rats since MDD is often accompanied by anxiety. The % distance travelled in the open arms, % time spent in the open arms and % number of open arm entries was similar across genotypes (Fig. 3).

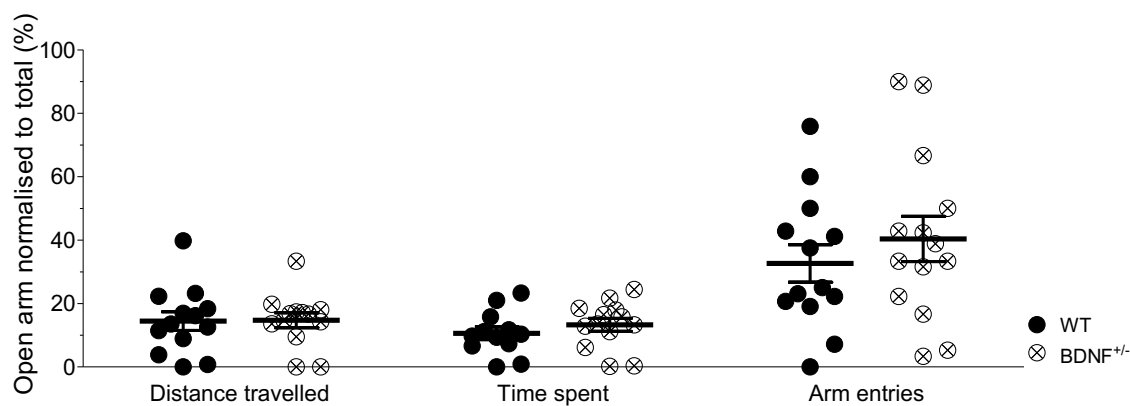


Figure 3: Behavioural parameters in the EPM. Distanced travelled in the open arm, time spent in the open arm and number of open arm entries normalised to total distance travelled, duration of experiment and total number of arm entries, respectively. Group mean (\pm SEM) and individual scores are shown.

However, WT rats (10.37 ± 3.13 m) travelled a significantly greater total distance ($t_{(25)} = 2.37$, $P = 0.026$) in the EPM than BDNF^{+/-} animals (7.56 ± 3.04 m). This effect is likely due to WT rats (7.32 m \pm 2.61 m) travelling a greater distance compared to BDNF^{+/-} rats (5.17 ± 2.55 m) in the closed ($t_{(25)} = 2.16$, $P = 0.041$), but not in the open arms. A trend in number of entries to the closed ($U = 1.779$, $P = 0.075$), but not open arms, was observed between WT (14.46 ± 7.11 entries, *Median* = 11) and BDNF^{+/-} animals (9.08 ± 5.25 entries, *Median* = 8). A trend ($t_{(25)} = 1.98$, $P = 0.059$, data log-transformed) in rearing

behaviour was observed with WT animals (6.42 ± 10.12 s, *Median* = 11.8) spending more time rearing than their BDNF^{+/-} littermates (10.79 ± 8.25 s, *Median* = 9.05). Time spent head dipping into open arms was similar between groups. WT rats (2.77 ± 3.00 boli, *Median* = 2) produced significantly more faecal boli ($U = 2.371$, $P = 0.018$) than BDNF^{+/-} rats (0.31 ± 0.63 boli, *Median* = 0) during testing on the EPM. Body weight of the animals, which can influence behaviour in the EPM, was not significantly different between genotypes at the time of testing. In sum, the primary readouts of the EPM suggest equal anxiety-like behaviour between genotypes, but decreased locomotor activity in the BDNF^{+/-} rats.

BDNF^{+/-} rats show increased anxiety-like behaviour in open field test

The open field (OF) test assesses the conflict between anxiety-related behaviour (fear of open and lit areas) and a desire to explore. Furthermore, the OF allows assessment of locomotor activity, which could be a cofounder for behavioural testing, such as the FST (Slattery & Cryan 2012). BDNF^{+/-} rats (*Median* = 2.10 s) spent less time in the centre than their WT littermates (*Median* = 9.70 s; $t_{(12,13)} = 3.31$, $P = 0.006$; Fig. 4a). BDNF^{+/-} rats (21.29 ± 16.97 s) also spent less time in the middle zone ($t_{(25)} = 2.28$, $P = 0.031$) than WT rats (41.29 ± 27.70 s). Accordingly, BDNF^{+/-} rats (574.59 s \pm 21.14, *Median* = 574.85) spent more time in the outer zone ($t_{(16,39)} = -2.38$, $P = 0.030$) than WT animals (540.76 ± 47.05 s, *Median* = 564.80). However, WT animals travelled a greater total distance ($t_{(25)} = 3.21$, $P = 0.004$) than BDNF^{+/-} animals in the OF test (Fig. 4b) suggesting decreased locomotor activity in BDNF^{+/-} animals than in WT rats. Since locomotor activity could interfere with the time spent in a zone, % distance travelled in each zone was analysed. Similarly to time spent in a zone, BDNF^{+/-} rats (*Median* = 2.60%) travelled less in the centre zone ($U = 1.99$, $P = 0.047$; Fig. 4c) than their WT littermates (*Median* = 4.55%); and less distance in the middle zone ($t_{(25)} = 2.36$, $P = 0.027$; BDNF^{+/-}: $10.31 \pm 6.68\%$; WT: $16.19 \pm 6.27\%$). Subsequently, BDNF^{+/-} rats ($86.95 \pm 8.90\%$) travelled more in the peripheral zone ($t_{(25)} = -2.47$, $P = 0.021$) compared to WT rats ($78.38 \pm 9.10\%$). These data support greater anxiety-like behaviour in the BDNF^{+/-} rats than in their WT littermates. No significant difference between genotypes was found for number of faecal boli in the OF.

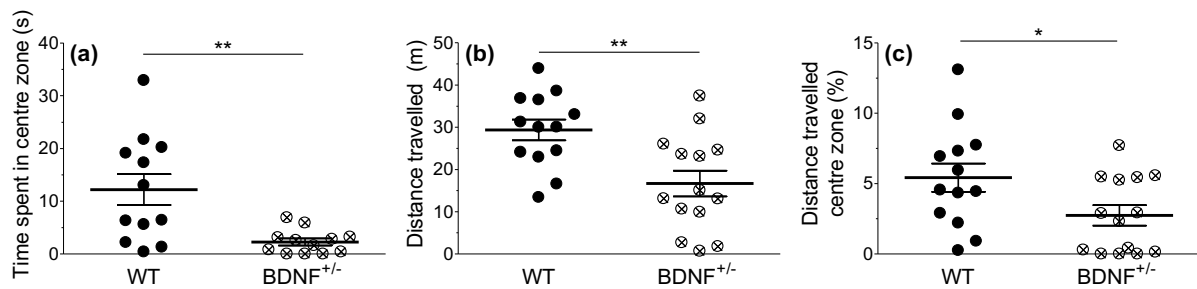


Figure 4: Open field behaviour. (a) Time spent in centre zone of the OF. (b) Total distance travelled in the OF. (c) Percentage distance travelled in centre zone. Significant differences between genotypes are indicated with * $P < 0.05$, ** $P < 0.01$. Individual results and group mean (\pm SEM) are displayed.

BDNF^{+/-} rats exhibit normal cognitive performance

BDNF^{+/-} rats exhibit normal working memory

The alternation ratio (number of sequentially alternating arm entries normalised to the possible alternation score), a readout of spatial working memory, did not differ between the genotypes (WT: $70.97 \pm 7.54\%$; BDNF^{+/-}: $67.13 \pm 16.89\%$). Results were not confounded by the non-significant difference in total number of arm entries between WT (21.31 ± 7.02 entries) and BDNF^{+/-} (15.57 ± 8.68 entries) rats. No difference in distance travelled in the Y-maze was observed between the two groups (WT: 19.32 ± 4.84 m; BDNF^{+/-}: 17.42 ± 7.60 m). Hence, this data suggest no difference in working memory or locomotor activity between BDNF^{+/-} and WT rats.

BDNF^{+/-} rats show normal performance in the Morris water maze

Spatial learning and memory performance, as well as perseverance behaviour was assessed in the MWM task because cognitive deficits have been frequently reported in depression. BDNF^{+/-} rats showed a similar learning curve as WT controls. Both groups improved performance over time by decreasing their latency to find the platform in the water [$(F_{4,55,27}) = 13.55$, $P < 0.0001$; Fig. 5a]. When the platform was removed the day after acquisition, BDNF^{+/-} and WT controls showed comparable performance for % time in target quadrant (WT: $34.33 \pm 10.51\%$; BDNF^{+/-}: $37.00 \pm 7.53\%$), number of platform crossings (WT: 2.4 ± 2.07 crossings; BDNF^{+/-}: 1.3 ± 1.16 crossings), thigmotaxis (WT: $15.4 \pm 8.99\%$; BDNF^{+/-}: $21 \pm 12.58\%$), and swim speed (WT: 21.60 ± 3.85 cm/sec; BDNF^{+/-}: 22.63 ± 1.92 cm/sec). Both groups improved performance during reversal learning [$(F_{2,27}) = 18.68$, $P < 0.0001$], but no difference in reversal learning latency was found between groups (Fig. 5b). WT ($39.00 \pm 7.93\%$) and BDNF^{+/-} ($39.20 \pm 13.03\%$) rats spent similar time in the original target quadrant during reversal learning. Thus, BDNF^{+/-} rats display intact spatial learning and memory as well as normal reversal learning, i.e. absence of perseverative behaviour.

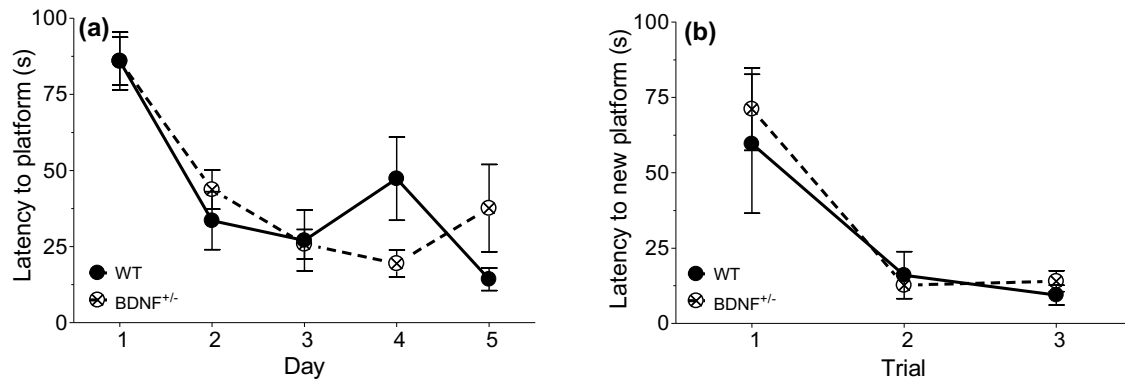


Figure 5: Morris water maze test. (a) Learning phase of finding the hidden platform (mean of two trials per day). (b) Reversal learning of finding the platform in a new location.

Locomotor activity

As many of the tests in behavioural mazes can be confounded by the general locomotor activity of the rats, it is important to determine if there is an alteration in general activity between genotypes. There was no difference in the total distance travelled in the Y-maze (section 3.3.1) or swim speed in the water maze (section 3.3.2) suggesting similar locomotor activity across genotypes. However, BDNF^{+/-} rats moved less in the OF (Fig. 4b) and in the EPM (section 3.2.1) compared to the WT controls. Thus, when altered locomotor activity between genotypes was present in tasks, it was accounted for in the respective analysis.

Gene expression

To test whether reduced BDNF levels alter expression patterns of a selection of genes thought to underpin the depressive phenotype, we investigated expression of genes involved in affective disorders and relevant for an appropriate stress response in the PFC and HPC of naïve WT and BDNF^{+/-} rats. Gene expression was upregulated in the PFC of BDNF^{+/-} rats compared to WT animals for GR ($t_{(17)} = -2.30$, $P = 0.035$), *Nrg1* ($t_{(17)} = -2.25$, $P = 0.038$) and *Disc1* ($t_{(17)} = -4.71$, $P = 0.0002$), displayed in Fig. 6. There were no significant differences between the mRNA levels of MR, *Crh*, *Fkbp5* and *Gsk3b* in the PFC of WT and BDNF^{+/-} rats (Supplementary Table 1). In the HPC, *Fkbp5* mRNA expression was reduced in BDNF^{+/-} animals compared to WT animals ($t_{(9.45)} = 3.09$, $P = 0.012$; Fig. 6). However, no significant difference in HPC mRNA expression was identified for GR, MR, *Crh*, *Nrg1*, *Disc1* and *Gsk3b* between WT and BDNF^{+/-} rats (Supplementary Table 1).

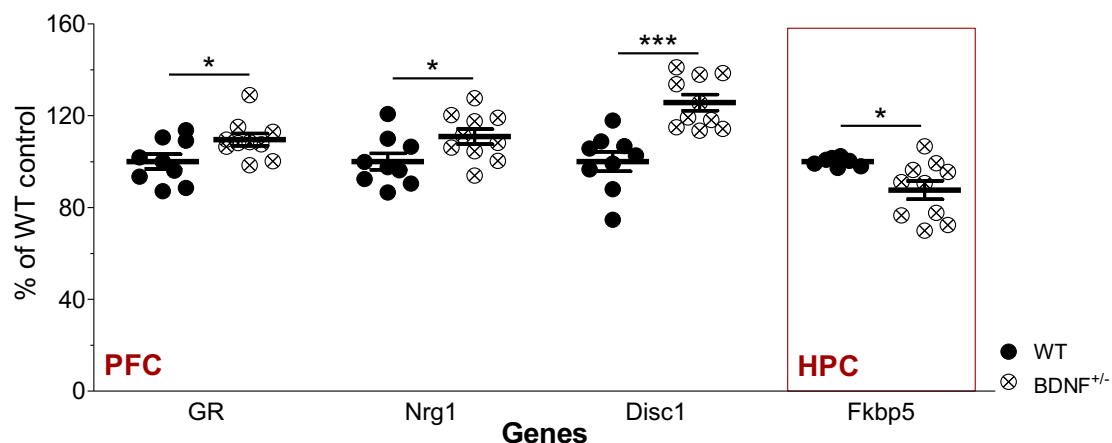


Figure 6. Gene expression. Presented are the individual and group (\pm SEM) gene expression levels (as % of WT group mean) from the prefrontal cortex (PFC) and hippocampus (HPC). Statistical significance is indicated with * $P < 0.05$, *** $P < 0.001$.

Discussion

In the present study, we showed that BDNF^{+/-} rats display depressive-like behaviour, signs of anxiety, and altered expression of genes associated with mood disorders in the PFC and HPC compared to WT animals. However, behavioural despair and cognition was similar for BDNF^{+/-} and WT littermates.

Depressive-like behaviour was assessed by testing for anhedonia, a core symptom of MDD. Sucrose consumption is frequently used to evaluate the hedonic state of an animal (Willner 1997). In the present study, BDNF^{+/-} rats consumed less sucrose solution in favour of a higher water intake compared to WT controls. Hence, reduced BDNF levels resulted in an anhedonic-like state and, thus, BDNF^{+/-} rats exhibit a depressive-like phenotype. It is possible that changes in sucrose preference may be the result of altered gustatory signalling or altered metabolic signalling in BDNF^{+/-} rats. However, equal food intake between the groups in our experiment does not support this. Furthermore, BDNF^{+/-} rats display changes in hedonic state in non-gustatory tasks (St. Laurent *et al.* 2013) and studies altering BDNF levels extraneous to the gustatory system observe changes in sucrose consumption as well (Schmidt & Duman 2010; Taliaz *et al.* 2010). Thus, BDNF^{+/-} rats express an anhedonic-like phenotype, which is in accordance with the literature (Schmidt & Duman 2010; Taliaz *et al.* 2010; Ye *et al.* 2011).

The NIH test evaluates the conflict between avoidance of open and lit areas and the desire for consuming a food reward (Dulawa & Hen 2005), reflecting depressive-like and/or anxiety-like behaviour. There was no difference between BDNF^{+/-} rats and WT controls regarding the time to collect the reward. However, all WT rats consumed the reward, whereas 20% of the BDNF^{+/-} group did not.

1 This might indicate increased anxiety or, alternatively, decreased motivation or sensitivity for the
2 reward in BDNF^{+/-} rats, consistent with the anhedonic-like phenotype that we found in the SPT.
3 Interestingly, Schmidt & Duman (2010) observed a shortened collection latency in the NIH test in WT
4 mice after peripherally administering BDNF, but unaltered behaviour in the sucrose consumption test
5 in these mice, suggesting an anxiolytic effect of BDNF administration with no effect on the hedonic
6 state. These opposite effects compared to the present study might be due to differences in
7 methodology, such as the nature of the reward or the duration of testing, as well as peripheral
8 administration versus knockdown (KD) of BDNF. Lower BDNF levels throughout life may alter
9 developmental processes and allow compensatory mechanisms to emerge, whereas acute changes in
10 BDNF do not succumb to such effects, and thus, the different approaches possibly lead to divergent
11 results. Also the model, mouse versus rat, could affect how BDNF influences behaviour, since mice do
12 not naturally express peripheral BDNF, which can influence brain gene expression and affective
13 behaviour (Schmidt & Duman 2010). The importance of the model is further emphasized by the findings
14 that temporal KD of BDNF in the dentate gyrus of rats (Taliaz *et al.* 2010) but not mice (Adachi *et al.*
15 2008) during adulthood resulted in a decrease of sucrose consumption. Finally, reduced reward
16 sensitivity in BDNF^{+/-} rats was also shown in a test of cocaine seeking behaviour (St. Laurent *et al.* 2013),
17 strengthening the results of the present study.

18 In this study, no difference in anxiety-related behaviour was found in the EPM. In mouse studies
19 using the EPM, peripheral BDNF administration was shown to have an anxiolytic effect (Schmidt &
20 Duman 2010) but comparison of BDNF^{+/-} to WT mice generated inconsistent results (MacQueen *et al.*
21 2001; Chen *et al.* 2006). However, another study with BDNF^{+/-} rats, failed to observe altered anxiety
22 behaviour in the EPM (Gururajan *et al.* 2014) hence substantiating our results. Nevertheless, in the
23 present study, BDNF^{+/-} rats spent less time in the centre or middle area of the OF, and significantly more
24 time in the periphery than their WT littermates. Although this behaviour was accompanied with
25 decreased locomotor activity in BDNF^{+/-} rats, the % distance travelled in each zone reflected the findings
26 of time spent in a specific zone. Our OF results are supported by another study (Gururajan *et al.* 2014),
27 in which BDNF^{+/-} rats spent less time in the centre of the OF and showed decreased locomotor activity.
28 This suggests that the phenotype observed in the OF is not due to the stress of the FST carried out 24
29 h prior to OF testing. However, to confirm the anxiety-like phenotype, further tests of anxiety, such as
30 the light-dark box, should be carried out to exclude the possibility that task-dependent adaptive traits
31 have occurred in the BDNF^{+/-} rats. Thus, findings in BDNF^{+/-} rats support anxiety-like behaviour in the
32 OF, but not in the EPM in accordance with the literature.

33 In the present study, BDNF^{+/-} rats did not show behavioural alterations compared to controls in
34 the FST, which is a common test used in preclinical depression research. However, the FST was

1 developed to assess antidepressant drug efficacy rather than depression symptomatology and is
2 sensitive to acute antidepressant treatment while only chronic treatment is efficacious in MDD patients
3 (Slattery & Cryan 2012). Therefore, the FST may not be the most appropriate test for assessing the
4 permanent effects of genetic manipulations on depressive-like behaviour.

5 Cognitive impairments are often seen in MDD patients (Rock *et al.* 2014) and reduced BDNF
6 levels have been implicated with impaired spatial short-term memory in rats (Gururajan *et al.* 2015).
7 Similarly, spatial working memory was also impaired in response to stress exposure in a preclinical MDD
8 model (Henningsen *et al.* 2009). We found that spatial working memory was intact in the BDNF^{+/-} group
9 compared to WT rats as examined in the SAB test. Furthermore, these results are supported by our
10 finding that BDNF^{+/-} rats displayed intact spatial learning and memory as well as normal reversal learning
11 in the MWM test. A limitation of our MWM study is the small sample size of WT rats used. While a
12 larger sample size would provide a greater level of confidence in our conclusions, the performance of
13 WT rats in our study is comparable to those reported in other studies (Spencer *et al.* 1995; Vorhees &
14 Williams 2006), hence we tentatively conclude that spatial cognition is not impaired in BDNF^{+/-} rats.

15 To understand the neurobiological underpinnings of the behavioural alterations observed in
16 the BDNF^{+/-} rats, we investigated mRNA levels of key genes associated with affective disorders. Reduced
17 BDNF levels in our model partially mimic the consequence of stress exposure (Smith *et al.* 1995) and,
18 thus, expression of glucocorticoid-regulated genes might be effected in our model. The PFC and HPC
19 are key regions affected both by stress as well as in psychiatric disorders (Sheline 2000; Rogers *et al.*
20 2004; Czéh & Lucassen 2007; Dias-Ferreira *et al.* 2009). Furthermore BDNF mRNA levels were found
21 decreased in depressed suicide victims in these regions (Dwivedi *et al.* 2003). In the PFC, we found that
22 GR mRNA is increased, in combination with *Nrg1* and *Disc1*, in BDNF^{+/-} rats compared to WT controls.

23 Viral Knockdown of GR in the PFC is associated with stress hyper-responsivity, reduced stress-
24 induced affective behaviour (McKlveen *et al.* 2013), and increased sensitivity to antidepressant
25 treatment (Hussain & Jacobson 2015). However, in the non-stressed situation the plasma
26 corticosterone levels were normal in these rats. We therefore postulate that the increased GR
27 expression in BDNF^{+/-} rats may be a compensatory mechanism to retain normal control of the HPA axis
28 and GR signalling in the PFC. This interpretation is supported by the finding that BDNF^{+/-} have a similar
29 HPA axis response to WT rats during mild stress exposure (Harris *et al.* 2016).

30 *NRG1* was identified as a susceptibility gene in neuropsychiatric diseases in humans, it drives
31 neuronal plasticity and is associated with cognitive impairments (Duffy *et al.* 2010). However, in the
32 chronic unpredictable stress (CUS) model of depression in rats, NRG1 protein was significantly
33 increased in the PFC (Dang *et al.* 2016). This increased PFC NRG1 expression parallels the results found

1 in our BDNF^{+/-} model and implies increased PFC *Nrg1* expression may contribute to the observed
2 depressive-like phenotype in our model.

3 In schizophrenia, bipolar disorder and MDD, translocation and loss of function of *DISC1* was
4 shown (Millar 2000; Lipina *et al.* 2011; Sigurdsson & Duvarci 2016). Overexpression of *Disc1* in the
5 mouse ventral HPC resulted in a depressive-like phenotype (Sauer *et al.* 2015). In our study, we found
6 that *Disc1* expression was elevated in the PFC of the BDNF^{+/-} rats. Viral knockdown of *Disc1* solely in the
7 PFC increased susceptibility to stress-induced PFC dysfunction (Gamo *et al.* 2013), which may explain
8 why patients with *DISC1* mutations are especially vulnerable to stress. Conversely, when *DISC1* is
9 overexpressed, mimicking a failure of *DISC1* proteostasis, characteristics of mental disorders are found
10 in behaviour, brain structure and in synaptic processes including membrane trafficking and synaptic
11 organisation (Sialana *et al.* 2018). Therefore, PFC *Disc1* overexpression in BDNF^{+/-} rats is potentially
12 consistent with underpinning an increased risk of schizophrenia and depression. *DISC1* regulates GSK3B
13 and altered expression pattern in the latter one was associated with neuropsychiatric diseases, such as
14 MDD and anxiety disorder (Ochs *et al.* 2015). Furthermore, reduced *Gsk3b* expression can be found in
15 the HPC following stress exposure in mice (Brydges *et al.* 2014), whereas altered GSK3B in the PFC
16 affects mood and is suggested relevant for the antidepressant response (DeI'Guidice *et al.* 2015).
17 However, in this study *Gsk3b* expression was not altered in the BDNF^{+/-} rat brain.

18 The hippocampus is a region of the brain known to be important for regulation of the HPA axis
19 and also is very sensitive to the action of glucocorticoids, which affect cognitive and affective
20 behaviours. BDNF^{+/-} rats exhibited normal expression of GR, but a decrease in hippocampal *Fkbp5*
21 mRNA. FKBP5 is a heat shock protein 90 co-chaperone and the complex competes with glucocorticoids
22 for binding to GR and, thus, modifies GR sensitivity and its function as a transcription factor (Binder
23 2009; Schmidt *et al.* 2012). Furthermore, GR activity increases FKBP5 expression, providing an ultra
24 short feedback loop for GR sensitivity (Binder 2009). Polymorphisms in *FKBP5* leading to an increased
25 expression of the gene were found in MDD patients and associated with a prolonged stress response
26 (Binder 2009). However, as HPA axis response (Harris *et al.* 2016) and hippocampal GR, MR and *Crh*
27 mRNA levels are normal in the BDNF^{+/-} rats, the mechanistic relevance of the decreased *Fkbp5* mRNA
28 expression remains to be determined. The unexpected finding that expression of key genes, known to
29 be regulated in depressive-like phenotypes, were not regulated in the hippocampus of BDNF^{+/-} rats is
30 surprising. However, as the hippocampus is a large heterogeneous structure (Fanselow & Dong 2010),
31 it is possible that using whole hippocampal samples in this study precludes detecting subtle differences
32 in gene expression levels that may only be present in specific hippocampal subregions.

33 We have previously demonstrated that BDNF^{+/-} rats exhibit 30-70% reduced brain and plasma
34 levels of BDNF protein (Harris *et al.* 2016), but due to the ZFN technology used to engineer this genetic

modification, this is not reflected in a change of BDNF mRNA levels. Unfortunately, this precludes determination of correlations between regional brain BDNF mRNA levels in BDNF^{+/-} rats with behavioural outcomes or key genes measured in this study.

Overall, we have shown that a genetically induced reduction of BDNF levels lead to a depressive-like phenotype as well as to alterations in expression levels of genes that are relevant for psychiatric disorders. In future studies, testing of female rats should be included due to sex differences observed in MDD prevalence in humans (Van de Velde *et al.* 2010). Furthermore, a combination of stress and genetic manipulation might be ideal to provoke a more differentiated phenotype. However, the present study established a solid basis for future research, with the rat as a better model for preclinical studies than the abundantly studied mouse, due the similarity of BDNF expression in rats and humans and the reproducibility of findings in the rat but not in the mouse. Moreover, our study suggests a link of decreased BDNF levels with the MDD core symptom of anhedonia. It is also demonstrated that changes in BDNF signalling result in altered expression of *Disc1*, *Nrg1*, GR and *Fkbp5* genes, relevant in affective disorders and a healthy stress response. Thus, the present study adds to the complex field of entangling the role of BDNF in the development and pathology of MDD.

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7 **Conflict of interest**

8 The authors declare no conflict of interests in relation to the current study.

Supplementary

Supplementary Table 1: Gene expression. Displayed are the normalised gene expression levels as % WT control mean (group mean \pm standard deviation) of the prefrontal cortex (PFC) and the hippocampus (HPC). *indicates statistically significant differences between groups corrected with the Benjamini-Hochberg procedure.

<i>Brain region</i>	<i>Gene</i>	<i>WT</i>	<i>BDNF^{+/-}</i>
PFC	GR*	100 \pm 9.6	109.6 \pm 8.5
	MR	100 \pm 5.2	106.6 \pm 13.6
	<i>Crh</i>	100 \pm 17.2	111.0 \pm 16.2
	<i>Fkbp5</i>	100 \pm 10.2	92.9 \pm 11.9
	<i>Nrg1</i> *	100 \pm 10.7	110.9 \pm 10.3
	<i>Disc1</i> *	100 \pm 12.7	125.7 \pm 11.1
	<i>Gsk3b</i>	100 \pm 11.0	106.2 \pm 8.2
HPC	GR	100 \pm 6.6	105.5 \pm 12.5
	MR	100 \pm 9.7	101.6 \pm 9.3
	<i>Crh</i>	100 \pm 12.9	91.8 \pm 14.0
	<i>Fkbp5</i> *	100 \pm 1.8	87.6 \pm 12.6
	<i>Nrg1</i>	100 \pm 11.3	93.8 \pm 11.3
	<i>Disc1</i>	100 \pm 9.4	99.7 \pm 15.6
	<i>Gsk3b</i>	100 \pm 5.0	104.5 \pm 6.0