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Brain Derived Neurotrophic Factor Deficiency is Associated with Cognitive Impairment and Elevated Phospholipase A2 Activity in Plasma of Mice

--Manuscript Draft--

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	Berko Milleit
Abstract:	Decreased levels of Brain-Derived Neurotrophic Factor (BDNF) are a common finding in schizophrenia. Another well-documented protein linked to schizophrenia is intracellular Ca 2+ -independent Phospholipase (PLA2). However, the potential association between PLA2 and BDNF with regard to schizophrenia has yet to be examined. In the present study, male and female BDNF knockout mice, a possible genetic model of schizophrenia, were exposed to prenatal stress and tested in the nest test, open field test and T-maze. Following behavioral tests, whole brain and plasma samples were harvested to measure the activity of PLA2. BDNF knockout mice showed cognitive deficits in the T-maze. Furthermore, there was a quadratic association of PLA2 with performance in the open field test. Moreover, BDNF deficiency and female sex were associated with elevated plasma PLA2 levels. The cognitive impairment of BDNF heterozygous mice as well as their increased PLA2 activity in plasma is consistent with findings in schizophrenia patients. The particular elevation of PLA2 activity in females may partly explain sex differences of clinical symptoms in schizophrenia (e.g. age of onset, severity of symptoms). Additionally, PLA2 was significantly correlated with body and adrenal weight after weaning, whereby the latter emphasizes the possible connection of PLA2 with steroidogenesis.
Response to Reviewers:	Reviewer #2: I thank the authors for having revised their manuscript. However, main issues were still not addressed: Abstract I would suggest using past tense in describing the results. Response: Thank you very much for this hint! We described the results in past tense now. First lines: BDNF is simply presented as decreased in schizophrenia. Thus, presenting PLA2 as "another well-documented protein promoting neuronal development" does not

make any sense.
Response: We fully agree with the reviewer and changed the passage into: “another well-documented protein linked to schizophrenia “ and deleted the following sentence: “Its activity has been shown to be increased in schizophrenia.” (please see line 42-43 of the marked manuscript).

Line 25: please use the acronym for PLA2
Response: We are grateful for this hint and used the acronym for PLA2 (now line 48 of the marked manuscript). Moreover, we also used the acronym BDNF in line 46 of the abstract as it was also already introduced before.

In the abstract it is still not clear what findings were collected in both sexes and what in females only.
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Introduction

Lines 47-54 It is true that the BDNF Val66Met polymorphism has been linked to cognition, but this is not directly related with schizophrenia. Similarly, the fact that BDNF is epigenetically regulated has no obvious connection with schizophrenia.
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"Indeed, Smesny et al. (2010) detected an association between PLA2 activity and structural changes…". Maybe, "Accordingly, Smesny et al….".
Response: Thank you! We changed the text according to your suggestion (please see line 87 of the revised manuscript)

Methods/Results

The considered experimental groups must be clearly described.
Here we have three independent factors: genotype, sex and stress.
Thus, I would expect to see the results in the resulting 8 groups: 1- males BDNFwt not stressed, 2- males BDNFwt stressed, 3- males BDNF+/- not stressed, 4- males BDNF +/- stressed, 5- females BDNFwt not stressed, 6- females BDNFwt stressed, 7- females BDNF+/- not stressed, 8- females BDNF +/- stressed. Moreover, the number of animals in each of these 8 experimental groups must be given.
What is the reason why the authors instead decided to analyze these factors independently? This is never stated, nor discussed.
Moreover, the authors can not perform an ANOVA on the selected 6 groups, because they are reanalyzing the same data, organized in different columns. Thus, the values in different columns are not independent.
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In order to verify our method and statistical analysis, we consulted our statistical department (Frau Dr. Iris Reinhard).
Overall, according to the expert there was no mistake in the statistical analysis as a three-factorial ANOVA is by far the best way to analyze the given data (with three independent factors). Doing a three-factorial ANOVA automatically leads to the analysis of the eight groups you listed above, so no group is missing in the analysis. Focusing mainly on the groups or - as we did - on the factors is in principle neither right nor wrong, as they represent just two sides of one coin.
However, we missed to describe some important statistical procedures in the methods

	part so that it might not be clear for the reader what we did exactly. We are sorry for that and added the missing information as specified in the following point-by-point answer! We agree with the reviewer that the groups were not clearly described. To fully clear this crucial issue, we added one table with the factorial groups and number of animals per factor (please see table 1a) as well as one table with the experimental groups and number of animals per experimental group (please see table 1b). The three factors were not analyzed independently, but all possible interaction effects were analyzed too. We are sorry that we did not describe this in the statistical passage in the first place! Please see line 232-234 for our correction. We never performed an ANOVA on 6 groups, but always on the eight groups you mentioned. We just focused on the three factors with two parameters each (resulting in 6 possible expressions of the factors) and misleadingly named these factorial expressions as experimental groups in one of the former revisions of the manuscript. This was certainly confusing and we deeply apologize for that! There are several reasons why we are still convinced that it has more advantages to show the factors (and not the groups) in our figures: -As there are no interaction effects between the factors, the presentation of the factors is much more informative than would be the presentation of the groups -We referred to the factors (not the groups) in the formulation of our hypotheses -For the reader it is much clearer to find the factors in figures than the groups (in our opinion the reader wants to know e.g. if the genotype has more impact on certain parameters and not if group a has more impact than group b) -In all our figures you see the three factors with two expressions each which already results in 6 box-whisker-plots per figure. If we decided to show all eight experimental groups there would be even two more box-whisker plots per figure. In our eyes the figures would become far too overcrowded and confusing this way. Discussion The text must be fully revised according to the results obtained after the re-analysis of data. Response: As a re-analysis of the data was not necessary (please see our statement above), the discussion was not revised.

Monday, 8th of November 2021

Dear Professor Sanes,

Please find enclosed our revised manuscript entitled “Brain Derived Neurotrophic Factor Deficiency is Associated with Cognitive Impairment and Elevated Phospholipase A2 Activity in Plasma of Mice”.

Thank you very much for your remarks and the reviewer’s comments on our manuscript. We assiduously have checked the raised methodological issues with our statistical department and hope that we could clear up the misunderstandings in our point-by-point response to the reviewer. We are very grateful for the chance to submit our manuscript again to your highly renowned journal Neuroscience!

This manuscript describes original work, has never been published before and is not under consideration by any other journal. All authors approved the revised manuscript and this re-submission. They declare no conflict of interest in relation to the work submitted.

Thank you for receiving our revised manuscript and considering it for publication. We appreciate your time and look very forward to your response.

With kind regards,

Michaela Schmidt

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Medical Faculty of Mannheim
University of Heidelberg
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Highlights

- BDNF heterozygous mice display deficits in the T-maze test.
- There is a quadratic association of PLA2 with performance in the open field test.
- BDNF deficiency and female sex are associated with elevated plasma PLA2 levels.
- PLA2 is significantly correlated with adrenal and body weight.

Brain Derived Neurotrophic Factor Deficiency is Associated with Cognitive Impairment and Elevated Phospholipase A2 Activity in Plasma of Mice

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Key words: polyunsaturated fatty acids, phospholipase A2, schizophrenia, mouse model, BDNF.

Abstract

Decreased levels of Brain-Derived Neurotrophic Factor (BDNF) are a common finding in schizophrenia. Another well-documented protein linked to schizophrenia is intracellular Ca^{2+}-independent Phospholipase (PLA2). However, the potential association between PLA2 and BDNF with regard to schizophrenia has yet to be examined.

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Introduction

The pathogenesis of schizophrenia has yet to be elucidated. One hypothesis is that a malfunction of neurotrophic factors during brain development leads to structural disorganisation of decisive neuronal networks (Thome et al., 1998). Subsequently, the brain is no longer able to adapt to challenging situations and is more vulnerable to neurotoxic damage (Mamounas et al., 1995). One of the most-examined neurotrophins is Brain-Derived Neurotrophic Factor (BDNF), which maintains neurons and mediates the plasticity of dopaminergic, cholinergic, and serotonergic neurons in the central nervous system (Angelucci et al., 2005).

There are many findings in animal models that support the BDNF hypothesis of schizophrenia. For example, rats prenatally exposed to the antiproliferative agent MAM (Methylazoxymethanol) manifested behavioural deficits and significant decreases of BDNF in the hippocampus (Fiore et al., 2002). Interestingly, Ashe et al. (2002) and Lipska et al. (2001) both showed a significant reduction of BDNF mRNA in the prefrontal cortex and hippocampus of animals that received neonatal lesions of the ventral hippocampus.

In human studies, decreased BDNF concentrations in cortical and hippocampal areas of schizophrenic patients have been documented (Durany et al., 2001). Moreover, there is a reduction of BDNF-positive neurons in the brain (Iritani et al., 2003) and lower levels of BDNF in the serum of patients suffering from schizophrenia (Toyooka et al., 2002). Furthermore, BDNF gene polymorphisms have been found to be associated with schizophrenia in several studies (Angelucci, Brenè and Mathé, 2005).

In addition to BDNF, Phospholipase A2 (PLA2) is involved in brain maturation, memory formation, and synaptic remodelling, and has also been associated with schizophrenia (Berger et al., 2006). In the membrane phospholipid hypothesis of schizophrenia, the postulated pathomechanism of structural brain changes is an increased phospholipid turnover and breakdown in cell membranes and myelin sheaths (Law et al., 2006). Accordingly, Smesny et al. (2010) detected an association between PLA2 activity and

structural changes in the left prefrontal cortex and bilateral thalamus of first-episode patients. Jensen et al. (2004) and Fukuzako et al. (1994) observed increased phospholipid breakdown products (phosphodiesters) using 31P-MR-spectroscopy in groups of patients possessing marked positive symptoms which supports this assumption.

Intracellular Ca^{2+}-independent Phospholipase has been found to be increased in plasma and serum of patients suffering from schizophrenia in several independent studies (e.g. Berger et al., 2006; Fenton et al., 2000; Law et al., 2006). Possibly as a consequence of increased PLA2 activity, polyunsaturated fatty acids (PUFAs) and phospholipids are reduced in post-mortem brains of patients with schizophrenia (Horrobin et al., 1991; Yao et al., 2000). Supplementation with PUFAs has successfully ameliorated schizophrenia-like symptoms in animal models (Armando et al., 2020; Crupi et al., 2013; Ribeiro et al., 2019; Zugno et al., 2015) and several clinical studies (Amminger et al., 2010; Assisi et al., 2006; Knöchel et al., 2015; Smesny et al., 2014).

However, despite the relatively similar effects on brain development and schizophrenia, the possible association of BDNF and PLA2 has not yet been examined. In order to assess the influence of a life-long genetically-mediated reduction of BDNF on the regulation of PLA2, we chose BDNF heterozygous C57BL/6N mice and their wild-type littermates as subjects in our study. In addition, prenatal stress is a crucial environmental factor involved in the development of schizophrenia in humans (e.g. Khashan et al., 2008; van Os and Selten, 1998) as well as psychosis-like expressions in animals (Koenig et al., 2005; Meyer and Feldon, 2010). Therefore, we exposed pregnant females carrying BDNF heterozygous offspring to restraint stress according to Schmidt et al. (2017), thus establishing a gene × environment experimental setting.

Our first hypothesis was an inverse relation of BDNF and PLA2, e.g. an elevated PLA2 activity in BDNF heterozygous animals. Secondly, we expected an increase of behavioral deficits associated with lower BDNF levels and higher PLA2 activity.

Materials and Methods

Animals

Acclimatized female $BDNF^{+/+}$ and male $BDNF^{+/-}$ mice on a C57BL/6N background were used for breeding. $BDNF^{+/-}$ animals were originally generated as described by MacQueen et al. (2001) and were genotyped with PCR as recommended by the Jackson Labs (Bar Harbour, Maine, USA). All animals were housed individually in conventional type II macrolon cages, maintained at a constant temperature (22 °C ± 1 °C) on a 12 hour light–dark cycle (lights on from 0700h) with food and water *ad libitum* (Chourbaji et al., 2012). Vaginal plugs were checked on a daily basis and defined as embryonic day 0 (E0) when positive. In the last trimester from E13 to E17, prenatal restraint stress was applied by putting the dams into a transparent 250 ml glass cylinder filled up to a height of 5 mm with cold tap water, whilst being exposed to bright light (6000 lx) three times a day (between 0800h and 1000h, 1200h and 1400h, as well as 1600h and 1800h) for 45 minutes per session (Schmidt et al., 2017). Control dams were left undisturbed in their cages.

On postnatal day 1–4, the biological C57BL/6N dams were substituted with experienced NMRI (Naval Medical Research Institute) foster dams in a cross-fostering design. The transfer into the nest of the lactating foster dam took place within 15 minutes.

After weaning at four weeks of age, one heterozygous or wild-type male or female per litter was chosen for the behavioral experiments in order to prevent litter effects (Chapman and Stern, 1979). Mice were housed individually in macrolon type II cages with sawdust bedding and cellulose as nesting material, under a reversed 12h day-night cycle (lights on from 1900h-0700h). The animals were allowed to adapt to this environment for at least 14 days before the start of experiments, and were supplied with food and water *ad libitum*. Please see table 1a and 1b for the number of animals per factor and experimental group.

Sampling

The body weights of mice were taken at 5, 8, 12, 16 and 20 weeks of age. Mice were decapitated at 20 weeks of age between 0800h and 1100h and trunk blood was collected within 30 seconds after removal from the cage. These whole blood samples were mixed with an anticoagulant and stored on ice until they were centrifuged at 5000g for 10 minutes in order to obtain plasma. Brains were removed, cleaned from blood in ice-cold 0.9% NaCl solution, then dissected on ice to obtain tissue samples from different brain regions. The remaining brain material consisting of white and gray matter was stored at -80 °C for PLA2 activity analysis in the brain. Additionally, the adrenal glands were removed, weighed and stored at -80 °C.

Behavioral analysis of adult offspring behavior

At age 15–17 weeks, 36 female and 40 male mice were tested behaviourally in the dark phase, i.e., in the active phase. Mice were acclimatized to the experimental room for at least 15 minutes prior to each experiment and then tested by an investigator who was blind to genotype and prenatal stress condition. The order of the tests followed earlier recommendations, ranking the tests from least stressful to more stressful (Mallien et al., 2020). Mice were sacrificed 3–5 weeks after the last experiment at 20 weeks of age.

Open field test

To evaluate locomotor and exploratory behavior, mice were individually placed into a white, open arena measuring 50×50 cm^2 under dimmed light conditions (25 lx). Activity monitoring was performed for 15 min using a video camera system (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVison 1.96 (Noldus Information Technology, Wageningen, the Netherlands) as described earlier (Lima-Ojeda et al., 2013).

Nest test

To assess general cognitive functioning and well-being via nest quality, the nesting material was removed

from the home cages at 0800h and a standard cotton nestlet (PLEXX, Arnheim) was introduced. The nest-building performance was rated after 5h and 24h with scores described elsewhere (Mallien et al., 2020).

T-maze

The T-maze test is a spatial working memory paradigm that analyzes the animals' ability to recognize and differentiate between a new unknown and a familiar compartment. The T-shaped maze is constructed of white plastic, with two 20-cm-long arms extending at right angles from a 40-cm-long alley. The arms and the alley have a width of 10 cm and are surrounded by 25-cm-high walls. Light intensity was 25 lx (Zueger et al., 2005).

The test consisted of two phases, with an intertrial interval of 60 minutes, during which the animals were returned to their home cage but remained in the experimental room. During an 8-min acquisition phase, one of the short arms was closed. In the 3-min retention phase, mice had access to all three arms. The number of visits and time spent in each of the short arms were assessed for the retention phase.

All procedures complied with the regulations covering animal experimentation within the EU (European Communities Council Directive 2010/63/EU). They were conducted in accordance with the institutions' animal care and use guidelines and approved by the national and local authorities (Regierungspräsidium Karlsruhe).

Intracellular Ca^{2+}-independent Phospholipase (PLA2)

Measurement in plasma

PLA2 activity was analyzed using a continuous kinetic fluorometric assay previously described (Smesny et al., 2011) but with some modifications. To exclusively quantify PLA2, a calcium-depleted environment was established by adding the Ca^{2+}-chelator ethylene glycol tetraacetic acid (EGTA) (Carl Roth,

Karlsruhe, Germany) to the reaction and using 0.05 M HEPES-buffer (pH 7.4) (Biochrom Ltd., Cambridge, UK) prepared with HPLC-grade water (Thermo Fisher Scientific Inc., Waltham, MA, USA). As the bee venom PLA2 (Sigma-Aldrich, Inc., St. Louis, MO, USA) used for calibration was Ca^{2+}-dependent, the reference standard was dissolved and diluted respectively in HEPES buffer containing 1 mM Ca^{2+}, while no EGTA was added to wells of the standard curve. In addition, a coagulation control N (Technoclone, Vienna, Austria) obtained from the citrate plasma of healthy donors was added to adapt the kinetic reaction of the bee venom PLA2 with the BODIPY® FL dye-labeled sn-2 acyl chain and the dinitrophenyl quencher group of the of the fluorescent substrate PED6 (Hendrickson et al., 1999) to the one seen in plasma samples.

To determine PLA2 activity, a black µ-clear 96-well microplate (Greiner bio-one, Kremsmünster, Austria) was filled with 5 µL plasma samples, 5 µL EGTA and 180 µL HEPES buffer, while 5 µL standard solution or Ca^{2+}-HEPES buffer (blank) was mixed with 5 µL coagulation control N and 180 µL HEPES buffer for the for the PLA2-standard curve. After recording baseline values, 10 µL of PED6 solution (dissolved in hygroscopic dimethyl sulfoxide (Sigma-Aldrich, Inc., St. Louis, MO, USA) to obtain a 200 mM stock solution) was injected via the reagent dispenser and the fluorescence intensity (λ_{Ex} 485 nm/ λ_{Em} 538 nm) was measured for about 50 s using the NOVOstar® Galaxy (BMG LABTECH, Ortenberg, Germany). Moreover, two intra-assay variations were carried along for control of each assay. For verification of all test compounds, the recovery of aliquoted standard concentrations (50, 100 and 200 U/mL) was measured using the coagulation control N. Also, a control of each sample was executed by adding 5 µL 15.86 µg/mL (R)-Bromenol lactone (BEL) (Cayman Chemicals, Ann Arbor, MI, USA) to actively inhibit the Ca^{2+}-independent PLA2 activity. The PLA2 activity is proportional to the fluorescent intensity resulting from the elimination of the intramolecular quenching effect of the dinitrophenyl group and was calculated using the blank-corrected area under the curve from the point of the PED6-injection. By means of the standard curve, PLA2 activities were calculated according to the linear regression and expressed as U/mL. However, results were normalized to 1 mg/mL protein content of the plasma sample.

Measurement of PLA2 in brain tissue

Initially, the murine white and gray matter was homogenized by transferring the sample into 2 mL tubes containing 1.4/2.8 mm Precellys ceramic beads (VWR International, Radnor, PA, USA) to which cold tissue homogenization buffer (20 mM HEPES with 1 mM EGTA, 210 mM Mannitol, 70 mM Sucrose; pH 7.2) was added containing cOmplete™ EDTA-free Protease Inhibitor (Roche/Sigma-Aldrich, Inc., St. Louis, MO, USA). Samples were homogenized at 6,500 rpm for 10 s using the Precellys 24-Dual Homogenisator (VWR International, Radnor, PA, USA), and subsequently centrifuged at 10,000 rpm and 4°C for 5 min in order to collect the clear supernatant, which was stored at -80° C until further analyses, while homogenates were considered stabile for one month.

Tissue PLA2 activity was analyzed following the plasma PLA2 procedure with minor adjustments. These involved the bee venom PLA2 for calibration, which was solved and diluted in BSA-Ca^{2+}-HEPES buffer containing 1 mM Ca^{2+} and 60 g/L BSA. Thus, a 96-well microplate was filled with 5 µL standard solution or BSA-Ca^{2+}-HEPES buffer (blank) and 185 µL HEPES buffer before initiating the reaction with 10 µL PED6 substrate.

Statistical Analysis

Based on the 3×2 factorial design, all behavioral tests were analyzed using the three between subject-factors “prenatal stress”, “genotype” and “sex”.

All ANOVAs described below were conducted using the General Linear Model (GLM). Whenever the assumptions of parametric analysis were not met by graphical examination of homoscedasticity and outliers, raw data were transformed according to the Box-Cox-method (Box and Cox, 1964) and/or outliers were eliminated. The model chosen for all ANOVAs included all possible three-way- and two-way-interactions. There was no three-way interaction and except one two-way interaction that is described in the results section, all other interactions also clearly did not reach the level of statistical significance.

All subtests of the open field test and nest test, as well as body weights, were analyzed by three-way repeated measurements ANOVA with different time points as the within-subject factor, whereby the time points for the open field test consisted in the first vs second five minutes of the test, for the nest test the first five hours vs 24 hours of the test and for the body weights 5, 8, 12, 16 and 20 weeks of age. In case of a significant two-way-interaction or main effect, an additional stratified analysis using t-tests was performed. A MANOVA with subsequent univariate two-way ANOVAs and t-tests was used for the analysis of the T-maze. A three-way univariate ANOVA was performed for weight of adrenals and phospholipase A2 in plasma with subsequent t-tests in case of a significant main or interaction effect.

Linear and non-linear associations were tested with the linear Pearson product-moment coefficient and quadratic regression, respectively.

$\alpha = 0.05$ was used as the level of statistical significance in all tests. The statistical analyses were performed using the SPSS 21.0 software package for Windows.

Results

Body and adrenal weight

BDNF is known to influence the metabolic homeostasis in the body. We therefore determined whether BDNF knockout had an effect on body weight in the present sample. Body weight steadily increased significantly from birth to the age of 20 weeks ($p<0.001$, $F_{4,65}=327.854$). Males were heavier than females ($p<0.001$, $F_{1,68}=44.640$) and $BDNF^{+/-}$ animals weighed significantly more than wild-types ($p<0.001$, $F_{1,68}=27.523$; table 2). Prenatal stress did not have any influence on body and adrenal weight.

As previously shown (Bielohuby et al., 2007), adrenal weight of our sample was significantly higher in females than in males (sex $p<0.001$, $F_{1,67}=307.621$ with 1.48±0.54 mg (mean ± standard deviation) for males and 3.34±0.49 mg for females. Neither genotype ($p=0.305$, $F_{1,67}=1.068$, with 2.37±1.08 mg for wild-types and 2.36±1.07 mg for $BDNF^{+/-}$ nor prenatal stress ($p=0.365$, $F_{1,67}=0.832$; with 2.43±1.07 mg (no prenatal stress) and 2.3±1.08 mg (prenatal stress) did result in any difference in adrenal weight.

Open field test

The animals performed as observed earlier in all subtests of the open field test. Over time, total distance moved ($F_{1,68}=14.713$, $p<0.001$), movement ($F_{1,68}=28.774$, $p<0.001$) and mean velocity ($F_{1,68}=14.110$, $p<0.001$) decreased, whereas distance to walls ($F_{1,68}=16.076$, $p<0.001$) and center time ($F_{1,68}=6.556$, $p=0.013$) increased. Overall, females moved a greater distance ($F_{1,68}=5.343$, $p=0.024$) and displayed more mean velocity ($F_{1,68}=5.099$, $p=0.027$; data not shown) than males.

There was a significant time×genotype effect regarding total distance moved ($F_{1,68}=4.635$, $p=0.035$) and mean velocity ($F_{1,68}=4.771$, $p=0.032$), indicating that BDNF heterozygote mice moved a shorter distance and slower than wild-types within the first five minutes of the test (fig. 1A and fig. 1B). In the second half of the test, from minute 6–10, total distance moved and mean velocity of both groups were not significantly different. For the subtest "center time" we found a significant sex×genotype interaction effect

($F_{1,68}$=4.725, p=0.033). Male wild-types and female BDNF heterozygote animals spent less time in the center of the arena than female wild-types and male heterozygotes (fig. 1C).

Prenatal stress did not have any influence on the animals' performance in the subtests of the open field test (total distance moved: $F_{1,68}$=1.530, p=0.220; distance to walls: $F_{1,68}$=1.155, p=0.286; center time: $F_{1,68}$=1.121, p=0.293; movement: $F_{1,68}$=2.092, p=0.153; mean velocity: $F_{1,68}$=1.564, p=0.215, data not shown).

Nest test

There was a significant effect of sex over time (time×sex: $F_{1,68}$=5.795, p=0.019) and time ($F_{1,68}$=164.628, p<0.001) on the performance of animals in the nest test, indicating that results improved after 24 h compared to 5 h. Sex was also significant ($F_{1,68}$=4.341, p=0.041). Subsequent t-tests revealed that males displayed significantly better nest building qualities in the first 5 h of the test compared to females (T(65.751)= -3.082, p=0.003, fig. 2). After 24 h there was no significant difference regarding nest building performance between the sexes (T(74)= -0.484, p=0.630). There was no significant difference between prenatally stressed vs non-stressed animals after 5 or 24 h (5 hrs: $F_{1,68}$=0.126, p=0.723; 24 h: $F_{1,68}$=0.257, p=0.614). BDNF heterozygote and wild-type mice also did not display any difference regarding their performance in the nest test (5hrs: $F_{1,68}$=2.544, p=0.115; 24 hrs: $F_{1,68}$=0.246, p=0.622).

T-maze

There was a significant main effect showing that BDNF heterozygous animals spent significantly less time in the new arm of the T-maze than wild-types ($F_{1,68}$=4.851, p=0.031), whereas there was no difference between these two groups of animals regarding time spent in the old arm of the T-maze ($F_{1,68}$=0.242, p=0.624). This result was confirmed by subsequent t-tests (time in new arm: T(74)=2.184, p=0.016; time in old arm: (T(74)= -0.530, p=0.299)). Prenatal stress (old arm: $F_{1,68}$=1.044, p=0.311; new arm: $F_{1,68}$=1.461, p=0.231) and sex (old arm: $F_{1,68}$=0.039, p=0.844; new arm: $F_{1,68}$=0.142, p=0.707; fig. 3) did not play any role regarding the time spent in the old vs. new arm of the T-maze.

PLA2 Activity in Plasma

As PLA2 in the white and gray matter of the whole brain yielded levels below detection threshold, only the plasma levels are shown here.

Genotype ($F_{1,64}$=8.860, p=0.004) and sex ($F_{1,64}$=14.834, p<0.001) had a significant overall effect on PLA2 activity in the plasma of our animals. These two main effects were confirmed by subsequent t-tests. BDNF heterozygous mice as well as female mice showed a significantly higher PLA2 activity in their plasma compared to wild-types (T(70)= -2.791, p=0.004) and male animals (T(65.359)=4.054, p<0.001, fig. 4), respectively.

Prenatal stress did not have a significant effect on PLA2 plasma activity levels ($F_{1,64}$=0.506, p=0.480).

Phospholipase A2 activity in plasma significantly correlated with adrenal weight, which was corrected by the body weight (r=0.319, p=0.006, fig. 5). Additionally, there was a significant negative correlation of phospholipase A2 in plasma with body weight at the age of 5 (r=- 0.307, p=0.009) and 8 weeks (r= -0.241, p=0.041, fig. 6A and 6B).

Additionally, PLA2 showed a significant quadratic association with "total distance moved from 5–10 minutes" ($F_{2,69}$=5.311, p=0.007, fig. 7A) and "mean velocity 5–10 minutes" of the open field test ($F_{2,69}$=5.054, p=0.009, fig. 7B). For both subtests this quadratic association reached significance in BDNF heterozygous animals (total distance moved: $F_{2,30}$=3.991, p=0.029, R^2=0.210; mean velocity: $F_{2,30}$=3.873, p=0.032, R^2=0.205) but not wild-type animals (total distance moved: $F_{2,36}$=2.073, p=0.141, R^2=0.103; mean velocity: $F_{2,36}$=1.964, p=0.155, R^2=0.98).

Discussion

Here we showed that BNDF heterozygous animals spent significantly less time in the new arm of the T-maze than their wild-type littermates. This indicates a lower cognitive performance in a classical working memory test and is recognized as a possible expression of a typical schizophrenia-like symptom (Castañé et al., 2015; Fujioka et al., 2014; Lander et al., 2019; Nikiforuk, 2018). In the open field test, BDNF deficient mice moved a significantly shorter distance and significantly slower than their wild-type littermates. These results might be interpreted as a correlate of negative symptoms, but are possibly confounded by an increased anxiety (Yee and Singer, 2013). Additionally, we cannot exclude the possibility that the size of our open field arena produced anxiety in the animals or that the knock-out is responsible for habituation deficits. However, phospholipase A2 showed a quadratic association with the subtest "total distance moved" and "velocity" of the open field test for BDNF heterozygous animals only. Thus, BDNF heterozygous mice with the lowest and highest PLA2 activity in plasma displayed the highest locomotor activity in the open field test, typically interpreted as a positive schizophrenia-like symptom (Nikiforuk, 2018). Similarly, McNamara et al. (2017) determined PLA2 using the same method employed in the present study and detected a quadratic association between phospholipase A2 activity and white matter changes in adolescents. The authors concluded that both higher and lower PLA2 activity is associated with lower white matter myelination. As we found this relation only in BDNF heterozygous animals, it is possible that BDNF is involved in the complex interplay between PLA2 and white matter maturation.

In accordance with our hypothesis, PLA2 activity was significantly increased in BDNF heterozygous animals. Although PLA2 and BDNF are both linked to the development of the brain and schizophrenic illness, their interaction has never been examined in a knockout model. Some previous studies have suggest an association between the metabolism of omega-3 PUFA, BDNF and neuronal function. Dong et al. (2018) demonstrated that supplementation with eicosapentaenoic acid (EPA), an omega-3 PUFA, up-regulated BDNF levels in the rat hippocampus. Keleshian et al. (2014) reported that in omega-3 PUFA deprived rats, NMDA induced a significant reduction of BDNF levels in the brain. Recently, a randomized

clinical trial by Pawełczyk et al. (2019) in schizophrenia patients showed that a six-month supplementation with omega-3 PUFA significantly increased peripheral BDNF levels. The authors speculated that this shift in BDNF may have been triggered by the activation of intracellular signaling pathways, including transcription factors such as cAMP-reactive element binding protein, in response to omega-3 PUFA.

Most interestingly, there is evidence that both PLA2 and BDNF are linked to the dopaminergic system. The Omega-6 PUFA arachidonic acid – a metabolite of PLA2 – is essential for dopaminergic signaling (Berger, Smesny and Amminger, 2006). A deficiency of PUFAs – amongst others - leads to a reduced dopamine concentration and lower number of D2 receptors in the frontal lobe (Zimmer et al., 2000) and an increased concentration of dopamine in the limbic system (Delion et al., 1994).

Interestingly, BDNF heterozygous mice display higher tones of dopamine in the dorsal striatum (Birbeck et al., 2014; Bosse et al., 2012). They also show less cognitive flexibility than wild-type mice (Parikh et al., 2016), which corresponds to our behavioral data.

The relationship described above between PLA2 and BDNF needs to be further specified with regard to sex-specific aspects. For the first time, we demonstrated significantly increased PLA2 levels in female C57BL/6N mice. In their review, Wu et al. (2013) outlined numerous human and animal studies indicating that estrogen correlates with BDNF signaling and thus also influences dopaminergic and NMDA receptor activity. According to the authors, this might explain the ubiquitous sex differences in schizophrenia age of onset, symptoms and treatment response. Our finding occurred predominantly in the subgroups of wild-types and prenatally non-stressed mice. Thus it seems likely that the increase of PLA2 in females is not directly associated with BDNF heterozygosity or prenatal stress but directly influenced by sex hormones.

Indeed, there are a few investigations demonstrating a sex-dependent regulation of fatty acids. For instance, Kitson et al. (2010) described several studies in human and animal studies that demonstrated an elevated production of docosahexaenoic acid – also an omega-3 fatty acid – in females. The authors suggested two potential molecular mechanisms for this finding: 1.) estrogen increases the activity of ERK-MAPK and protein kinase A, which phosphorylates and increases the activity of PPARα, and/or 2.)

estrogen increases the intracellular concentration of both PUFA and eicosanoids, PPARα ligands, via elevated Ca^{2+}-PLA2 and COX-2 activities, resulting in activation of PPARα. This second mechanism of molecular regulation is completely consistent with our findings. There is one clinical examination describing a possible sex-dependent regulation of PLA2. McNamara et al. (2017) showed a significant relationship of PLA2 activity, long-chain unsaturated fatty acids and white matter microstructure in males but not females. The authors also suggested a possible role of sex hormones in fatty acid homeostasis for white matter maturation.

As there is evidence of basic adrenal overactivity and metabolic disturbance in schizophrenia (Ryan et al., 2004; Sullivan et al., 2015; Thakore, 2004), we determined adrenal and body weights of our animals and found a positive correlation between PLA2 and adrenal weight. Although little is known about the role of PLA2 in steroidogenesis (Bollag, 2016), there are suggestions that inhibitors of PLA2 activity, such as quinacrine, reduce AngII-stimulated aldosterone production in glomerulosa cells and exogenous PLA2 leads to transient steroidogenesis (Kojima et al., 1985). Furthermore, PLA2 inhibitors reduce cortisol secretion from fasciculata/reticularis cells following ACTH production. In contrast, Andreis et al. (1999) found that the PLA2 inhibitor AACOCF3 increases basal aldosterone and corticosterone secretion in rats and humans. It also leads to more cortisol production in response to ACTH and the release of other steroid intermediates, suggesting that that PLA2 inhibits early steps in steroidogenesis. Stimulation as well as inhibition (possibly as a compensatory mechanism) of steroidogenesis by PLA2 may lead to adrenal tissue growth and could explain our finding that adrenal weight is positively correlated to PLA2 activity. We assume that the hypothalamic-pituitary axis is activated via anxiety and stress caused by the onset of positive symptoms of schizophrenia. As this process also causes neurotoxic effects, it is likely that PLA2 is subsequently activated as a maintenance enzyme on the membrane level.

There was a negative correlation of PLA2 with body weight directly after weaning at 5 weeks of age. At 8 weeks of age this correlation was still significant, but was not detectable after week 12. Forlenza et al. (2002) found a peak PLA2 activity in embryonic rat brains at embryonic day 18 that decreased towards birth. Yang et al. (1999) detected a slightly different development of PLA2 in rat brains. PLA2 reached its

peak after birth at 12 weeks of age. It is possible that the smallest and most underdeveloped offspring in our study produced the highest PLA2 levels to accelerate neurodevelopment via increased synaptogenesis, glial proliferation and myelination.

Finally, we used plasma as a peripheral parameter of PLA2 activity, as several studies showed that peripheral and central PLA2 activity is strongly correlated (Chen et al., 2008; Gattaz et al., 1996; Rapoport, 2008; Smesny et al., 2008). Our aim was to demonstrate the correlation between peripheral and central PLA2 in our samples. Unfortunately, our data in brain tissue appeared to be below the detection threshold. In the literature, there are no studies examining PLA2 activity in mouse brain, but PLA2 has been measured in rat brain (e.g. Schaeffer et al., 2005). It is likely that the amount of PLA2 in mouse brain is too small to be detectable with the antibody or analysis system used here. Further research is necessary to clarify this issue.

Here we demonstrated for the first time a significantly elevated PLA2 level in BDNF heterozygous mice. This reflects a possible association of these two parameters in the development of schizophrenia. Moreover, the increased PLA2 activity in females may reflect a sex-specific course of schizophrenia. The correlation between adrenal weight and PLA2 suggests a connection of PLA2 with steroidogenesis and may reflect the association between the acute positive symptomatic onset of schizophrenic psychosis, stress, hormonal stress response and structural changes at the membrane level.

Conflict of Interest

The authors declare no conflict of interest.

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References

Amminger GP, Schäfer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, Mackinnon A, McGorry PD, et al. (2010) Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiat 67:146-154.

Andreis PG, Buttazzi P, Tortorella C, De Caro R, Aragona F, Neri G, Nussdorfer GG (1999) The inhibitor of phospholipase-A2, AACOCF3, stimulates steroid secretion by dispersed human and rat adrenocortical cells. Life Sci 64:1287-1294.

Angelucci F, Brenè S, Mathé AA (2005) BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatr 10:345-352.

Armando M, Ciampoli M, Padula MC, Amminger P, De Crescenzo F, Maeder J, Schneider M, Schaer M, et al. (2020) Favorable effects of omega-3 polyunsaturated fatty acids in attentional control and conversion rate to psychosis in 22q11.2 deletion syndrome. Neuropharmacology 168:107995.

Ashe PC, Chlan-Fourney J, Juorio AV, Li XM (2002) Brain-derived neurotrophic factor (BDNF) mRNA in rats with neonatal ibotenic acid lesions of the ventral hippocampus. Brain Res 956:126-135.

Assisi A, Banzi R, Buonocore C, Capasso F, Di Muzio V, Michelacci F, Renzo D, Tafuri G, et al. (2006) Fish oil and mental health: the role of n-3 long-chain polyunsaturated fatty acids in cognitive development and neurological disorders. Int Clin Psychopharm 21:319-336.

Berger GE, Smesny S, Amminger GP (2006) Bioactive lipids in schizophrenia. Int Rev Psychiatr 18:85-98.

Bielohuby M, Herbach N, Wanke R, Maser-Gluth C, Beuschlein F, Wolf E, Hoeflich A (2007) Growth analysis of the mouse adrenal gland from weaning to adulthood: time- and gender-dependent alterations of cell size and number in the cortical compartment. Am J Physiol-Endoc M 293:E139-146.

Birbeck JA, Khalid M, Mathews TA (2014) Potentiated striatal dopamine release leads to hyperdopaminergia in female brain-derived neurotrophic factor heterozygous mice. ACS Chem Neurosci 5:275-281.

Bollag WB (2016) Role of phospholipases in adrenal steroidogenesis. J Endocrinol 229:R29-41.

Bosse KE, Maina FK, Birbeck JA, France MM, Roberts JJ, Colombo ML, Mathews TA (2012) Aberrant striatal dopamine transmitter dynamics in brain-derived neurotrophic factor-deficient mice. J Neurochem 120:385-395.

Box GEP, Cox DR (1964) An analysis of transformations. J R Stat Soc B 26:211-252.

Castañé A, Santana N, Artigas F (2015) PCP-based mice models of schizophrenia: differential behavioral, neurochemical and cellular effects of acute and subchronic treatments. Psychopharmacology (Berl) 232:4085-4097.

Chapman RH, Stern JM (1979) Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male rat offspring: Implications of litter effects for prenatal studies. Dev Psychobiol 12:255-267.

Chen CT, Green JT, Orr SK, Bazinet RP (2008) Regulation of brain polyunsaturated fatty acid uptake and turnover. Prostag Leukotr Ess 79:85-91.

Chourbaji S, Hortnagl H, Molteni R, Riva MA, Gass P, Hellweg R (2012) The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system. Neuroscience 220:267-276.

Crupi R, Marino A, Cuzzocrea S (2013) n-3 fatty acids: role in neurogenesis and neuroplasticity. Curr Med Chem 20:2953-2963.

Delion S, Chalon S, Hérault J, Guilloteau D, Besnard JC, Durand G (1994) Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 124:2466-2476.

Dong Y, Xu M, Kalueff AV, Song C (2018) Dietary eicosapentaenoic acid normalizes hippocampal omega-3 and 6 polyunsaturated fatty acid profile, attenuates glial activation and regulates BDNF function in a rodent model of neuroinflammation induced by central interleukin-1β administration. Eur J Nutr 57:1781-1791.

Durany N, Michel T, Zöchling R, Boissl KW, Cruz-Sánchez FF, Riederer P, Thome J (2001) Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophr Res 52:79-86.

Fenton WS, Hibbeln J, Knable M (2000) Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biol Psychiat 47:8-21.

Fiore M, Korf J, Antonelli A, Talamini L, Aloe L (2002) Long-lasting effects of prenatal MAM treatment on water maze performance in rats: associations with altered brain development and neurotrophin levels. Neurotoxicol Teratol 24:179-191.

Forlenza OV, Schaeffer EL, Gattaz WF (2002) Phospholipase A2 activity in rat embryonic brain and in primary cultures of cortical neurons. J Neural Transm (Vienna) 109:623-631.

Fujioka R, Nii T, Iwaki A, Shibata A, Ito I, Kitaichi K, Nomura M, Hattori S, et al. (2014) Comprehensive behavioral study of mGluR3 knockout mice: implication in schizophrenia related endophenotypes. Mol Brain 7:31.

Fukuzako H, Takeuchi K, Ueyama K, Fukuzako T, Hokazono Y, Hirakawa K, Yamada K, Hashiguchi T, et al. (1994) 31P magnetic resonance spectroscopy of the medial temporal lobe of schizophrenic patients with neuroleptic-resistant marked positive symptoms. Eur Arch Psy Clin N 244:236-240.

Gattaz WF, Cairns NJ, Levy R, Förstl H, Braus DF, Maras A (1996) Decreased phospholipase A2 activity in the brain and in platelets of patients with Alzheimer's disease. Eur Arch Psy Clin N 246:129-131.

Hendrickson HS, Hendrickson EK, Johnson ID, Farber SA (1999) Intramolecularly quenched BODIPY-labeled phospholipid analogs in phospholipase A(2) and platelet-activating factor acetylhydrolase assays and in vivo fluorescence imaging. Anal Biochem 276:27-35.

Horrobin DF, Manku MS, Hillman H, Iain A, Glen M (1991) Fatty acid levels in the brains of schizophrenics and normal controls. Biol Psychiat 30:795-805.

Iritani S, Niizato K, Nawa H, Ikeda K, Emson PC (2003) Immunohistochemical study of brain-derived neurotrophic factor and its receptor, TrkB, in the hippocampal formation of schizophrenic brains. Prog Neuropsychopharmacol Biol Psychiat 27:801-807.

Jensen JE, Miller J, Williamson PC, Neufeld RW, Menon RS, Malla A, Manchanda R, Schaefer B, et al. (2004) Focal changes in brain energy and phospholipid metabolism in first-episode schizophrenia: 31P-MRS chemical shift imaging study at 4 Tesla. Brit J Psychiat 184:409-415.

Keleshian VL, Kellom M, Kim HW, Taha AY, Cheon Y, Igarashi M, Rapoport SI, Rao JS (2014) Neuropathological responses to chronic NMDA in rats are worsened by dietary n-3 PUFA deprivation but are not ameliorated by fish oil supplementation. PLoS One 9:e95318.

Khashan AS, Abel KM, McNamee R, Pedersen MG, Webb RT, Baker PN, Kenny LC, Mortensen PB (2008) Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. Arch Gen Psychiat 65:146-152.

Kitson AP, Stroud CK, Stark KD (2010) Elevated production of docosahexaenoic acid in females: potential molecular mechanisms. Lipids 45:209-224.

Knöchel C, Voss M, Grüter F, Alves GS, Matura S, Sepanski B, Stäblein M, Wenzler S, et al. (2015) Omega 3 Fatty Acids: Novel Neurotherapeutic Targets for Cognitive Dysfunction in Mood Disorders and Schizophrenia? Curr Neuropharmacol 13:663-680.

Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, Hercher E, Brady DL (2005) Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav Brain Res 156:251-261.

Kojima I, Kojima K, Rasmussen H (1985) Possible role of phospholipase A2 action and arachidonic acid metabolism in angiotensin II-mediated aldosterone secretion. Endocrinology 117:1057-1066.

Lander SS, Khan U, Lewandowski N, Chakraborty D, Provenzano FA, Mingote S, Chornyy S, Frigerio F, et al. (2019) Glutamate Dehydrogenase-Deficient Mice Display Schizophrenia-Like Behavioral Abnormalities and CA1-Specific Hippocampal Dysfunction. Schizophrenia Bull 45:127-137.
Law MH, Cotton RG, Berger GE (2006) The role of phospholipases A2 in schizophrenia. Mol Psychiatr 11:547-556.
Lima-Ojeda JM, Vogt MA, Pfeiffer N, Dormann C, Köhr G, Sprengel R, Gass P, Inta D (2013) Pharmacological blockade of GluN2B-containing NMDA receptors induces antidepressant-like effects lacking psychotomimetic action and neurotoxicity in the perinatal and adult rodent brain. Prog Neuro-Psychoph 45:28-33.
Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR (2001) BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. Eur J Neurosci 14:135-144.
MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav Neurosci 115:1145-1153.
Mallien AS, Häger C, Palme R, Talbot SR, Vogt MA, Pfeiffer N, Brandwein C, Struve B, et al. (2020) Systematic analysis of severity in a widely used cognitive depression model for mice. Lab Animal 54:40-49.
Mamounas LA, Blue ME, Siuciak JA, Altar CA (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 15:7929-7939.
McNamara RK, Szeszko PR, Smesny S, Ikuta T, DeRosse P, Vaz FM, Milleit B, Hipler UC, et al. (2017) Polyunsaturated fatty acid biostatus, phospholipase A2 activity and brain white matter microstructure across adolescence. Neuroscience 343:423-433.
Meyer U, Feldon J (2010) Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog Neurobiol 90:285-326.
Nikiforuk A (2018) Assessment of cognitive functions in animal models of schizophrenia. Pharmacol Rep 70:639-649.
Parikh V, Naughton SX, Yegla B, Guzman DM (2016) Impact of partial dopamine depletion on cognitive flexibility in BDNF heterozygous mice. Psychopharmacology (Berl) 233:1361-1375.
Pawełczyk T, Grancow-Grabka M, Trafalska E, Szemraj J, Żurner N, Pawełczyk A (2019) An increase in plasma brain derived neurotrophic factor levels is related to n-3 polyunsaturated fatty acid efficacy in first episode schizophrenia: secondary outcome analysis of the OFFER randomized clinical trial. Psychopharmacology (Berl) 236:2811-2822.
Rapoport SI (2008) Arachidonic acid and the brain. J Nutr 138:2515-2520.
Ribeiro BMM, Chaves Filho AJM, Costa D, de Menezes AT, da Fonseca ACC, Gama CS, Moura Neto V, de Lucena DF, et al. (2019) N-3 polyunsaturated fatty acids and clozapine abrogates poly I: C-induced immune alterations in primary hippocampal neurons. Prog Neuro-Psychoph 90:186-196.
Ryan MC, Sharifi N, Condren R, Thakore JH (2004) Evidence of basal pituitary-adrenal overactivity in first episode, drug naïve patients with schizophrenia. Psychoneuroendocrino 29:1065-1070.
Schaeffer EL, Bassi F, Jr., Gattaz WF (2005) Inhibition of phospholipase A2 activity reduces membrane fluidity in rat hippocampus. J Neural Transm (Vienna) 112:641-647.
Schmidt M, Lapert F, Brandwein C, Deuschle M, Kasperk C, Grimsley JM, Gass P (2017) Prenatal stress changes courtship vocalizations and bone mineral density in mice. Psychoneuroendocrino 75:203-212.
Smesny S, Stein S, Willhardt I, Lasch J, Sauer H (2008) Decreased phospholipase A2 activity in cerebrospinal fluid of patients with dementia. J Neural Transm (Vienna) 115:1173-1179.
Smesny S, Milleit B, Nenadic I, Preul C, Kinder D, Lasch J, Willhardt I, Sauer H, et al. (2010) Phospholipase A2 activity is associated with structural brain changes in schizophrenia. Neuroimage 52:1314-1327.

Smesny S, Kunstmann C, Kunstmann S, Willhardt I, Lasch J, Yotter RA, Proffitt TM, Kerr M, et al. (2011) Phospholipase A_2 activity in first episode schizophrenia: associations with symptom severity and outcome at week 12. World J Biol Psychia 12:598-607.

Smesny S, Milleit B, Hipler UC, Milleit C, Schafer MR, Klier CM, Holub M, Holzer I, et al. (2014) Omega-3 fatty acid supplementation changes intracellular phospholipase A2 activity and membrane fatty acid profiles in individuals at ultra-high risk for psychosis. Mol Psychiatr 19:317-324.

Sullivan EL, Riper KM, Lockard R, Valleau JC (2015) Maternal high-fat diet programming of the neuroendocrine system and behavior. Horm Behav 76:153-161.

Thakore JH (2004) Metabolic disturbance in first-episode schizophrenia. Brit J Psychiat Suppl 47:S76-79.

Thome J, Foley P, Riederer P (1998) Neurotrophic factors and the maldevelopmental hypothesis of schizophrenic psychoses. Review article. J Neural Transm (Vienna) 105:85-100.

Toyooka K, Asama K, Watanabe Y, Muratake T, Takahashi M, Someya T, Nawa H (2002) Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. Psychiat Res 110:249-257.

van Os J, Selten JP (1998) Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. Brit J Psychiat 172:324-326.

Wu YC, Hill RA, Gogos A, van den Buuse M (2013) Sex differences and the role of estrogen in animal models of schizophrenia: interaction with BDNF. Neuroscience 239:67-83.

Yang HC, Mosior M, Ni B, Dennis EA (1999) Regional distribution, ontogeny, purification, and characterization of the Ca2+-independent phospholipase A2 from rat brain. J Neurochem 73:1278-1287.

Yao JK, Leonard S, Reddy RD (2000) Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophr Res 42:7-17.

Yee BK, Singer P (2013) A conceptual and practical guide to the behavioural evaluation of animal models of the symptomatology and therapy of schizophrenia. Cell Tissue Res 354:221-246.

Zimmer L, Delpal S, Guilloteau D, Aïoun J, Durand G, Chalon S (2000) Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. Neurosci Lett 284:25-28.

Zueger M, Urani A, Chourbaji S, Zacher C, Roche M, Harkin A, Gass P (2005) Olfactory bulbectomy in mice induces alterations in exploratory behavior. Neurosci Lett 374:142-146.

Zugno AI, Chipindo H, Canever L, Budni J, Alves de Castro A, Bittencourt de Oliveira M, Heylmann AS, Gomes Wessler P, et al. (2015) Omega-3 fatty acids prevent the ketamine-induced increase in acetylcholinesterase activity in an animal model of schizophrenia. Life Sci 121:65-69.

Brain Derived Neurotrophic Factor Deficiency is Associated with Cognitive Impairment and Elevated Phospholipase A2 Activity in Plasma of Mice

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Abstract

Decreased levels of Brain-Derived Neurotrophic Factor (BDNF) are a common finding in schizophrenia. Another well-documented protein ~~promoting neuronal development~~ linked to schizophrenia is intracellular Ca^{2+}-independent Phospholipase (PLA2). ~~Its activity has been shown to be increased in schizophrenia.~~ However, the potential association between PLA2 and BDNF with regard to schizophrenia has yet to be examined.

In the present study, male and female BDNF knockout mice, a possible genetic model of schizophrenia, were exposed to prenatal stress and tested in the nest test, open field test and T-maze. Following behavioral tests, whole brain and plasma samples were harvested to measure the activity of PLA2. BDNF knockout mice showed cognitive deficits in the T-maze. Furthermore, there was a quadratic association of PLA2 with performance in the open field test. Moreover, BDNF deficiency and female sex were associated with elevated plasma PLA2 levels. The cognitive impairment of BDNF heterozygous mice as well as their increased PLA2 activity in plasma is consistent with findings in schizophrenia patients. The particular elevation of PLA2 activity in females may partly explain sex differences of clinical symptoms in schizophrenia (e.g. age of onset, severity of symptoms). Additionally, PLA2 was significantly correlated with ~~adrenal~~ body and ~~body~~adrenal weight after weaning~~.~~, whereby the latter emphasizes the possible connection of PLA2 with steroidogenesis.

Introduction

The pathogenesis of schizophrenia has yet to be elucidated. One hypothesis is that a malfunction of neurotrophic factors during brain development leads to structural disorganisation of decisive neuronal networks (Thome et al., 1998). Subsequently, the brain is no longer able to adapt to challenging situations and is more vulnerable to neurotoxic damage (Mamounas et al., 1995). One of the most-examined neurotrophins is Brain-Derived Neurotrophic Factor (BDNF), which maintains neurons and mediates the plasticity of dopaminergic, cholinergic, and serotonergic neurons in the central nervous system (Angelucci et al., 2005).

There are many findings in animal models that support the BDNF hypothesis of schizophrenia. For example, rats prenatally exposed to the antiproliferative agent MAM (Methylazoxymethanol) manifested behavioural deficits and significant decreases of BDNF in the hippocampus (Fiore et al., 2002). Interestingly, Ashe et al. (2002) and Lipska et al. (2001) both showed a significant reduction of BDNF mRNA in the prefrontal cortex and hippocampus of animals that received neonatal lesions of the ventral hippocampus.

In human studies, decreased BDNF concentrations in cortical and hippocampal areas of schizophrenic patients have been documented (Durany et al., 2001). Moreover, there is a reduction of BDNF-positive neurons in the brain (Iritani et al., 2003) and lower levels of BDNF in the serum of patients suffering from schizophrenia (Toyooka et al., 2002). Furthermore, BDNF gene polymorphisms have been found to be associated with schizophrenia in several studies (Angelucci, Brenè and Mathé, 2005). ~~The influence of the BDNF Val66Met polymorphism on cognition has been extensively documented (e.g. Beste et al., 2011; Richter Schmidinger et al., 2011). More recent studies have shown that BDNF expression is partially regulated by epigenetic mechanisms (Martinowich et al., 2003; Ursini et al., 2016). This makes BDNF an interesting candidate molecule for the potential triggering of schizophrenia (Di Carlo et al., 2019).~~

In addition to BDNF, Phospholipase A2 (PLA2) is involved in brain maturation, memory formation, and synaptic remodelling, and has also been associated with schizophrenia (Berger et al., 2006). In the membrane phospholipid hypothesis of schizophrenia, the postulated pathomechanism of structural brain changes is an increased phospholipid turnover and breakdown in cell membranes and myelin sheaths (Law et al., 2006). Accordingly, Smesny et al. (2010) detected an association between PLA2 activity and structural changes in the left prefrontal cortex and bilateral thalamus of first-episode patients. Jensen et al. (2004) and Fukuzako et al. (1994) observed increased phospholipid breakdown products (phosphodiesters) using 31P-MR-spectroscopy in groups of patients possessing marked positive symptoms which supports this assumption.

Intracellular Ca^{2+}-independent Phospholipase has been found to be increased in plasma and serum of patients suffering from schizophrenia in several independent studies (e.g. Berger et al., 2006; Fenton et al., 2000; Law et al., 2006). Possibly as a consequence of increased PLA2 activity, polyunsaturated fatty acids (PUFAs) and phospholipids are reduced in post-mortem brains of patients with schizophrenia (Horrobin et al., 1991; Yao et al., 2000). Supplementation with PUFAs has successfully ameliorated schizophrenia-like symptoms in animal models (Armando et al., 2020; Crupi et al., 2013; Ribeiro et al., 2019; Zugno et al., 2015) and several clinical studies (Amminger et al., 2010; Assisi et al., 2006; Knöchel et al., 2015; Smesny et al., 2014).

However, despite the relatively similar effects on brain development and schizophrenia, the possible association of BDNF and PLA2 has not yet been examined. In order to assess the influence of a life-long genetically-mediated reduction of BDNF on the regulation of PLA2, we chose BDNF heterozygous C57BL/6N mice and their wild-type littermates as subjects in our study. In addition, prenatal stress is a crucial environmental factor involved in the development of schizophrenia in humans (e.g. Khashan et al., 2008; van Os and Selten, 1998) as well as psychosis-like expressions in animals (Koenig et al., 2005; Meyer and Feldon, 2010). Therefore, we exposed pregnant females carrying BDNF heterozygous

offspring to restraint stress according to Schmidt et al. (2017), thus establishing a gene × environment experimental setting.

Our first hypothesis was an inverse relation of BDNF and PLA2, e.g. an elevated PLA2 activity in BDNF heterozygous animals. Secondly, we expected an increase of behavioral deficits associated with lower BDNF levels and higher PLA2 activity.

Materials and Methods

Animals

Acclimatized female BDNF$^{+/+}$ and male BDNF$^{+/-}$ mice on a C57BL/6N background were used for breeding. BDNF$^{+/-}$ animals were originally generated as described by MacQueen et al. (2001) and were genotyped with PCR as recommended by the Jackson Labs (Bar Harbour, Maine, USA). All animals were housed individually in conventional type II macrolon cages, maintained at a constant temperature (22 °C ± 1 °C) on a 12 hour light–dark cycle (lights on from 0700h) with food and water *ad libitum* (Chourbaji et al., 2012). Vaginal plugs were checked on a daily basis and defined as embryonic day 0 (E0) when positive. In the last trimester from E13 to E17, prenatal restraint stress was applied by putting the dams into a transparent 250 ml glass cylinder filled up to a height of 5 mm with cold tap water, whilst being exposed to bright light (6000 lx) three times a day (between 0800h and 1000h, 1200h and 1400h, as well as 1600h and 1800h) for 45 minutes per session (Schmidt et al., 2017). Control dams were left undisturbed in their cages.

On postnatal day 1–4, the biological C57BL/6N dams were substituted with experienced NMRI (Naval Medical Research Institute) foster dams in a cross-fostering design. The transfer into the nest of the lactating foster dam took place within 15 minutes.

After weaning at four weeks of age, one heterozygous or wild-type male or female per litter was chosen for the behavioral experiments in order to prevent litter effects (Chapman and Stern, 1979). Mice were housed individually in macrolon type II cages with sawdust bedding and cellulose as nesting material, under a reversed 12h day-night cycle (lights on from 1900h-0700h). The animals were allowed to adapt to this environment for at least 14 days before the start of experiments, and were supplied with food and water *ad libitum*. Please see table 1a and 1b for the number of animals per factor and experimental group.

Sampling

The body weights of mice were taken at 5, 8, 12, 16 and 20 weeks of age. Mice were decapitated at 20 weeks of age between 0800h and 1100h and trunk blood was collected within 30 seconds after removal from the cage. These whole blood samples were mixed with an anticoagulant and stored on ice until they were centrifuged at 5000g for 10 minutes in order to obtain plasma. Brains were removed, cleaned from blood in ice-cold 0.9% NaCl solution, then dissected on ice to obtain tissue samples from different brain regions. The remaining brain material consisting of white and ~~grey~~gray matter was stored at -80 °C for PLA2 activity analysis in the brain. Additionally, the adrenal glands were removed, weighed and stored at -80 °C.

Behavioral analysis of adult offspring behavior

At age 15–17 weeks, 36 female and 40 male mice were tested behaviourally in the dark phase, i.e., in the active phase. Mice were acclimatized to the experimental room for at least 15 minutes prior to each experiment and then tested by an investigator who was blind to genotype and prenatal stress condition. The order of the tests followed earlier recommendations, ranking the tests from least stressful to more stressful (Mallien et al., 2020). Mice were sacrificed 3–5 weeks after the last experiment at 20 weeks of age.

Open field test

To evaluate locomotor and exploratory behavior, mice were individually placed into a white, open arena measuring 50×50 cm^2 under dimmed light conditions (25 lx). Activity monitoring was performed for 15 min using a video camera system (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVison 1.96 (Noldus Information Technology, Wageningen, the Netherlands) as described earlier (Lima-Ojeda et al., 2013).

Nest test

To assess general cognitive functioning and well-being via nest quality, the nesting material was removed

from the home cages at 0800h and a standard cotton nestlet (PLEXX, Arnheim) was introduced. The nest-building performance was rated after 5h and 24h with scores described elsewhere (Mallien et al., 2020).

T-maze

The T-maze test is a spatial working memory paradigm that analyzes the animals' ability to recognize and differentiate between a new unknown and a familiar compartment. The T-shaped maze is constructed of white plastic, with two 20-cm-long arms extending at right angles from a 40-cm-long alley. The arms and the alley have a width of 10 cm and are surrounded by 25-cm-high walls. Light intensity was 25 lx (Zueger et al., 2005).

The test consisted of two phases, with an intertrial interval of 60 minutes, during which the animals were returned to their home cage but remained in the experimental room. During an 8-min acquisition phase, one of the short arms was closed. In the 3-min retention phase, mice had access to all three arms. The number of visits and time spent in each of the short arms were assessed for the retention phase.

All procedures complied with the regulations covering animal experimentation within the EU (European Communities Council Directive 2010/63/EU). They were conducted in accordance with the institutions' animal care and use guidelines and approved by the national and local authorities (Regierungspräsidium Karlsruhe).

Intracellular Ca^{2+}-independent Phospholipase (PLA2)

Measurement in plasma

PLA2 activity was analyzed using a continuous kinetic fluorometric assay previously described (Smesny et al., 2011) but with some modifications. To exclusively quantify PLA2, a calcium-depleted environment was established by adding the Ca^{2+}-chelator ethylene glycol tetra–acetic acid (EGTA) (Carl Roth,

Karlsruhe, Germany) to the reaction and using 0.05 M HEPES-buffer (pH 7.4) (Biochrom Ltd., Cambridge, UK) prepared with HPLC-grade water (Thermo Fisher Scientific Inc., Waltham, MA, USA). As the bee venom PLA2 (Sigma-Aldrich, Inc., St. Louis, MO, USA) used for calibration was Ca^{2+}-dependent, the reference standard was dissolved and diluted respectively in HEPES buffer containing 1 mM Ca^{2+}, while no EGTA was added to wells of the standard curve. In addition, a coagulation control N (Technoclone, Vienna, Austria) obtained from the citrate plasma of healthy donors was added to adapt the kinetic reaction of the bee venom PLA2 with the BODIPY® FL dye-labeled sn-2 acyl chain and the dinitrophenyl quencher group of the of the fluorescent substrate PED6 (Hendrickson et al., 1999) to the one seen in plasma samples.

To determine PLA2 activity, a black µ-clear 96-well microplate (Greiner bio-one, Kremsmünster, Austria) was filled with 5 µL plasma samples, 5 µL EGTA and 180 µL HEPES buffer, while 5 µL standard solution or Ca^{2+}-HEPES buffer (blank) was mixed with 5 µL coagulation control N and 180 µL HEPES buffer for the for the PLA2-standard curve. After recording baseline values, 10 µL of PED6 solution (dissolved in hygroscopic dimethyl sulfoxide (Sigma-Aldrich, Inc., St. Louis, MO, USA) to obtain a 200 mM stock solution) was injected via the reagent dispenser and the fluorescence intensity (λ_{Ex} 485 nm/ λ_{Em} 538 nm) was measured for about 50 s using the NOVOstar® Galaxy (BMG LABTECH, Ortenberg, Germany). Moreover, two intra-assay variations were carried along for control of each assay. For verification of all test compounds, the recovery of aliquoted standard concentrations (50, 100 and 200 U/mL) was measured using the coagulation control N. Also, a control of each sample was executed by adding 5 µL 15.86 µg/mL (R)-Bromenol lactone (BEL) (Cayman Chemicals, Ann Arbor, MI, USA) to actively inhibit the Ca^{2+}-independent PLA2 activity. The PLA2 activity is proportional to the fluorescent intensity resulting from the elimination of the intramolecular quenching effect of the dinitrophenyl group and was calculated using the blank-corrected area under the curve from the point of the PED6-injection. By means of the standard curve, PLA2 activities were calculated according to the linear regression and expressed as U/mL. However, results were normalized to 1 mg/mL protein content of the plasma sample.

Measurement of PLA2 in brain tissue

Initially, the murine white and gray matter was homogenized by transferring the sample into 2 mL tubes containing 1.4/2.8 mm Precellys ceramic beads (VWR International, Radnor, PA, USA) to which cold tissue homogenization buffer (20 mM HEPES with 1 mM EGTA, 210 mM Mannitol, 70 mM Sucrose; pH 7.2) was added containing cOmplete™ EDTA-free Protease Inhibitor (Roche/Sigma-Aldrich, Inc., St. Louis, MO, USA). Samples were homogenized at 6,500 rpm for 10 s using the Precellys 24-Dual Homogenisator (VWR International, Radnor, PA, USA), and subsequently centrifuged at 10,000 rpm and 4°C for 5 min in order to collect the clear supernatant, which was stored at -80° C until further analyses, while homogenates were considered stabile for one month.

Tissue PLA2 activity was analyzed following the plasma PLA2 procedure with minor adjustments. These involved the bee venom PLA2 for calibration, which was solved and diluted in BSA-Ca^{2+}-HEPES buffer containing 1 mM Ca^{2+} and 60 g/L BSA. Thus, a 96-well microplate was filled with 5 µL standard solution or BSA-Ca^{2+}-HEPES buffer (blank) and 185 µL HEPES buffer before initiating the reaction with 10 µL PED6 substrate.

Statistical Analysis

Based on the 3×2 factorial design, all behavioral tests were analyzed using the three between subject-factors "prenatal stress", "genotype" and "sex".

All ANOVAs described below were conducted using the General Linear Model (GLM). Whenever the assumptions of parametric analysis were not met by graphical examination of homoscedasticity and outliers, raw data were transformed according to the Box-Cox-method (Box and Cox, 1964) and/or outliers were eliminated. The model chosen for all ANOVAs included all possible three-way- and two-way-interactions. There was no three-way interaction and except one two-way interaction that is described in the results section, all other interactions also clearly did not reach the level of statistical significance.

All subtests of the open field test and nest test, as well as body weights, were analyzed by three-way repeated measurements ANOVA with different time points as the within-subject factor~~,~~ whereby the time points for the open field test consisted in the first vs second five minutes of the test, for the nest test the first five hours vs 24 hours of the test and for the body weights 5, 8, 12, 16 and 20 weeks of age. In case of a significant two-way-interaction or main effect, ~~possible main effects were determined by~~ an additional stratified analysis using t-tests was performed. A MANOVA with subsequent univariate two-way ANOVAs and ~~subsequent~~ t-tests was ~~performed~~ used for the analysis of the T-maze. A ~~T~~three-way univariate ANOVA was performed for weight of adrenals and phospholipase A2 in plasma with subsequent t-tests in case of a significant main or interaction effect.

Linear and non-linear associations were tested with the linear Pearson product-moment coefficient and quadratic regression, respectively.

~~A p value <~~ $\alpha =$ 0.05 was used as the level of statistical significance in all tests. The statistical analyses were performed using the SPSS 21.0 software package for Windows.

Results

Body and adrenal weight

BDNF is known to influence the metabolic homeostasis in the body. We therefore determined whether BDNF knockout had an effect on body weight in the present sample. Body weight steadily increased significantly from birth to the age of 20 weeks ($p<0.001$, $F_{4,65}=327.854$). Males were heavier than females ($p<0.001$, $F_{1,68}=44.640$) and $BDNF^{+/-}$ animals weighed significantly more than wild-types ($p<0.001$, $F_{1,68}=27.523$; table 2). Prenatal stress did not have any influence on body and adrenal weight.

As previously shown (Bielohuby et al., 2007), adrenal weight of our sample was significantly higher in females than in males (sex $p<0.001$, $F_{1,67}=307.621$ with 1.48±0.54 mg (mean ± standard deviation) for males and 3.34±0.49 mg for females. Neither genotype ($p=0.305$, $F_{1,67}=1.068$, with 2.37±1.08 mg for wild-types and 2.36±1.07 mg for $BDNF^{+/-}$ nor prenatal stress ($p=0.365$, $F_{1,67}=0.832$; with 2.43±1.07 mg (no prenatal stress) and 2.3±1.08 mg (prenatal stress) did result in any difference in adrenal weight.

Open field test

The animals performed as observed earlier in all subtests of the open field test. Over time, total distance moved ($F_{1,68}=14.713$, $p<0.001$), movement ($F_{1,68}=28.774$, $p<0.001$) and mean velocity ($F_{1,68}=14.110$, $p<0.001$) decreased, whereas distance to walls ($F_{1,68}=16.076$, $p<0.001$) and center time ($F_{1,68}=6.556$, $p=0.013$) increased. Overall, females moved a greater distance ($F_{1,68}=5.343$, $p=0.024$) and displayed more mean velocity ($F_{1,68}=5.099$, $p=0.027$; data not shown) than males.

There was a significant time×genotype effect regarding total distance moved ($F_{1,68}=4.635$, $p=0.035$) and mean velocity ($F_{1,68}=4.771$, $p=0.032$), indicating that BDNF heterozygote mice moved a shorter distance and slower than wild-types within the first five minutes of the test (fig. 1A and fig. 1B). In the second half of the test, from minute 6–10, total distance moved and mean velocity of both groups were not significantly different. For the subtest "center time" we found a significant sex×genotype interaction effect

($F_{1,68}$=4.725, p=0.033). Male wild-types and female BDNF heterozygote animals spent less time in the center of the arena than female wild-types and male heterozygotes (fig. 1C).

Prenatal stress did not have any influence on the animals' performance in the subtests of the open field test (total distance moved: $F_{1,68}$=1.530, p=0.220; distance to walls: $F_{1,68}$=1.155, p=0.286; center time: $F_{1,68}$=1.121, p=0.293; movement: $F_{1,68}$=2.092, p=0.153; mean velocity: $F_{1,68}$=1.564, p=0.215, data not shown).

Nest test

There was a significant effect of sex over time (time×sex: $F_{1,68}$=5.795, p=0.019) and time ($F_{1,68}$=164.628, p<0.0010) on the performance of animals in the nest test, indicating that results improved after 24 h compared to 5 h. Sex was also significant ($F_{1,68}$=4.341, p=0.041). Subsequent t-tests revealed that males displayed significantly better nest building qualities in the first 5 h of the test compared to females (T(65.751)= -3.082, p=0.003, fig. 2). After 24 h there was no significant difference regarding nest building performance between the sexes (T(74)= -0.484, p=0.630). There was no significant difference between prenatally stressed vs non-stressed animals after 5 or 24 h (5 hrs: $F_{1,68}$=0.126, p=0.723; 24 h: $F_{1,68}$=0.257, p=0.614). BDNF heterozygote and wild-type mice also did not display any difference regarding their performance in the nest test (5hrs: $F_{1,68}$=2.544, p=0.115; 24 hrs: $F_{1,68}$=0.246, p=0.622).

T-maze

There was a significant main effect showing that BDNF heterozygous animals spent significantly less time in the new arm of the T-maze than wild-types ($F_{1,68}$=4.851, p=0.031), whereas there was no difference between these two groups of animals regarding time spent in the old arm of the T-maze ($F_{1,68}$=0.242, p=0.624). This result was confirmed by subsequent t-tests (time in new arm: T(74)=2.184, p=0.016; time in old arm: (T(74)= -0.530, p=0.299)). Prenatal stress (old arm: $F_{1,68}$=1.044, p=0.311; new arm: $F_{1,68}$=1.461, p=0.231) and sex (old arm: $F_{1,68}$=0.039, p=0.844; new arm: $F_{1,68}$=0.142, p=0.707; fig. 3) did not play any role regarding the time spent in the old vs. new arm of the T-maze.

PLA2 Activity in Plasma

As PLA2 in the white and gray matter of the whole brain yielded levels below detection threshold, only the plasma levels are shown here.

Genotype ($F_{1,64}$=8.860, p=0.004) and sex ($F_{1,64}$=14.834, p<0.0010) had a significant overall effect on PLA2 activity in the plasma of our animals. These two main effects were confirmed by subsequent t-tests. BDNF heterozygous mice as well as female mice showed a significantly higher PLA2 activity in their plasma compared to wild-types (T(70)= -2.791, p=0.004) and male animals (T(65.359)=4.054, p<0.0010, fig. 4), respectively.

Prenatal stress did not have a significant effect on PLA2 plasma activity levels ($F_{1,64}$=0.506, p=0.480).

Phospholipase A2 activity in plasma significantly correlated with adrenal weight, which was corrected by the body weight (r=0.319, p=0.006, fig. 5). Additionally, there was a significant negative correlation of phospholipase A2 in plasma with body weight at the age of 5 (r=- 0.307, p=0.009) and 8 weeks (r= -0.241, p=0.041, fig. 6A and 6B).

Additionally, PLA2 showed a significant quadratic association with "total distance moved from 5–10 minutes" ($F_{2,69}$=5.311, p=0.007, fig. 7A) and "mean velocity 5–10 minutes" of the open field test ($F_{2,69}$=5.054, p=0.009, fig. 7B). For both subtests this quadratic association reached significance in BDNF heterozygous animals (total distance moved: $F_{2,30}$=3.991, p=0.029, R^2=0.210; mean velocity: $F_{2,30}$=3.873, p=0.032, R^2=0.205) but not wild-type animals (total distance moved: $F_{2,36}$=2.073, p=0.141, R^2=0.103; mean velocity: $F_{2,36}$=1.964, p=0.155, R^2=0.98).

Discussion

Here we showed that BNDF heterozygous animals spent significantly less time in the new arm of the T-maze than their wild-type littermates. This indicates a lower cognitive performance in a classical working memory test and is recognized as a possible expression of a typical schizophrenia-like symptom (Castañé et al., 2015; Fujioka et al., 2014; Lander et al., 2019; Nikiforuk, 2018). In the open field test, BDNF deficient mice moved a significantly shorter distance and significantly slower than their wild-type littermates. These results might be interpreted as a correlate of negative symptoms, but are possibly confounded by an increased anxiety (Yee and Singer, 2013). Additionally, we cannot exclude the possibility that the size of our open field arena produced anxiety in the animals or that the knock-out is responsible for habituation deficits. However, phospholipase A2 showed a quadratic association with the subtest "total distance moved" and "velocity" of the open field test for BDNF heterozygous animals only. Thus, BDNF heterozygous mice with the lowest and highest PLA2 activity in plasma displayed the highest locomotor activity in the open field test, typically interpreted as a positive schizophrenia-like symptom (Nikiforuk, 2018). Similarly, McNamara et al. (2017) determined PLA2 using the same method employed in the present study and detected a quadratic association between phospholipase A2 activity and white matter changes in adolescents. The authors concluded that both higher and lower PLA2 activity is associated with lower white matter myelination. As we found this relation only in BDNF heterozygous animals, it is possible that BDNF is involved in the complex interplay between PLA2 and white matter maturation.

In accordance with our hypothesis, PLA2 activity was significantly increased in BDNF heterozygous animals. Although PLA2 and BDNF are both linked to the development of the brain and schizophrenic illness, their interaction has never been examined in a knockout model. Some previous studies have suggest an association between the metabolism of omega-3 PUFA, BDNF and neuronal function. Dong et al. (2018) demonstrated that supplementation with eicosapentaenoic acid (EPA), an omega-3 PUFA, up-regulated BDNF levels in the rat hippocampus. Keleshian et al. (2014) reported that in omega-3 PUFA deprived rats, NMDA induced a significant reduction of BDNF levels in the brain. Recently, a randomized

clinical trial by Pawełczyk et al. (2019) in schizophrenia patients showed that a six-month supplementation with omega-3 PUFA significantly increased peripheral BDNF levels. The authors speculated that this shift in BDNF may have been triggered by the activation of intracellular signaling pathways, including transcription factors such as cAMP-reactive element binding protein, in response to omega-3 PUFA.

Most interestingly, there is evidence that both PLA2 and BDNF are linked to the dopaminergic system. The Omega-6 PUFA arachidonic acid – a metabolite of PLA2 – is essential for dopaminergic signaling (Berger, Smesny and Amminger, 2006). A deficiency of PUFAs – amongst others - leads to a reduced dopamine concentration and lower number of D2 receptors in the frontal lobe (Zimmer et al., 2000) and an increased concentration of dopamine in the limbic system (Delion et al., 1994).

Interestingly, BDNF heterozygous mice display higher tones of dopamine in the dorsal striatum (Birbeck et al., 2014; Bosse et al., 2012). They also show less cognitive flexibility than wild-type mice (Parikh et al., 2016), which corresponds to our behavioral data.

The relationship described above between PLA2 and BDNF needs to be further specified with regard to sex-specific aspects. For the first time, we demonstrated significantly increased PLA2 levels in female C57BL/6N mice. In their review, Wu et al. (2013) outlined numerous human and animal studies indicating that estrogen correlates with BDNF signaling and thus also influences dopaminergic and NMDA receptor activity. According to the authors, this might explain the ubiquitous sex differences in schizophrenia age of onset, symptoms and treatment response. Our finding occurred predominantly in the subgroups of wild-types and prenatally non-stressed mice. Thus it seems likely that the increase of PLA2 in females is not directly associated with BDNF heterozygosity or prenatal stress but directly influenced by sex hormones.

Indeed, there are a few investigations demonstrating a sex-dependent regulation of fatty acids. For instance, Kitson et al. (2010) described several studies in human and animal studies that demonstrated an elevated production of docosahexaenoic acid – also an omega-3 fatty acid – in females. The authors suggested two potential molecular mechanisms for this finding: 1.) estrogen increases the activity of ERK-MAPK and protein kinase A, which phosphorylates and increases the activity of PPARα, and/or 2.)

estrogen increases the intracellular concentration of both PUFA and eicosanoids, PPARα ligands, via elevated Ca^{2+}-PLA2 and COX-2 activities, resulting in activation of PPARα. This second mechanism of molecular regulation is completely consistent with our findings. There is one clinical examination describing a possible sex-dependent regulation of PLA2. McNamara et al. (2017) showed a significant relationship of PLA2 activity, long-chain unsaturated fatty acids and white matter microstructure in males but not females. The authors also suggested a possible role of sex hormones in fatty acid homeostasis for white matter maturation.

As there is evidence of basic adrenal overactivity and metabolic disturbance in schizophrenia (Ryan et al., 2004; Sullivan et al., 2015; Thakore, 2004), we determined adrenal and body weights of our animals and found a positive correlation between PLA2 and adrenal weight. Although little is known about the role of PLA2 in steroidogenesis (Bollag, 2016), there are suggestions that inhibitors of PLA2 activity, such as quinacrine, reduce AngII-stimulated aldosterone production in glomerulosa cells and exogenous PLA2 leads to transient steroidogenesis (Kojima et al., 1985). Furthermore, PLA2 inhibitors reduce cortisol secretion from fasciculata/reticularis cells following ACTH production. In contrast, Andreis et al. (1999) found that the PLA2 inhibitor AACOCF3 increases basal aldosterone and corticosterone secretion in rats and humans. It also leads to more cortisol production in response to ACTH and the release of other steroid intermediates, suggesting that that PLA2 inhibits early steps in steroidogenesis. Stimulation as well as inhibition (possibly as a compensatory mechanism) of steroidogenesis by PLA2 may lead to adrenal tissue growth and could explain our finding that adrenal weight is positively correlated to PLA2 activity. We assume that the hypothalamic-pituitary axis is activated via anxiety and stress caused by the onset of positive symptoms of schizophrenia. As this process also causes neurotoxic effects, it is likely that PLA2 is subsequently activated as a maintenance enzyme on the membrane level.

There was a negative correlation of PLA2 with body weight directly after weaning at 5 weeks of age. At 8 weeks of age this correlation was still significant, but was not detectable after week 12. Forlenza et al. (2002) found a peak PLA2 activity in embryonic rat brains at embryonic day 18 that decreased towards birth. Yang et al. (1999) detected a slightly different development of PLA2 in rat brains. PLA2 reached its

peak after birth at 12 weeks of age. It is possible that the smallest and most underdeveloped offspring in our study produced the highest PLA2 levels to accelerate neurodevelopment via increased synaptogenesis, glial proliferation and myelination.

Finally, we used plasma as a peripheral parameter of PLA2 activity, as several studies showed that peripheral and central PLA2 activity is strongly correlated (Chen et al., 2008; Gattaz et al., 1996; Rapoport, 2008; Smesny et al., 2008). Our aim was to demonstrate the correlation between peripheral and central PLA2 in our samples. Unfortunately, our data in brain tissue appeared to be below the detection threshold. In the literature, there are no studies examining PLA2 activity in mouse brain, but PLA2 has been measured in rat brain (e.g. Schaeffer et al., 2005). It is likely that the amount of PLA2 in mouse brain is too small to be detectable with the antibody or analysis system used here. Further research is necessary to clarify this issue.

Here we demonstrated for the first time a significantly elevated PLA2 level in BDNF heterozygous mice. This reflects a possible association of these two parameters in the development of schizophrenia. Moreover, the increased PLA2 activity in females may reflect a sex-specific course of schizophrenia. The correlation between adrenal weight and PLA2 suggests a connection of PLA2 with steroidogenesis and may reflect the association between the acute positive symptomatic onset of schizophrenic psychosis, stress, hormonal stress response and structural changes at the membrane level.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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References

Amminger GP, Schäfer MR, Papageorgiou K, Klier CM, Cotton SM, Harrigan SM, Mackinnon A, McGorry PD, et al. (2010) Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch Gen Psychiat 67:146-154.

Andreis PG, Buttazzi P, Tortorella C, De Caro R, Aragona F, Neri G, Nussdorfer GG (1999) The inhibitor of phospholipase-A2, AACOCF3, stimulates steroid secretion by dispersed human and rat adrenocortical cells. Life Sci 64:1287-1294.

Angelucci F, Brenè S, Mathé AA (2005) BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatr 10:345-352.

Armando M, Ciampoli M, Padula MC, Amminger P, De Crescenzo F, Maeder J, Schneider M, Schaer M, et al. (2020) Favorable effects of omega-3 polyunsaturated fatty acids in attentional control and conversion rate to psychosis in 22q11.2 deletion syndrome. Neuropharmacology 168:107995.

Ashe PC, Chlan-Fourney J, Juorio AV, Li XM (2002) Brain-derived neurotrophic factor (BDNF) mRNA in rats with neonatal ibotenic acid lesions of the ventral hippocampus. Brain Res 956:126-135.

Assisi A, Banzi R, Buonocore C, Capasso F, Di Muzio V, Michelacci F, Renzo D, Tafuri G, et al. (2006) Fish oil and mental health: the role of n-3 long-chain polyunsaturated fatty acids in cognitive development and neurological disorders. Int Clin Psychopharm 21:319-336.

Berger GE, Smesny S, Amminger GP (2006) Bioactive lipids in schizophrenia. Int Rev Psychiatr 18:85-98.

Bielohuby M, Herbach N, Wanke R, Maser-Gluth C, Beuschlein F, Wolf E, Hoeflich A (2007) Growth analysis of the mouse adrenal gland from weaning to adulthood: time- and gender-dependent alterations of cell size and number in the cortical compartment. Am J Physiol-Endoc M 293:E139-146.

Birbeck JA, Khalid M, Mathews TA (2014) Potentiated striatal dopamine release leads to hyperdopaminergia in female brain-derived neurotrophic factor heterozygous mice. ACS Chem Neurosci 5:275-281.

Bollag WB (2016) Role of phospholipases in adrenal steroidogenesis. J Endocrinol 229:R29-41.

Bosse KE, Maina FK, Birbeck JA, France MM, Roberts JJ, Colombo ML, Mathews TA (2012) Aberrant striatal dopamine transmitter dynamics in brain-derived neurotrophic factor-deficient mice. J Neurochem 120:385-395.

Box GEP, Cox DR (1964) An analysis of transformations. J R Stat Soc B 26:211-252.

Castañé A, Santana N, Artigas F (2015) PCP-based mice models of schizophrenia: differential behavioral, neurochemical and cellular effects of acute and subchronic treatments. Psychopharmacology (Berl) 232:4085-4097.

Chapman RH, Stern JM (1979) Failure of severe maternal stress or ACTH during pregnancy to affect emotionality of male rat offspring: Implications of litter effects for prenatal studies. Dev Psychobiol 12:255-267.

Chen CT, Green JT, Orr SK, Bazinet RP (2008) Regulation of brain polyunsaturated fatty acid uptake and turnover. Prostag Leukotr Ess 79:85-91.

Chourbaji S, Hortnagl H, Molteni R, Riva MA, Gass P, Hellweg R (2012) The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system. Neuroscience 220:267-276.

Crupi R, Marino A, Cuzzocrea S (2013) n-3 fatty acids: role in neurogenesis and neuroplasticity. Curr Med Chem 20:2953-2963.

Delion S, Chalon S, Hérault J, Guilloteau D, Besnard JC, Durand G (1994) Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 124:2466-2476.

Dong Y, Xu M, Kalueff AV, Song C (2018) Dietary eicosapentaenoic acid normalizes hippocampal omega-3 and 6 polyunsaturated fatty acid profile, attenuates glial activation and regulates BDNF function in a rodent model of neuroinflammation induced by central interleukin-1β administration. Eur J Nutr 57:1781-1791.

Durany N, Michel T, Zöchling R, Boissl KW, Cruz-Sánchez FF, Riederer P, Thome J (2001) Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophr Res 52:79-86.

Fenton WS, Hibbeln J, Knable M (2000) Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biol Psychiat 47:8-21.

Fiore M, Korf J, Antonelli A, Talamini L, Aloe L (2002) Long-lasting effects of prenatal MAM treatment on water maze performance in rats: associations with altered brain development and neurotrophin levels. Neurotoxicol Teratol 24:179-191.

Forlenza OV, Schaeffer EL, Gattaz WF (2002) Phospholipase A2 activity in rat embryonic brain and in primary cultures of cortical neurons. J Neural Transm (Vienna) 109:623-631.

Fujioka R, Nii T, Iwaki A, Shibata A, Ito I, Kitaichi K, Nomura M, Hattori S, et al. (2014) Comprehensive behavioral study of mGluR3 knockout mice: implication in schizophrenia related endophenotypes. Mol Brain 7:31.

Fukuzako H, Takeuchi K, Ueyama K, Fukuzako T, Hokazono Y, Hirakawa K, Yamada K, Hashiguchi T, et al. (1994) 31P magnetic resonance spectroscopy of the medial temporal lobe of schizophrenic patients with neuroleptic-resistant marked positive symptoms. Eur Arch Psy Clin N 244:236-240.

Gattaz WF, Cairns NJ, Levy R, Förstl H, Braus DF, Maras A (1996) Decreased phospholipase A2 activity in the brain and in platelets of patients with Alzheimer's disease. Eur Arch Psy Clin N 246:129-131.

Hendrickson HS, Hendrickson EK, Johnson ID, Farber SA (1999) Intramolecularly quenched BODIPY-labeled phospholipid analogs in phospholipase A(2) and platelet-activating factor acetylhydrolase assays and in vivo fluorescence imaging. Anal Biochem 276:27-35.

Horrobin DF, Manku MS, Hillman H, Iain A, Glen M (1991) Fatty acid levels in the brains of schizophrenics and normal controls. Biol Psychiat 30:795-805.

Iritani S, Niizato K, Nawa H, Ikeda K, Emson PC (2003) Immunohistochemical study of brain-derived neurotrophic factor and its receptor, TrkB, in the hippocampal formation of schizophrenic brains. Prog Neuropsychopharmacol Biol Psychiat 27:801-807.

Jensen JE, Miller J, Williamson PC, Neufeld RW, Menon RS, Malla A, Manchanda R, Schaefer B, et al. (2004) Focal changes in brain energy and phospholipid metabolism in first-episode schizophrenia: 31P-MRS chemical shift imaging study at 4 Tesla. Brit J Psychiat 184:409-415.

Keleshian VL, Kellom M, Kim HW, Taha AY, Cheon Y, Igarashi M, Rapoport SI, Rao JS (2014) Neuropathological responses to chronic NMDA in rats are worsened by dietary n-3 PUFA deprivation but are not ameliorated by fish oil supplementation. PLoS One 9:e95318.

Khashan AS, Abel KM, McNamee R, Pedersen MG, Webb RT, Baker PN, Kenny LC, Mortensen PB (2008) Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. Arch Gen Psychiat 65:146-152.

Kitson AP, Stroud CK, Stark KD (2010) Elevated production of docosahexaenoic acid in females: potential molecular mechanisms. Lipids 45:209-224.

Knöchel C, Voss M, Grüter F, Alves GS, Matura S, Sepanski B, Stäblein M, Wenzler S, et al. (2015) Omega 3 Fatty Acids: Novel Neurotherapeutic Targets for Cognitive Dysfunction in Mood Disorders and Schizophrenia? Curr Neuropharmacol 13:663-680.

Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, Hercher E, Brady DL (2005) Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. Behav Brain Res 156:251-261.

Kojima I, Kojima K, Rasmussen H (1985) Possible role of phospholipase A2 action and arachidonic acid metabolism in angiotensin II-mediated aldosterone secretion. Endocrinology 117:1057-1066.

Lander SS, Khan U, Lewandowski N, Chakraborty D, Provenzano FA, Mingote S, Chornyy S, Frigerio F, et al. (2019) Glutamate Dehydrogenase-Deficient Mice Display Schizophrenia-Like Behavioral Abnormalities and CA1-Specific Hippocampal Dysfunction. Schizophrenia Bull 45:127-137.
Law MH, Cotton RG, Berger GE (2006) The role of phospholipases A2 in schizophrenia. Mol Psychiatr 11:547-556.
Lima-Ojeda JM, Vogt MA, Pfeiffer N, Dormann C, Köhr G, Sprengel R, Gass P, Inta D (2013) Pharmacological blockade of GluN2B-containing NMDA receptors induces antidepressant-like effects lacking psychotomimetic action and neurotoxicity in the perinatal and adult rodent brain. Prog Neuro-Psychoph 45:28-33.
Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR (2001) BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. Eur J Neurosci 14:135-144.
MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav Neurosci 115:1145-1153.
Mallien AS, Häger C, Palme R, Talbot SR, Vogt MA, Pfeiffer N, Brandwein C, Struve B, et al. (2020) Systematic analysis of severity in a widely used cognitive depression model for mice. Lab Animal 54:40-49.
Mamounas LA, Blue ME, Siuciak JA, Altar CA (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 15:7929-7939.
McNamara RK, Szeszko PR, Smesny S, Ikuta T, DeRosse P, Vaz FM, Milleit B, Hipler UC, et al. (2017) Polyunsaturated fatty acid biostatus, phospholipase A2 activity and brain white matter microstructure across adolescence. Neuroscience 343:423-433.
Meyer U, Feldon J (2010) Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog Neurobiol 90:285-326.
Nikiforuk A (2018) Assessment of cognitive functions in animal models of schizophrenia. Pharmacol Rep 70:639-649.
Parikh V, Naughton SX, Yegla B, Guzman DM (2016) Impact of partial dopamine depletion on cognitive flexibility in BDNF heterozygous mice. Psychopharmacology (Berl) 233:1361-1375.
Pawełczyk T, Grancow-Grabka M, Trafalska E, Szemraj J, Żurner N, Pawełczyk A (2019) An increase in plasma brain derived neurotrophic factor levels is related to n-3 polyunsaturated fatty acid efficacy in first episode schizophrenia: secondary outcome analysis of the OFFER randomized clinical trial. Psychopharmacology (Berl) 236:2811-2822.
Rapoport SI (2008) Arachidonic acid and the brain. J Nutr 138:2515-2520.
Ribeiro BMM, Chaves Filho AJM, Costa D, de Menezes AT, da Fonseca ACC, Gama CS, Moura Neto V, de Lucena DF, et al. (2019) N-3 polyunsaturated fatty acids and clozapine abrogates poly I: C-induced immune alterations in primary hippocampal neurons. Prog Neuro-Psychoph 90:186-196.
Ryan MC, Sharifi N, Condren R, Thakore JH (2004) Evidence of basal pituitary-adrenal overactivity in first episode, drug naïve patients with schizophrenia. Psychoneuroendocrino 29:1065-1070.
Schaeffer EL, Bassi F, Jr., Gattaz WF (2005) Inhibition of phospholipase A2 activity reduces membrane fluidity in rat hippocampus. J Neural Transm (Vienna) 112:641-647.
Schmidt M, Lapert F, Brandwein C, Deuschle M, Kasperk C, Grimsley JM, Gass P (2017) Prenatal stress changes courtship vocalizations and bone mineral density in mice. Psychoneuroendocrino 75:203-212.
Smesny S, Stein S, Willhardt I, Lasch J, Sauer H (2008) Decreased phospholipase A2 activity in cerebrospinal fluid of patients with dementia. J Neural Transm (Vienna) 115:1173-1179.
Smesny S, Milleit B, Nenadic I, Preul C, Kinder D, Lasch J, Willhardt I, Sauer H, et al. (2010) Phospholipase A2 activity is associated with structural brain changes in schizophrenia. Neuroimage 52:1314-1327.

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Smesny S, Kunstmann C, Kunstmann S, Willhardt I, Lasch J, Yotter RA, Proffitt TM, Kerr M, et al. (2011) Phospholipase A_2 activity in first episode schizophrenia: associations with symptom severity and outcome at week 12. World J Biol Psychia 12:598-607.

Smesny S, Milleit B, Hipler UC, Milleit C, Schafer MR, Klier CM, Holub M, Holzer I, et al. (2014) Omega-3 fatty acid supplementation changes intracellular phospholipase A2 activity and membrane fatty acid profiles in individuals at ultra-high risk for psychosis. Mol Psychiatr 19:317-324.

Sullivan EL, Riper KM, Lockard R, Valleau JC (2015) Maternal high-fat diet programming of the neuroendocrine system and behavior. Horm Behav 76:153-161.

Thakore JH (2004) Metabolic disturbance in first-episode schizophrenia. Brit J Psychiat Suppl 47:S76-79.

Thome J, Foley P, Riederer P (1998) Neurotrophic factors and the maldevelopmental hypothesis of schizophrenic psychoses. Review article. J Neural Transm (Vienna) 105:85-100.

Toyooka K, Asama K, Watanabe Y, Muratake T, Takahashi M, Someya T, Nawa H (2002) Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. Psychiat Res 110:249-257.

van Os J, Selten JP (1998) Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. Brit J Psychiat 172:324-326.

Wu YC, Hill RA, Gogos A, van den Buuse M (2013) Sex differences and the role of estrogen in animal models of schizophrenia: interaction with BDNF. Neuroscience 239:67-83.

Yang HC, Mosior M, Ni B, Dennis EA (1999) Regional distribution, ontogeny, purification, and characterization of the Ca2+-independent phospholipase A2 from rat brain. J Neurochem 73:1278-1287.

Yao JK, Leonard S, Reddy RD (2000) Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophr Res 42:7-17.

Yee BK, Singer P (2013) A conceptual and practical guide to the behavioural evaluation of animal models of the symptomatology and therapy of schizophrenia. Cell Tissue Res 354:221-246.

Zimmer L, Delpal S, Guilloteau D, Aïoun J, Durand G, Chalon S (2000) Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. Neurosci Lett 284:25-28.

Zueger M, Urani A, Chourbaji S, Zacher C, Roche M, Harkin A, Gass P (2005) Olfactory bulbectomy in mice induces alterations in exploratory behavior. Neurosci Lett 374:142-146.

Zugno AI, Chipindo H, Canever L, Budni J, Alves de Castro A, Bittencourt de Oliveira M, Heylmann AS, Gomes Wessler P, et al. (2015) Omega-3 fatty acids prevent the ketamine-induced increase in acetylcholinesterase activity in an animal model of schizophrenia. Life Sci 121:65-69.

Figure

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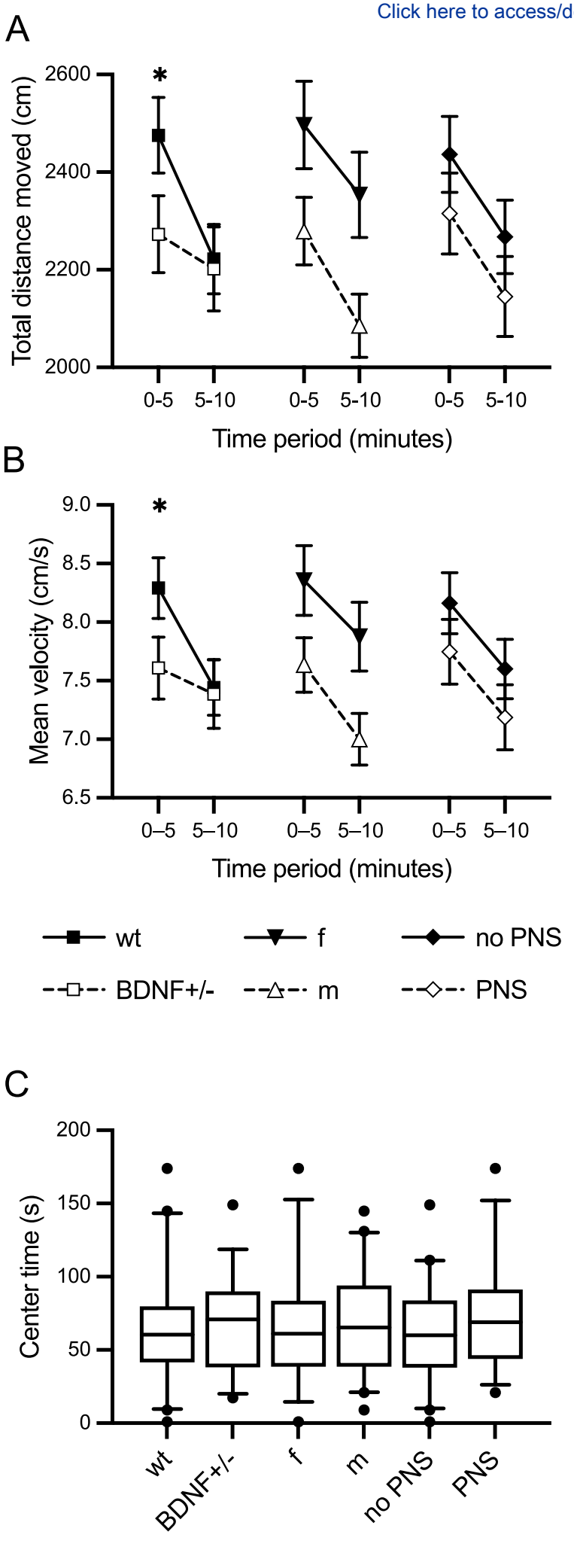

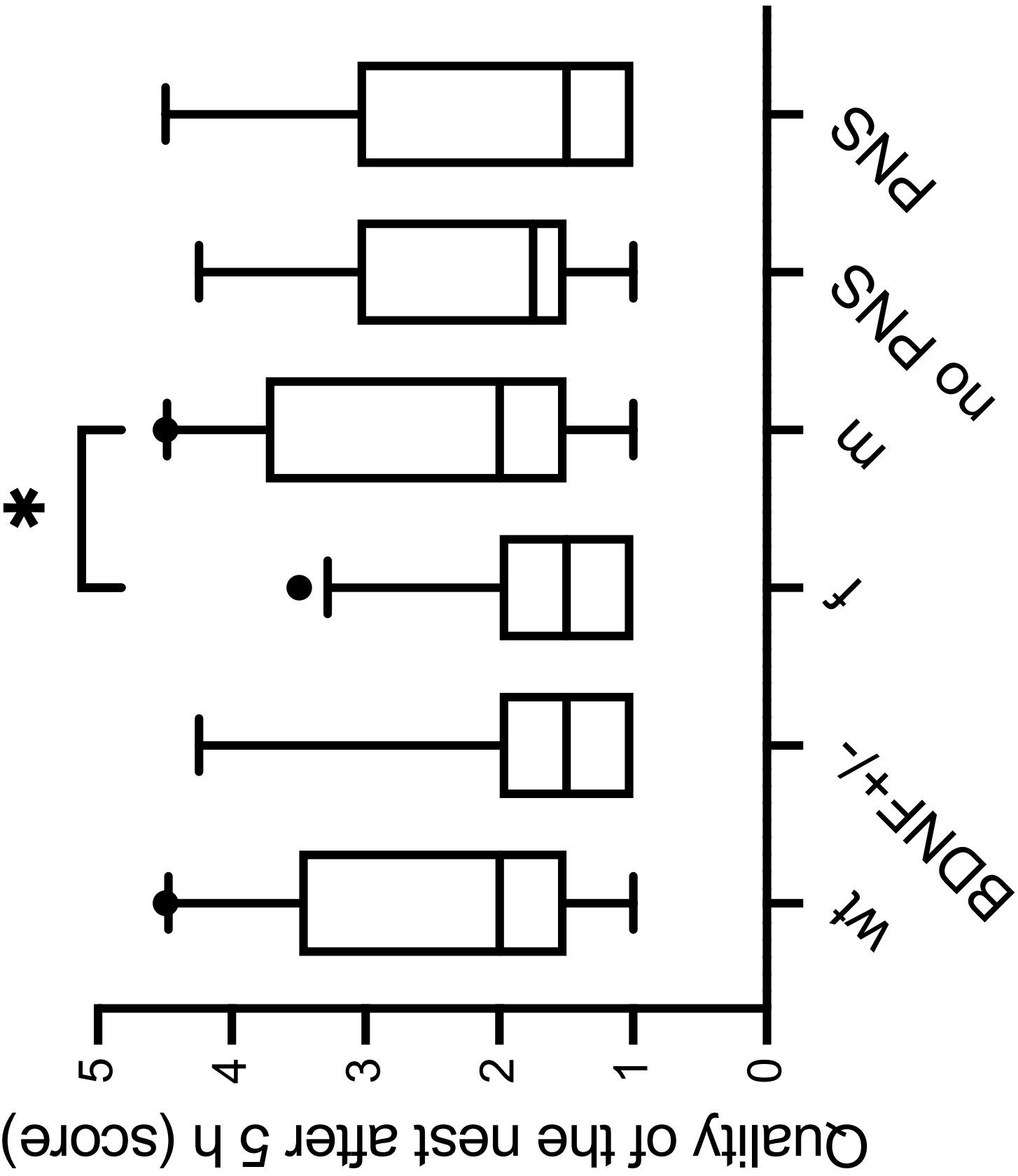

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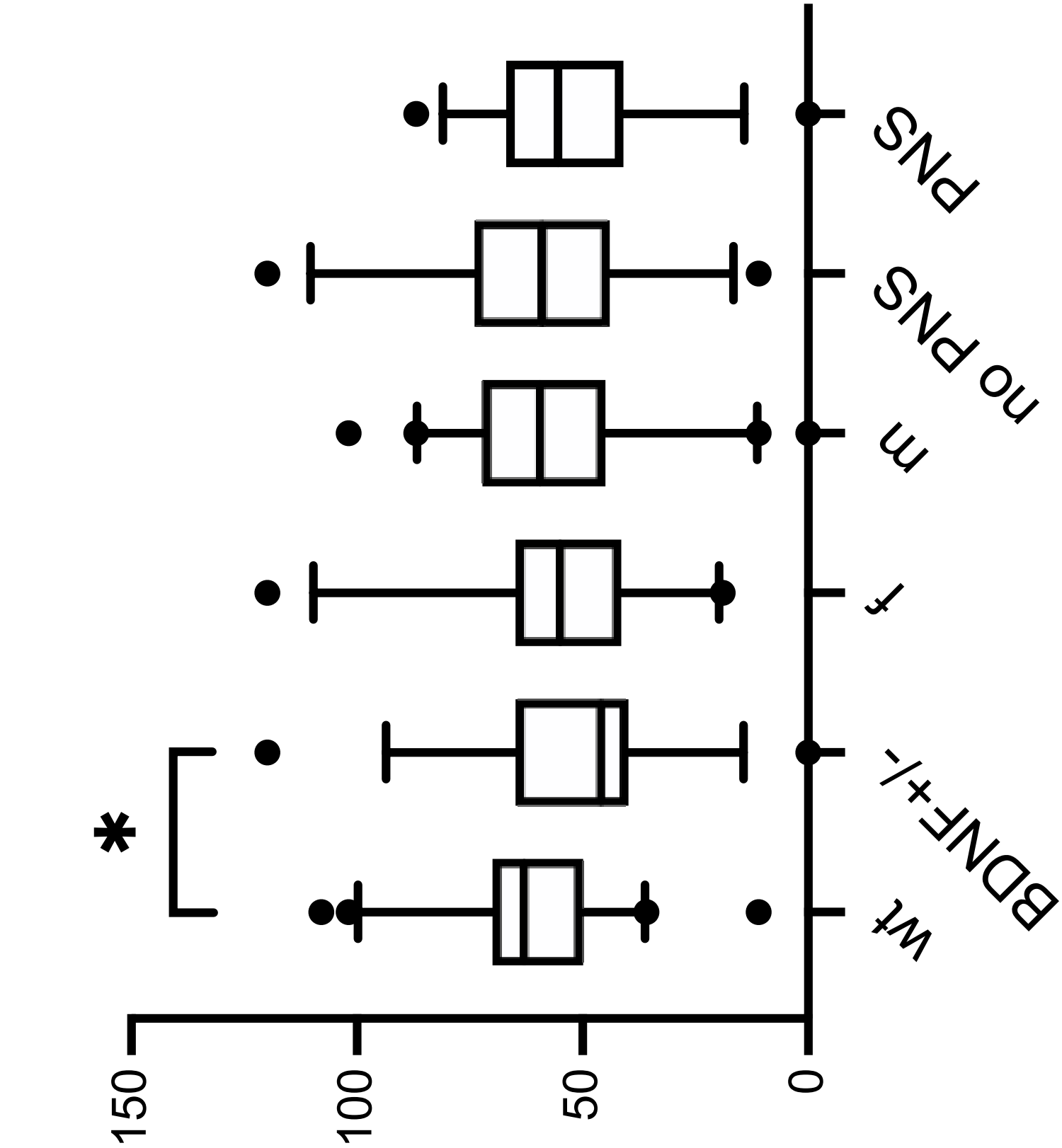

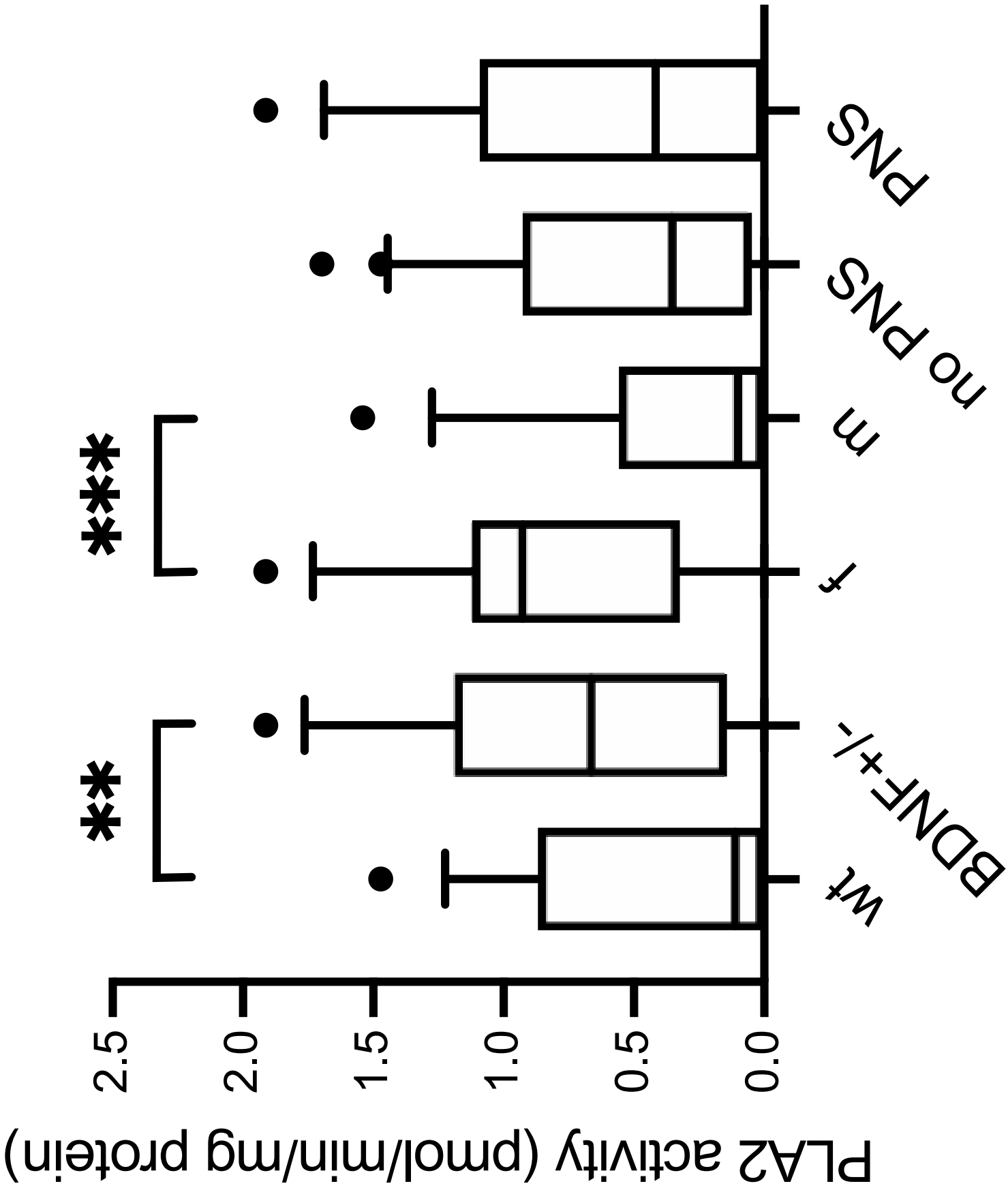

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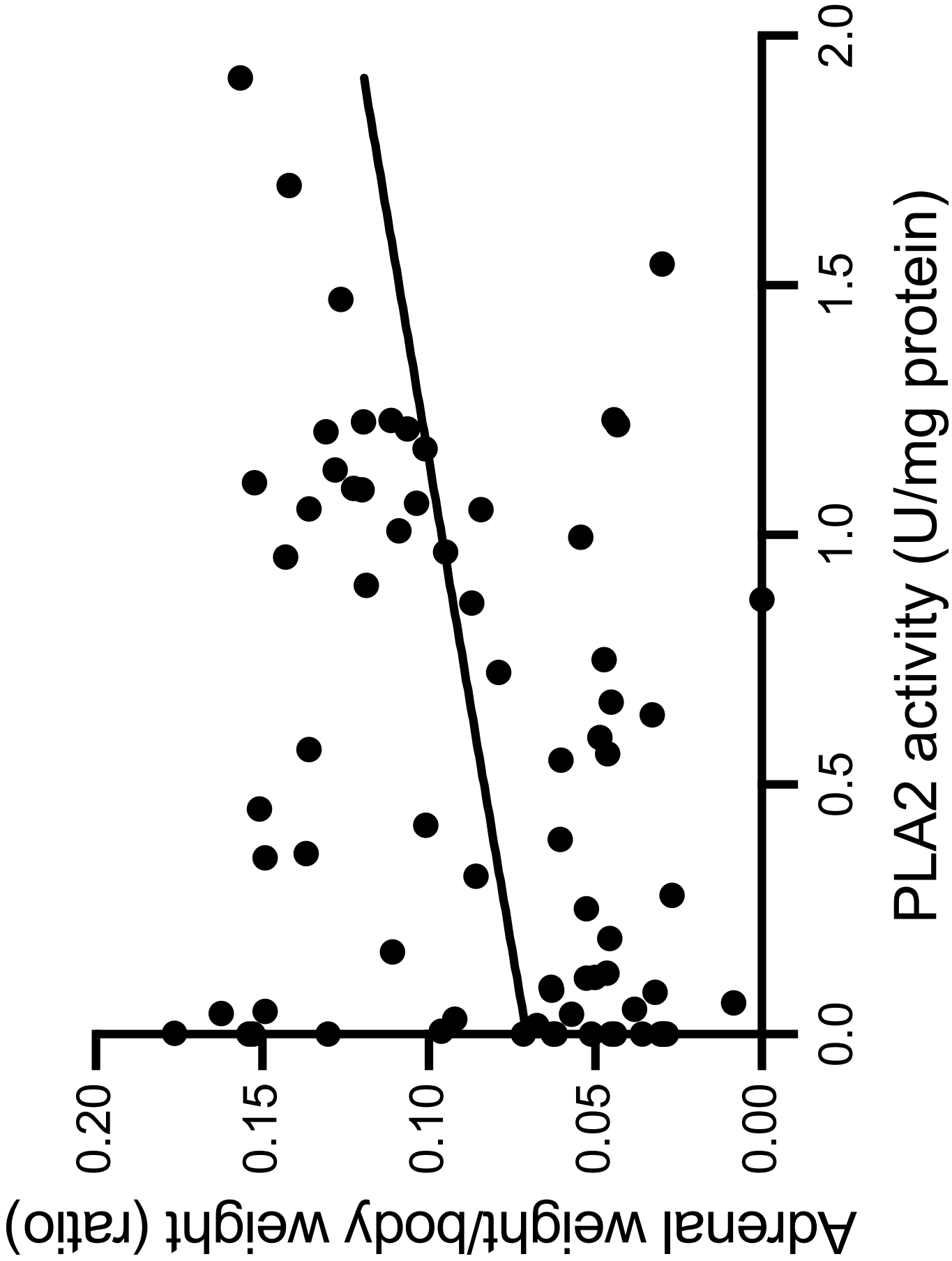

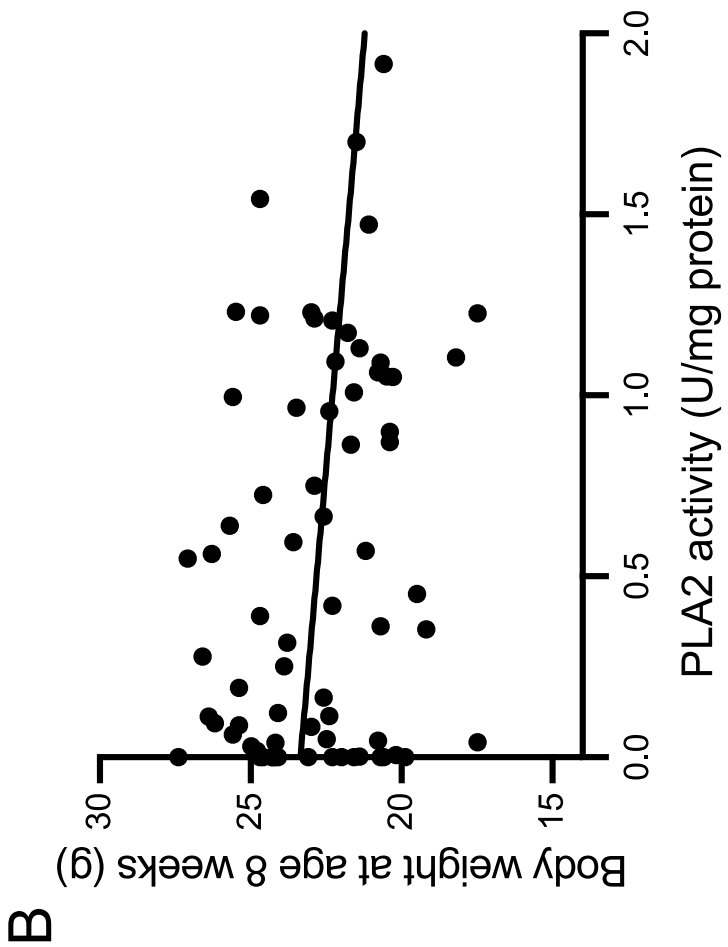

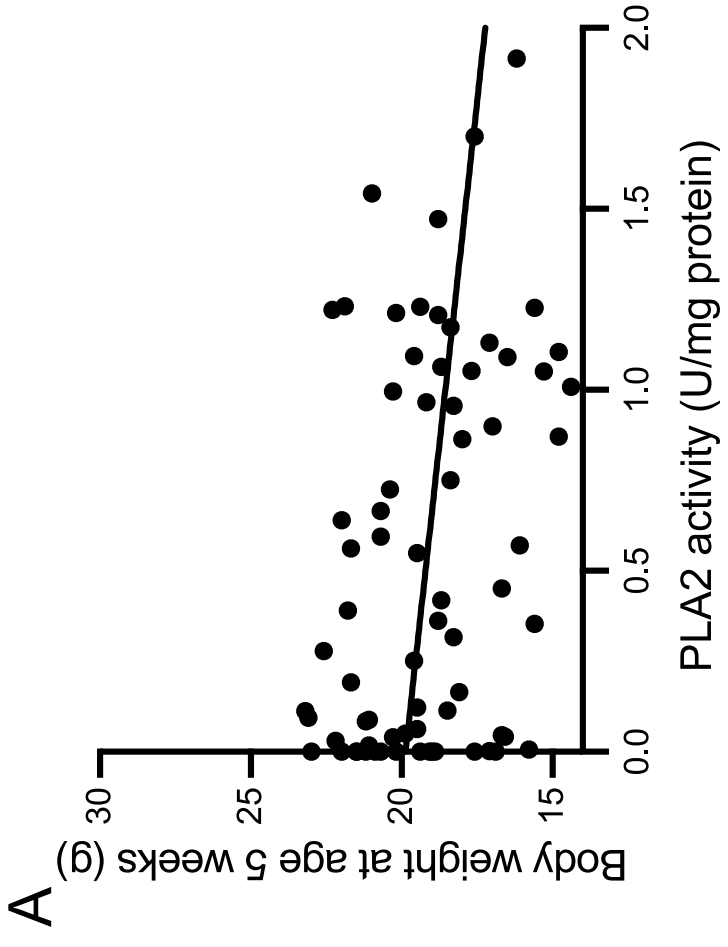

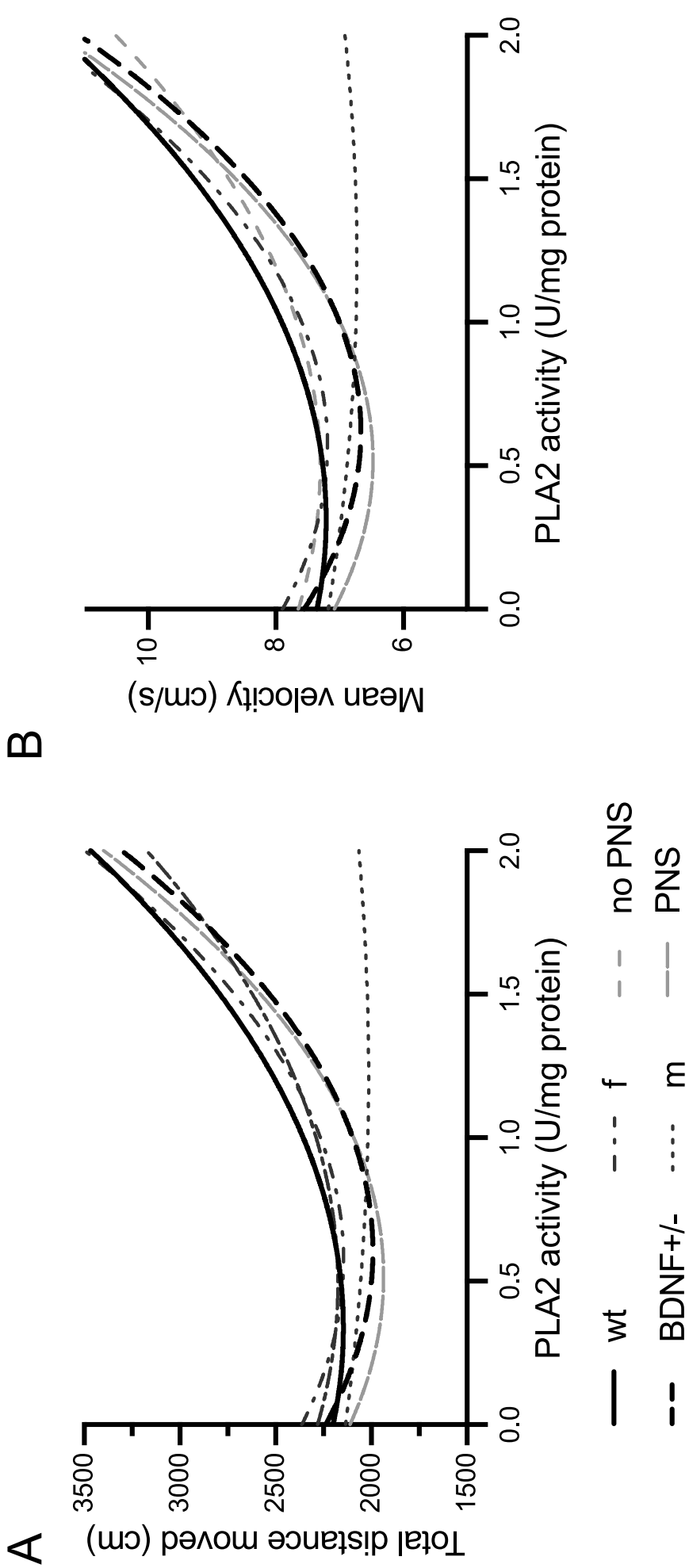

Figure 1: Open field test. A: Total distance moved (line charts with means and standard errors of the mean, SEM). BDNF heterozygous mice moved a significantly shorter distance than wild-type mice within the first five minutes of the test. B: Mean velocity (line charts, means, SEM). BDNF heterozygous mice moved significantly slower than wild-type mice within the first five minutes of the test. C: Center time (box-and-whisker plots). Male wild-type mice and female BDNF heterozygous animals spent less time in the center of the arena than female wild-type mice and male heterozygous animals.
wt: wild-type, BDNF+/-: BDNF heterozygote, m: male, f: female, no PNS: no prenatal stress, PNS: prenatal stress. Symbol coding according to legends in the figures. Boxes of all box-whisker plots: 25th to 75th percentile, whiskers: 5th to 95th percentile, outliers: •. * denotes statistical significance for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$.

Figure 2: Nest test. Males perform significantly better in the first five hours of the test than females. Box-whisker-plots with abbreviations and symbol coding according to fig. 1.

Figure 3: Time spent in the new arm of the T-maze. BDNF heterozygous animals spent significantly less time in the new arm of the T-maze. Box-whisker-plots with abbreviations and symbol coding according to fig. 1.

Figure 4: Phospholipase A2 (PLA2) activity in plasma. There was a significantly higher PLA2 activity in plasma of BDNF heterozygous and female mice compared to wild-type mice and male animals respectively. Box-whisker-plots with abbreviations and symbol coding according to fig. 1.

Figure 5: Significantly positive correlation of Phospholipase A2 (PLA2) activity in plasma with body weight adjusted adrenal weight. Scatter plot with linear regression line.

Figure 6: Significantly negative correlation of Phospholipase A2 (PLA2) activity in plasma with body weight at age 5 weeks (A) and 8 weeks (B). Scatter plot with linear regression line.

Figure 7: Quadratic regression of PLA2 plasma activity with “total distance moved” (A) and „mean velocity” (B) of the open field test is significant only for BDNF heterozygous animals. Quadratic regression lines.

Table 1a: Number of animals per factor.

Experimental Factors		Number of Animals
Sex	Male (m)	40
	Female (f)	36
Genotype	Wildtype (wt)	41
	$BDNF^{+/-}$	35
Prenatal Stress	No Prenatal Stress (no PNS)	42
	Prenatal Stress (PNS)	34

Table 1b: Number of animals per experimental group (sorted by factors sex, genotype and prenatal stress).

Sex	Genotype	Prenatal Stress	Number of Animals
Male	Wildtype	No	12
Female	Wildtype	No	11
Male	$BDNF^{+/-}$	No	10
Female	$BDNF^{+/-}$	No	9
Male	Wildtype	Yes	10
Female	Wildtype	Yes	8
Male	$BDNF^{+/-}$	Yes	8
Female	$BDNF^{+/-}$	Yes	8

Table 2: Body weights from week 5 to 20, depicted as mean ± standard deviation and results of the T-tests (T- and p-value).

Body weight	5 weeks	8 weeks	12 weeks	16 weeks	20 weeks
males	20.693 ± 1.4353	24.220 ± 1.8131	27.050 ± 2.1149	31.420 ± 4.2065	34.290 ± 5.6377
females	17.578 ± 1.7534	21.258 ± 1.6814	24.139 ± 2.2853	27.339 ± 3.8498	29.681 ± 5.4926
T-tests	T (74) = -8.507; p < 0.001	T (74) = -7.358; p < 0.001	T (74) = -5.767; p < 0.001	T (74) = -4.395; p < 0.001	T (74) = -3.602; p < 0.001
wild-types	18.832 ± 2.2961	22.117 ± 2.2478	25.073 ± 2.3316	27.759 ± 3.2543	29.198 ± 3.6772
BDNF+/-	19.669 ± 2.0839	23.637 ± 2.0837	26.371 ± 2.8102	31.511 ± 4.9553	35.514 ± 6.4256
T-tests	T (74) = -1.652; p = 0.052	T (74) = -3.038; p = 0.002	T (74) = -2.201; p = 0.016	T (57,069) = 3.831; p< 0.001	T (52,200)=-5.141; p < 0.001
No prenatal stress	18.969 ± 0.3413	22.619 ± 0.3798	25.490 ± 0.3969	29.071 ± 0.7263	31.614 ± 0.9600
Prenatal stress	19.524 ± 0.3839	23.062 ± 0.3549	25.894 ± 0.4659	30.000 ± 0.7312	32.715 ± 0.9849
T-tests	T (74) = -1.081; p = 0.283	T (74) = -0.836; p = 0.406	T (74) = 0.559; p = 0.509	T (74) = -0.892; p = 0.376	T (74) = -0.793; p = 0.430