Published in final edited form as:

Behav Brain Res. 2017 August 14; 332: 154–163. doi:10.1016/j.bbr.2017.05.039.

BDNF heterozygosity is associated with memory deficits and alterations in cortical and hippocampal EEG power

Phillip A. Geist¹, Brooke N. Dulka², Abigail Barnes^{1,2}, Michael Totty¹, and Subimal Datta^{1,2,3}

¹Department of Anesthesiology, Graduate School of Medicine, The University of Tennessee, 1924 Alcoa Highway, Knoxville, TN 37920

²Department of Psychology, College of Arts and Sciences, 1404 Circle Drive

³Program in Comparative and Experimental Medicine, 2407 River Drive, RM A102; The University of Tennessee, Knoxville, TN 37996

Abstract

Brain derived neurotrophic factor (BDNF) plays a pivotal role in structural plasticity, learning, and memory. Electroencephalogram (EEG) spectral power in the cortex and hippocampus has also been correlated with learning and memory. In this study, we investigated the effect of globally reduced BDNF levels on learning behavior and EEG power via BDNF heterozygous (KO) rats. We employed several behavioral tests that are thought to depend on cortical and hippocampal plasticity to varying degrees: novel object recognition, a test that is reliant on a variety of cognitive systems; contextual fear, which is highly hippocampal-dependent; and cued fear, which has been shown to be amygdala-dependent. We also examined the effects of BDNF reduction on cortical and hippocampal EEG spectral power via chronically implanted electrodes in the motor cortex and dorsal hippocampus. We found that BDNF KO rats were impaired in novelty recognition and fear memory retention, while hippocampal EEG power was decreased in slow waves and increased in fast waves. Interestingly, our results, for the first time, show sexual dimorphism in each of our tests. These results support the hypothesis that BDNF drives both cognitive plasticity and coordinates EEG activity patterns, potentially serving as a link between the two.

Keywords

Rats; cortical theta; hippocampal gamma; novelty recognition; fear acquisition; fear retention

1. Introduction

Brain derived neurotrophic factor (BDNF) has been shown to be essential for memory formation [1, 2]. When BDNF activity is obstructed, major deficits in learning and memory

Corresponding Author: All correspondence should be addressed to Subimal Datta, Department of Anesthesiology, Graduate School of Medicine, The University of Tennessee, 1924 Alcoa Highway, 5th Floor Research, Knoxville. TN 37920. sdatta1@utk.edu; Telephone: 865-305-8963; Fax: 865-305-6105.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

are readily apparent [3–6], and these deficits can be prevented or even rescued by introducing BDNF [3–8]. Furthermore, BDNF has also been associated with hippocampal plasticity, a major neural correlate of learning and memory [9, 10]. However, not all learning and memory tasks are hippocampal-dependent. For instance, while contextual fear conditioning strongly depends on the hippocampus [11, 12], cued fear conditioning depends more on the amygdala [11, 13]. Meanwhile, the involvement of the hippocampus in novel object recognition (NOR) remains highly contentious [14, 15]. Using varied learning tasks in BDNF studies allows investigators to examine the different aspects of BDNF's involvement in memory and cognition.

Electroencephalographic (EEG) power has been well established as a metric for cognitive ability in animals and humans [16–18]. Alterations across delta, theta, alpha, beta and gamma frequencies have been linked to a large variety of neuropsychiatric disorders that feature cognitive dysfunction [19–28]. EEG power has also been linked to learning and memory. For example, Sauseng et al. showed a correlation between theta oscillation activity between anterior and posterior brain regions and memory performance [29]. In animal models, hippocampal theta and gamma rhythms are closely linked to memory consolidation and long term potentiation [30, 31]. Interestingly, BDNF polymorphisms and local deletions of the BDNF gene have been tied to changes in EEG spectral power in both human subjects and brain slices in mice [32–34], further linking BDNF and cognition.

Research utilizing genetically modified BDNF animals has shown that the BDNF gene is essential to neural survival and differentiation [35]. Unfortunately, homozygous BDNF knockout animals do not live past two to three days. BDNF heterozygous (KO) knockout animals do not possess the same severe physical defects, but do show reduced levels of BDNF expression in the brain [36]. BDNF KO rats and mice are also less proficient at several experimental learning and memory tasks [37–40]. Altogether, the BDNF KO rodent model has proven to be a useful model for studying the role of BDNF in neural development, plasticity, and cognition. However, to our knowledge, no studies have investigated how BDNF heterozygosity affects EEG spectral power in freely moving rats. Additionally, no in-vivo studies have examined cortical and hippocampal EEG sex differences in BDNF heterozygous animals.

Using BDNF KO rats, the present study was designed to investigate the effects of globally reduced BDNF on male and female EEG power as well as performance in learning and memory tasks. We first compared the behavioral performance of wild-type and BDNF KO animals in a NOR paradigm. Next, we investigated the effects of BDNF heterozygosity on both amygdala-dependent cued fear memory and hippocampal-dependent contextual fear memory. Finally, using chronically implanted EEGs, we recorded and analyzed signals from the cortex and hippocampus of WT and BDNF KO animals. Given previous research on the role of BDNF in cognition, we hypothesize that BDNF KO animals will display deficits in learning tasks and cognition-related EEG power bands. The results of this study indicate that not only does BDNF affect both memory and EEG power, the effect is sexually dimorphic.

2. Materials and Methods

2.1. Animals

For Experiments 1 and 2, the subjects were 12 adult male and 12 adult female rats, weighing between 250–300g and housed individually in a home cage with *ad libitum* access to food and water. Half of the males and half of the females were wild type Sprague Dawley rats, while the other half were BDNF heterozygous rats (SAGE Labs, Boyertown, PA). Animals were divided into groups by strain and sex: WT males (n = 6), WT females (n = 6), BDNF KO males (n = 6), and BDNF KO females (n = 6). While in home cage housing, ambient temperatures were maintained at 25–27°C and animals were kept in a 12L:12D cycle (lights on at 6:00 am, lights off at 6:00 pm). Prior to any experimental procedures, animals were each handled 5 minutes for 5 consecutive days to habituate the animals to experimenter handling. Experiment 3 used identical animal distribution and housing conditions as Experiments 1 and 2.

2.2 Experimental Design

All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Tennessee Animal Care Committee (Protocol Number: 2349-UTK). All behavioral tests were performed during the animals' light phase. Experiment 1 was performed during the first week, at which point both groups underwent a four-day Novel Object Recognition paradigm to measure cognitive performance. Experiment 2 was performed during the second week, at which time animals underwent a four-day fear-conditioning paradigm to measure emotional memory retention. At the end of the second week, animals from Experiments 1 and 2 were euthanized via isoflurane overdose. Tail snips were collected from 3 WT and 3 BDNF KO animals, chosen at random, in order to genotype the BDNF gene.

All animals in Experiment 3 underwent stereotaxic surgery under sterile conditions. During surgery, animals were chronically implanted with cortical electroencephalogram (EEG) and hippocampal EEG electrodes (Fig. 1). After surgery, animals were monitored for a 7–10 recovery period. After surgical recovery, animals started daily electrophysiological recordings that continued for 1–2 weeks. At the conclusion of the recordings, rats were deeply anesthetized with ketamine-xylazine (100 mg/kg ketamine, 10 mg/kg xylazine) and perfused transcardially with normal saline and 4% paraformaldehyde. The brains were removed and processed for histological localization of electrode track and EEG recording sites. Tail snips were collected from 3 WT and 6 BDNF KO animals, chosen at random, in order to sequence the BDNF gene.

2.3 Experiment 1: Novel Object Recognition

The NOR procedure was performed as previously described [41]. Briefly, the NOR procedure consisted of two habituation days, a sample phase day, and a test phase. Each phase of the NOR procedure began on separate, sequential days at 10:00 am, when animals were transported from their housing room to the behavioral testing room. Each animal's behavior during all phases was videotaped from directly above the 60 × 60 cm chamber, to be analyzed at a later time. For the sample phase, two duplicates of an object were placed in

the chamber and each rat was allowed to explore for 10 minutes. The test phase was conducted 24 hours later. For the test phase, one of the duplicate familiar objects was replaced with a different, novel object and each rat was allowed to explore for 5 minutes. The placement of the objects was counterbalanced within groups, to minimize the confounding effects of location preference and olfactory cues. Familiarization with the sample objects was also counterbalanced, such that half of the animals in each group were familiarized with two replicates of Object A and tested with Object B, and half were familiarized with two replicates of Object B and tested with Object A. At every point in the procedure, the chamber and objects were cleaned with Quatricide after each animal, to remove olfactory cues.

2.3.1. Experiment 1 Analysis—Only the first two minutes of each video were scored, since this is the time period when animals are most sensitive to novelty and discrimination is detectable, as previously described [41–43]. All videos were scored using EthoVision XT software (Noldus Information Technology, Wageningen, The Netherlands), which automatically tracks the animal's nose-point and calculates exploration times as the amount of time the nose-point is < 2 cm from each object. These exploration times are used to calculate the Recognition Index (RI), which is a percentage defined as the amount of time spent exploring the novel object (T_N) divided by the time the spent exploring the familiar object (T_F) and the novel object: $RI = T_N / (T_N + T_F)$.

Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, California, USA). The total distance travelled by each group during the test phase was analyzed using oneway ANOVA with Tukey's multiple comparisons test, to investigate the effects of strain and sex on locomotive activity. One-way ANOVAs and Tukey's post-tests were also used to compare the object exploration times of all groups and the RI's of all groups, to investigate the effects of strain and sex on NOR performance. Statistical significance was determined when $p < 0.05$.

2.4. Experiment 2: Fear Conditioning Apparatus and Acquisition Procedure

As described previously [44, 45], a standard testing chamber ($30.5 \times 24.1 \times 21.0$ cm; Standard modular test chamber, ENV-008; Sound attenuating cubicle, ENV-022MD; Med Associates) constructed from aluminum side walls and Plexiglas rear wall, ceiling, and hinged front door was used. Prior to any fear conditioning, animals were habituated to the test chamber for 5 min each day for two consecutive days. On the day of the fear acquisition procedure, rats were placed inside the testing chamber for a baseline/adaptation period of 2 min. Following adaptation, rats received two neutral tones (1000 Hz; 30 s tone presentation; 1 min inter trial interval) followed 30 s later by the presentation of three conditioned stimuli (5000 Hz; 30 s tone presentation; 1 min inter trial intervals) that co-terminated with a 2-s scrambled foot shock (1.0 mA). Rats were removed from the testing chamber 2 min after the last trial. The testing chamber was cleaned and disinfected with Quatricide before and after each use.

2.4.1. Experiment 2: Cued and Contextual Fear Retention Testing—

Approximately 24 h after fear acquisition, rats were brought to the behavioral testing room

for fear retention testing. Rats received both cued and contextual fear retention testing, in a counterbalanced fashion. Contextual fear retention testing took place in the context of the cued fear acquisition procedure (Context A). Cued fear retention testing took place in an alternative context (Context B), which consisted of white plastic sidewalls and a white plastic floor. 4.5 hours of time elapsed between contextual and cued retention testing. For contextual fear retention testing, rats were placed inside the testing chamber (in Context A) for a period of 5 min. For cued fear retention testing, rats were placed inside the testing chamber (in Context B) for a period of 2 min, at which point rats were presented with one conditioned stimulus (5000 Hz; 30 s tone presentation) and were then removed from the testing chamber, which was cleaned and disinfected with Quatricide before and after each use to remove olfactory cues.

2.4.2. Experiment 2 Analysis—During fear memory acquisition training and retention testing, rat behavioral freeze responses were recorded and analyzed using customized computer software (Med Associates, Fairfax, VT). Freezing behavior was detected with infrared light and defined as the absence of any movement with the exception of breathing (sample rate: 30 frames/s; min freeze duration 15 frames). Freeze duration and bouts were automatically scored using VideoFreeze software (Med Associates). Freezing is the most commonly used behavioral measure to assess fear during training, extinction, and testing in rodents. A greater percentage of freezing during testing is interpreted as greater fear memory [46, 47]. Statistical analyses were performed using SigmaPlot 13.0 (Systat Software, Inc., San Jose, California, USA). Two-way repeated measures ANOVAs and Tukey's post-hoc tests were used to investigate differences in freezing behavior across the cued fear acquisition training and retention testing procedures. Statistical significance was determined when $p < 0.05$.

2.5. Experiment 3: Behaving Cortical and Hippocampal EEG Recordings

After 7–10 days of post-surgery recovery time, rats were habituated to the experimenter, the recording chamber (30 × 30 cm fiberglass cylinder), and freely moving EEG recording conditions for a minimum of five days. To begin the recording, each wired hub was mated to a dedicated EEG recording apparatus (Pinnacle Technologies Pinnacle 8409 rat commutator) that amplified the cortical and hippocampal EEG 100 times. Data was sampled at a rate of 1 kHz. A bandpass filter eliminated input outside of the range of interest (0.5–100 Hz) with Sirenia® Acquisition software (Pinnacle Technology Inc., Lawrence, Kansas, USA). Habituation was maintained under the a 12-hour light-dark cycle, free access to food and water, and freely moving conditions as similar to housing, and male and female recordings were performed a minimum of 24 hours apart. The recording chambers were thoroughly cleaned with Quatricide between animals. The habituation sessions were performed continuously for six hours from 9:00 AM to 3:00 PM. During recording, signal and video output were monitored from an adjacent room to ensure data quality and normal animal behavior. Experimental recordings were conducted in a manner identical to habituation.

2.5.1 Experiment 3 Analysis—For spectral power analysis, amplified and filtered EEG data were digitized at a sampling frequency of 200 Hz. Cortical and hippocampal digitized data were subjected to a Fast Fourier Transformation from 0.5–100 Hz with an interval of

0.2 Hz to determine the total power per hour for each six hour recording (Sirenia Sleep Pro, Pinnacle Technologies, Inc.). From the resulting data, delta (0.5–4.5 Hz), theta (4.6–10.0 Hz), alpha (10.1–14.0 Hz), beta (14.1–35 Hz), and gamma (35.1–100 Hz) frequencies were extracted. Data for each frequency were normalized as a percentage of the absolute power. Spectrograms were created using complex Morlet wavelet convolution with frequencies extracted from continuous data at integer intervals from 1 – 40 Hz. The number of wavelet cycles was set at 20 to increase frequency precision. To show changes in total power, resulting data were segmented into 10-second bins and averaged within subject group. A baseline subtraction was then performed to show changes in heterozygous versus wild type animals. Time-frequency power was calculated as the squared magnitude of the results of the complex convolution. Statistical tests of skewedness and homogeneity were measured to ensure data normality. Cortical and hippocampal power for each group was measured against every other group with a one-way ANOVA (IBM SPSS Version 22.0, IBM Corp. Armonk, NY). Bonferroni post-hoc tests were used to determine differences between individual groups, with a threshold of significance set at $P < 0.05$.

2.6 Determination of the BDNF Gene Sequence in Wild-Type and BDNF KO Rats

DNA was extracted from 4–6 mm tail snips (6 total WT and 9 total BDNF KO) using the DNEasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands), in accordance with the manufacturer's protocol. Extracted DNA was combined with the forward and reverse primers (SAGE labs, Boyertown, PA) and the AmpliTaq Gold PCR Master Mix (Applied Biosystems, Thermo Fisher, Foster City, CA) in order to amplify the BDNF gene using a PCR approach. The PCR conditions were an initial denaturation at 95°C for 5 min, followed by 95°C for 30 s, 60°C for 30 s, 35 cycles of 68°C for 40 s, and a final extension at 68°C for 5 min. PCR products were prepared for sequencing using Exo-SAP (Affectrix, Thermo Fisher, Foster City, CA). DNA samples were sent to the University of Tennessee Genomics Core for sequencing. The resulting sequence files were aligned and analyzed for percent agreement (99% for all the WT and BDNF KO samples) and deletion location using SeqMan Pro and MegAlign software (DNASTAR Inc., Madison, WI). Raw data was exported and a selected range of base-pairs were graphed using SigmaPlot.

3. Results

3.1 BDNF gene sequencing confirms knockout heterozygosity

In order to confirm that our BDNF KO rats were in fact heterozygous for the knockout gene, we analyzed the sequences of a random set of WT and BDNF KO rats. Our analyses confirmed a 7 base-pair deletion in the BDNF KO animals. The deletion point was detected by the presence of ambiguous bases, which indicated that two base-pairs were detected at one point – a feature of heterozygosity. Visualization of the appropriate base-pairs confirmed that one matched the WT, while the other corresponded to a point seven base-pairs downstream, indicating the desired deletion (Fig. 2).

3.2 BDNF heterozygosity affects test phase object exploration and has sex-specific effects on novel object recognition

To ensure that all groups showed equal amounts of object exploration during the sample phase, we compared the groups' exploration times of the two duplicate sample objects using one-factor ANOVAs. These analyses revealed no significant differences in exploration of the two duplicate objects (Object 1: $F_{(3,20)} = 2.6$, $p = 0.08$; Object 2: $F_{(3,20)} = 0.23$, $p = 0.88$), as shown in Figure 3A. One-factor ANOVAs of object exploration during the testing phase indicated significant differences between groups in time spent exploring the familiar ($F_{(3,20)} = 4.1$, $p = 0.02$) and novel ($F_{(3,20)} = 3.9$, $p = 0.02$) objects, shown in Figure 3B. Tukey's posttests revealed that KO males showed significantly less exploration of the familiar object than WT males ($p < 0.05$), while other groups did not differ significantly. Posttests of novel object exploration revealed that, although KO animals showed decreased exploration of the novel objects compared to their WT counterparts, these differences were insignificant. However, because no sex differences in exploration were detected at either time point, we collapsed the male and female groups of both genotypes and ran a one-factor ANOVA with repeated measures on total object exploration time, shown in Figure 3C. This analysis revealed significant differences between groups ($F_{(3,11)} = 5.8$, $p = 0.003$). Tukey's posttests revealed that WT animals spent significantly more time exploring objects during the test phase compared to their sample phase exploration ($p = 0.01$) and to the KO animals' test phase exploration ($p < 0.01$).

A one-factor ANOVA of Recognition Indices (RIs), shown in Figure 3D, indicated significant differences between groups in NOR performance ($F_{(3,20)} = 3.8$, $p = 0.03$). In fact, the average RI of the KO females was 50%, which is chance level performance. Also, Tukey's post-tests revealed they had significant recognition deficits compared to their WT counterparts ($p < 0.05$) while all the other groups performed above chance level. It should be noted that, although BDNF KO males did successfully discriminate between novel and familiar objects, their method of discrimination differed from the WT animals. While the WT animals increased exploration of the novel object, the BDNF KO males did not, and instead decreased exploration of the familiar object. These differences in exploratory behavior are highlighted in Figure 3F, which exemplifies how WT animals seemed to have more interest in objects during the test phase than the BDNF KO animals did. Since it is possible that these differences were due to differences in locomotor activity, we ran a one-factor ANOVA on locomotor activity of the BDNF KO and WT animals (Fig 3E). Although significant variation was detected ($F_{(3,20)} = 5.7$, $p = 0.005$), Tukey's posttests revealed the source of this variation to be between the two sexes in the BDNF KO animals ($p < 0.01$). BDNF KO males had significantly less locomotor activity than the BDNF KO females. No significant differences were found between the BDNF KO animals and their WT counterparts.

3.3. BDNF heterozygous animals display deficits in fear memory retention

BDNF heterozygosity is associated with reductions in global BDNF levels, thus we would expect that animals with a heterozygous genotype to exhibit deficits in fear learning. Indeed we found this to be the case. For females, as indicated by a two-way repeated measures ANOVA, there was a main effect of both genotype ($F_{(1,60)} = 8.76$, $p = 0.014$) and trial

($F_{(1,60)} = 13.17$, $p < 0.001$). There was also a significant genotype $\times$ trial interaction ($F_{(1,60)} = 2.63$, $p = 0.025$). Further analysis (Tukey's post-hoc tests) revealed that the percentage of time spent freezing, compared to wild type females, was significantly lower in heterozygous females during acquisition at the second and third conditioned stimulus tone (CS2 and CS3, respectively). However, freezing levels between heterozygous and wild type females was equivalent at the anticipatory time point (POST). Freezing was also significantly decreased in BDNF heterozygous females during both cued and contextual memory retention testing (Fig. 5). In males, as indicated by a two-way repeated measures ANOVA, there was a main effect of both genotype ($F_{(1,60)} = 5.89$, $P = 0.036$) and trial ($F_{(1,60)} = 14.98$, $p < 0.001$), and there was also a trend towards a significant genotype $\times$ trial interaction ($F_{(1,60)} = 2.23$, $p = 0.052$). Tukey's post-hoc analysis revealed that BDNF heterozygous males froze significantly less than wild type males at CS2, however freezing was equivalent at CS3 and POST. Furthermore, freezing was significantly decreased in heterozygous males during contextual, but not cued, memory retention testing. Overall, these results that BDNF heterozygosity results in marked deficits in fear memory retention in a sex specific manner.

3.4 Qualitative differences in hippocampal power between BDNF KO and wild type animals

As can be seen in the spectrograms, all hippocampal power bands showed major qualitative differences between genotypes and across sexes (Fig. 5). Females displayed obvious decreases in delta power, small decreases in theta power, small increases in alpha power, marked increases in beta power, and major increases in gamma power (Fig. 5A). Males showed an analogous but exaggerated pattern, with notable decreases in delta, marginal decreases in theta, and marked increases in alpha, beta, and gamma power (Fig. 5B).

3.5 Hippocampal delta and theta power reduction in BDNF KO animals

Quantitative analysis revealed the same trend. For both sexes, one-way analysis of variance exposed differences in delta, across all but one hour (hour one: $F_{(3,20)} = 4.97$, $p = 0.010$; two: $F_{(3,20)} = 5.52$, $p = 0.006$; three: $F_{(3,20)} = 6.05$, $p = 0.004$; four: $F_{(3,20)} = 6.12$, $p = 0.004$; six: $F_{(3,20)} = 3.40$, $p = 0.038$). Female BDNF KO animals displayed no differences in delta power compared to the wild types. However, male BDNF KO animals revealed significant deficits between groups in delta hours one ($F_{(3,20)} = 5.00$; $p = 0.01$), two ($F_{(3,20)} = 5.52$; $p = 0.006$), three ($F_{(3,20)} = 6.05$; $p = 0.004$), four ($F_{(3,20)} = 6.12$; $p = 0.004$), and six ($F_{(3,20)} = 3.40$; $p = 0.038$) (Fig. 6A). Neither males nor females showed significant differences in hippocampal theta. (Fig. 6B).

3.6 Hippocampal alpha, beta, and gamma power increase in BDNF KO animals

In contrast, hippocampal alpha and beta demonstrate the opposite trend, with BDNF KO males displaying increased power as compared to wild types (Fig. 7). Differences in hippocampal alpha were minimal across sexes in the alpha band (Fig. 7A). Hippocampal beta, however, showed a distinct effect. BDNF KO females again showed no significant differences. Male BDNF KO animals showed highly significant differences in hours one ($F_{(3,20)} = 6.44$; $P = 0.003$), two ($F_{(3,20)} = 6.85$ $P = 0.002$), three ($F_{(3,20)} = 5.00$; $P = 0.01$), four ($F_{(3,20)} = 6.25$; $P = 0.004$), and six ($F_{(3,20)} = 4.39$; $P = 0.016$) (Fig 7B). Gamma power shows the same pattern. Only BDNF KO males exhibited differences, increasing significantly in all six hours (hour one $F_{(3,20)} = 4.40$; $P = 0.016$), two $F_{(3,20)} = 5.63$ $P =$

0.006), three $F_{(3, 20)} = 4.22$; $P = 0.018$), four $F_{(3, 20)} = 6.03$; $P = 0.004$), five $F_{(3, 20)} = 3.54$; $P = 0.033$ and six $F_{(3, 20)} = 3.59$; $P = 0.032$) (Fig. 7C). These results indicate that a deficit in BDNF is not only highly disruptive to these hippocampal power bands, but also that male animals are selectively affected by these disruptions.

3.7 Cortical EEG Power is decreased in BDNF KO male theta band

Figure 8 summarizes our findings in cortical power. One-way analysis of variance showed no significant power differences between the wild type and BDNF KO or females and males in cortical delta in any of the six tested hours. Cortical theta, however, displayed a significant decrease from wild types in the male BDNF KO animals $F_{(3, 20)} = 3.11$; $p = 0.049$), but no effect in females. Finally, there were no noticeable differences between BDNF KO and wild type females or males in cortical alpha, beta, or gamma. Because only theta band displayed significant differences between BDNF KO and wild type animals, these bands are presented in a time-collapsed format.

4. Discussion

Our results indicate that BDNF KO rats display behavioral differences in a sex dependent manner, and that these changes may be due to underlying abnormal hippocampal and cortical EEG activity. Our specific conclusions are as follows: 1) BDNF KO females and males were differentially affected by reduced BDNF levels in a novel object recognition task; 2) BDNF KO animals show reduced interest in novel objects; 3) The retention of fear memories appears to be impaired by BDNF heterozygosity, and female and male KO rats display different degrees of memory retention; 4) Finally, cortical theta is decreased while hippocampal slow waves are suppressed and fast waves are increased in BDNF KO rats, suggesting abnormal information processing in these areas.

The present study, for the first time, provides evidence for sex differences in the behavioral effects of the BDNF KO genotype in rats. According to our results, male KO rats were able to discriminate the novel object from the familiar object, which matches previous literature [48]. However, female KO rats were not able to discriminate successfully, indicating a sex difference in NOR behavior. These results could be explained by the reduced locomotion seen in KO males compared to KO females. It is possible that the males simply did not explore as much as the females and therefore, by chance, reduced exploration of the familiar object. However, due to the counterbalancing of the location of the familiar object, this seems unlikely. Instead, it seems that female rat NOR performance is more affected by reduced BDNF levels than male rat performance. This is interesting in light of the fact that female rodents have been shown to have better NOR performance than males when the novel and familiar objects are similar – or, in other words, when discrimination between objects is more difficult [49]. Perhaps the neural or behavioral mechanisms underlying NOR are slightly different between the sexes, allowing females to have increased accuracy under some circumstances, but rendering them more sensitive to cellular or molecular alterations, particular in regards to BDNF expression.

It is important to mention that the present study found behavioral alterations in BDNF KO rats in the NOR paradigm, which has never before been reported [48, 50, 51]. Specifically,

the WT animals increased total object exploration during the test phase, but the KO animals did not. Studies that investigated lesioning of the perirhinal cortex, widely considered the most critical region for NOR, found that animals increased exploration of both the novel and familiar objects [52–54]. Investigators interpreted this behavior as a demonstration of novelty detection with an inability to discriminate or properly map the novel stimulus [52–54]. This is in direct contrast with our BDNF KO animals. Since the KO animals showed no increased object exploration, it is possible that they did not detect a novel stimulus. In fact, BDNF KO animals may have reduced interest in or sensitivity to novelty. This is supported by the fact that higher baseline levels of BDNF have been linked to novelty sensitivity [55, 56]. Taken together, these results imply that BDNF may be involved in multiple aspects of memory and cognition, which may not be limited to hippocampal plasticity.

In addition to deficits in object memory, our data suggest that BDNF heterozygosity is also associated with deficits in fear memory. Specifically, KO males and females display deficits in the retention, but not the acquisition, of conditioned fear. Despite selective deficits in freezing at some CSs, all animals showed equivalent freezing at the anticipatory time point (Post CS), which suggests that all animals acquired the fear memory. This would be in line with other studies of KO animals that also do not show an impairment in acquisition [57–59]. Reduced freezing during the CS presentations in the KO animals may be related to reduced BDNF-related pain perception [60]. We also found that, at testing, female KO rats have impaired cued and contextual memory retention, whereas male BDNF KO rats only have impaired contextual memory retention. Interestingly, Endres and Lessmann demonstrated that BDNF KO male mice showed an age-dependent deficit in fear learning, such that deficits in amygdala dependent-cued fear learning only occurs when mice are aged 3 months and older [59]. BDNF levels are known to decline with aging in male rats [61], perhaps this decline occurs earlier in females. Alternatively, this pattern of results might suggest that, in males, hippocampal-dependent tasks are more sensitive to BDNF knockdown than amygdala-dependent tasks. At any rate, a deficit in contextual memory in KO males is similar to previous studies using male KO mice [57, 58]. It is noteworthy that Liu et al. was able to rescue this deficit in contextual fear memory with intra-hippocampal infusions of exogenous BDNF [58]. In fact, BDNF mRNA is up-regulated in the CA1 layer of the hippocampus during contextual fear learning [62]. In conjunction with our EEG findings, these data suggest that knockdown of the BDNF gene is particularly deleterious to the hippocampal functioning and, thus, hippocampal dependent tasks.

The results of our EEG study show persistent male-specific effects. These perturbations may be a potential source of the anomalous behavioral results. The deficit in male cortical theta spectral power suggests a disruption of cortical coupling to other areas, as has been shown between cortical-hippocampal and cortical-thalamic interactions [62, 63]. Disruption of cortical information transfer could result in difficulties correctly recognizing temporal cues, short-term memory, and other cognitive functions [64]. Such a disruption would have a major effect on tests sensitive to novelty information as in NOR testing and in temporally linked events such as fear conditioning. However, BDNF KO males in NOR testing only showed differences in exploration rather than recognition, and while male BDNF KO rats did show selectively impaired contextual memory, females also showed a deficit without differences in EEG power.

While the cortical differences were limited to the theta band, male BDNF animals' hippocampal rhythms were markedly changed from those of our wild types. The general pattern of hippocampal changes across all bands was a decrease in low-frequency oscillations and an increase in high frequency oscillations. This finding immediately brings to mind the hierarchical model of oscillatory organization [65], in which lower frequency oscillations are responsible for regulating those of higher frequency. The tendency for slow wave oscillations to cover a relatively large area of the brain, sometimes encompassing multiple distinct functional areas, and for high frequency waves to be confined to specific functional areas lends credence to the theory that slow waves are responsible for basic behavioral processes such as attention, arousal, and homeostatic maintenance [66], while high frequency oscillations are involved in higher level cognitive function [67–69]. This general model links the behavioral testing results with our electrophysiological findings.

In relation to the specific alterations seen in our animals, delta oscillations are increased in sexual and food-based arousal states, both states that increase exploration and novelty seeking [66]. The decrease in delta found in our males may explain the decreased exploration in NOR testing. Interestingly, theta oscillations, which have previously been shown to play an essential part in spatial memory [70], were not significantly affected. However, it has been shown that gamma oscillations and theta oscillations are nested during memory tasks [71]. It is possible that the disparities found here represent a disruption of this coordinated activity. While alpha and beta have not been studied extensively, gamma oscillations in the hippocampus have been suggested to package relevant information into appropriate temporal categories. This allows for efficient assimilation of environmental information in the hippocampus [67]. Gamma power has also been shown to increase during learning paradigms. [72]. In our aberrant recordings, the improper coordination and assimilation of sensory and temporal information may be responsible for the deficits found in behavioral testing. In particular, contextual and object memories, which are reliant on hippocampal function [73], may be impaired by this disruption.

There are several limitations the authors would like to discuss. First, it is possible female deficits in behavioral tests are due to estrus cycle effects, which has been shown to effect BDNF levels [75]. In addition, the EEG power effects were not recorded in the same animals that underwent behavioral testing. Also, we did not record electromyogram data from our animals, preventing identification of sleep-wake states. However, given the persistence of the effects, and the documented deleterious effect of BDNF heterozygosity on hippocampal function [76], it seems unlikely that these findings are not somehow interrelated. Thus, we propose here that this interruption of hippocampal function impacts cognitive activity not only by altering neuroanatomical processes but also the delicate network of electrophysiological communication that coordinates behavior. Future research exploring the specific effects of behavioral testing on EEG power would provide a more robust test of our hypothesis.

5. Conclusions

In conclusion, BDNF heterozygosity results in marked deficits in object and fear memory, as well as male-specific alterations in cortical and hippocampal EEG activity. These changes in

EEG spectral power may indicate a differential mechanism of learning and memory deficits between male and female BDNF KO rats. If true, these findings could prove valuable to future research on sex-dependent learning.

Acknowledgments

This work was supported by the National Institutes of Health Research Grant (MH59839). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We would like to thank John G. Little, Jonathan E. Chambers, and Michael D. Oliver II for their technical contributions. We thank Dr. R. Eric Heidel for his statistical assistance. We thank Dr. Ralph Lydic, Dr. Helen Baghdoyan, and Dr. Robert Craft for critical discussions for this research.

References

1. Datta S, Siwek DF, Huang MP. Improvement of Two-Way Active Avoidance Memory Requires Protein Kinase A Activation and Brain-Derived Neurotrophic Factor Expression in the Dorsal Hippocampus. *J Mol Neurosci*. 2009; 38(3):257–264. [PubMed: 19418263]
2. Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH. BDNF is essential to promote persistence of long-term memory storage. *Proceedings of the National Academy of Sciences*. 2008; 105(7):2711–2716.
3. Rattiner LM, Davis M, French CT, Ressler KJ. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2004; 24(20):4796–806. [PubMed: 15152040]
4. Taylor SL, Stanek LM, Ressler KJ, Huhman KL. Differential brain-derived neurotrophic factor expression in limbic brain regions following social defeat or territorial aggression. *Behavioral neuroscience*. 2011; 125(6):911–20. [PubMed: 22122152]
5. Heldt SA, Zimmermann K, Parker K, Gaval M, Weinshenker D, Ressler KJ. BDNF deletion or TrkB impairment in amygdala inhibits both appetitive and aversive learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014; 34(7):2444–50. [PubMed: 24523535]
6. Dulka BN, Ford EC, Lee MA, Donnell NJ, Goode TD, Prosser R, Cooper MA. Proteolytic cleavage of proBDNF into mature BDNF in the basolateral amygdala is necessary for defeat-induced social avoidance. *Learning & Memory*. 2016; 23(4):156–160. [PubMed: 26980783]
7. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron*. 1996; 16(6):1137–1145. [PubMed: 8663990]
8. Radecki DT, Brown LM, Martinez J, Teyler TJ. BDNF protects against stress-induced impairments in spatial learning and memory and LTP. *Hippocampus*. 2005; 15(2):246–53. [PubMed: 15476265]
9. Pang PT, Teng HK, Woo ZENT, Sakata K, Zhen S, Teng KK, Yung W, Hempstead BL, Lu B. Cleavage of proBDNF by tPA/Plasmin is Essential for Long-Term Hippocampal Plasticity. *Science*. 2004; 306:487–491. [PubMed: 15486301]
10. Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience*. 2004; 20(10):2580–2590. [PubMed: 15548201]
11. Phillips R, LeDoux J. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral neuroscience*. 1992; 106(2):274. [PubMed: 1590953]
12. Maren S, Aharonov G, Fanselow MS. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural brain research*. 1997; 88(2):261–274. [PubMed: 9404635]
13. LaBar KS, Gatenby JC, Gore JC, LeDoux JE, Phelps EA. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron*. 1998; 20(5):937–945. [PubMed: 9620698]

14. Murray EA, Mishkin M. Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *Journal of Neuroscience*. 1998; 18(16):6568–6582. [PubMed: 9698344]
15. Oliveira AM, Hawk JD, Abel T, Havekes R. Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. *Learning & memory*. 2010; 17(3):155–160. [PubMed: 20189960]
16. Klimesch W. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Rev*. 1999; 29(2–3):169–195. [PubMed: 10209231]
17. Fitzgibbon SP, Pope KJ, Mackenzie L, Clark CR, Willoughby JO. Cognitive tasks augment gamma EEG power. *Clin Neurophysiol*. 2004; 115(8):1802–9. [PubMed: 15261859]
18. Buzsaki G, Wiesner J, Henriksen SJ, Gage FH. Long-Term Potentiation of Evoked and Spontaneous Neuronal-Activity in the Grafted Hippocampus. *Exp Brain Res*. 1989; 76(2):401–408. [PubMed: 2767191]
19. Ford JM, Roach BJ, Hoffman RS, Mathalon DH. The dependence of P300 amplitude on gamma synchrony breaks down in schizophrenia. *Brain Res*. 2008; 1235:133–142. [PubMed: 18621027]
20. Yener G, Guentekin B, Basar E. Event-related delta oscillatory responses of Alzheimer patients. *Eur J Neurol*. 2008; 15(6):540–547. [PubMed: 18410376]
21. Guntekin B, Saatci E, Yener G. Decrease of evoked delta, theta and alpha coherences in Alzheimer patients during a visual oddball paradigm. *Brain Res*. 2008; 1235:109–116. [PubMed: 18598686]
22. Jones KA, Porjesz B, Chorlian D, Rangaswamy M, Kamarajan C, Padmanabhapillai A, Stimus A, Begleiter H. S-transform time-frequency analysis of P300 reveals deficits in individuals diagnosed with alcoholism. *Clinical Neurophysiology*. 2006; 117(10):2128–2143. [PubMed: 16926113]
23. Winterer G, Enoch MA, White KV, Saylan M, Coppola R, Goldman D. EEG phenotype in alcoholism: increased coherence in the depressive subtype. *Acta Psychiatr Scand*. 2003; 108(1):51–60. [PubMed: 12807377]
24. Adler G, Brassen S, Jajcevic A. EEG coherence in Alzheimer's dementia. *J Neural Transm*. 2003; 110(9):1051–1058. [PubMed: 12928837]
25. Besthorn C, Forstl H, Geigerkabisch C, Sattel H, Gasser T, Schreitergasser U. Eeg Coherence in Alzheimer-Disease. *Electroen Clin Neuro*. 1994; 90(3):242–245.
26. Giannicola G, Marceglia S, Rossi L, Mrakic-Sposta S, Rampini P, Tamma F, Cogiamanian F, Barbieri S, Priori A. The effects of levodopa and ongoing deep brain stimulation on subthalamic beta oscillations in Parkinson's disease. *Exp Neurol*. 2010; 226(1):120–7. [PubMed: 20713047]
27. Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser M-B, Moser EI. Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature*. 2009; 462(7271):353–357. [PubMed: 19924214]
28. Basar-Eroglu C, Schmiedt-Fehr C, Mathes B, Zimmermann J, Brand A. Are oscillatory brain responses generally reduced in schizophrenia during long sustained attentional processing? *International Journal of Psychophysiology*. 2009; 71(1):75–83. [PubMed: 18725254]
29. Sauseng P, Klimesch W, Schabus M, Doppelmayr M. Fronto-parietal EEG coherence in theta and upper alpha reflect central executive functions of working memory. *International Journal of Psychophysiology*. 2005; 57(2):97–103. [PubMed: 15967528]
30. Buzsaki G. 2-Stage Model of Memory Trace Formation - a Role for Noisy Brain States. *Neuroscience*. 1989; 31(3):551–570. [PubMed: 2687720]
31. Larson J, Munkacsy E. Theta-burst LTP. *Brain Res*. 2015; 1621:38–50. [PubMed: 25452022]
32. Gatt JM, Kuan SA, Dobson-Stone C, Paul RH, Joffe RT, Kemp AH, Gordon E, Schofield PR, Williams LM. Association between BDNF Val66Met polymorphism and trait depression is mediated via resting EEG alpha band activity. *Biological psychology*. 2008; 79(2):275–84. [PubMed: 18721847]
33. Huang Y, Morozov A. Hippocampal deletion of BDNF gene attenuates gamma oscillations in area CA1 by up-regulating 5-HT₃ receptor. *PloS one*. 2011; 6(1):e16480. [PubMed: 21298058]
34. Zoon HF, Veth CP, Arns M, Drinkenburg WH, Talloen W, Peeters PJ, Kenemans JL. EEG alpha power as an intermediate measure between brain-derived neurotrophic factor Val66Met and depression severity in patients with major depressive disorder. *Journal of clinical*

- neurophysiology : official publication of the American Electroencephalographic Society. 2013; 30(3):261–7. [PubMed: 23733090]
35. Poo MM. Neurotrophins as synaptic modulators. *Nat Rev Neurosci.* 2001; 2(1):24–32. [PubMed: 11253356]
 36. Klug M, Hill RA, Choy KH, Kyrios M, Hannan AJ, van den Buuse M. Long-term behavioral and NMDA receptor effects of young-adult corticosterone treatment in BDNF heterozygous mice. *Neurobiology of disease.* 2012; 46(3):722–31. [PubMed: 22426399]
 37. Bartoletti A, Cancedda L, Reid SW, Tessarollo L, Porciatti V, Pizzorusso T, Maffei L. Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation. *Journal of Neuroscience.* 2002; 22(23):10072–10077. [PubMed: 12451106]
 38. Ikegaya Y, Ishizaka Y, Matsuki N. BDNF attenuates hippocampal LTD via activation of phospholipase C: implications for a vertical shift in the frequency-response curve of synaptic plasticity. *European Journal of Neuroscience.* 2002; 16(1):145–148. [PubMed: 12153539]
 39. Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *Journal of Neuroscience.* 2000; 20(18):7116–7121. [PubMed: 10995859]
 40. Linnarsson S, Bjorklund A, Ernfors P. Learning deficit in BDNF mutant mice. *European Journal of Neuroscience.* 1997; 9(12):2581–2587. [PubMed: 9517463]
 41. Barnes AK, Smith SB, Datta S. Beyond Emotional and Spatial Processes: Cognitive Dysfunction in a Depressive Phenotype Produced by Long Photoperiod Exposure. 2017; 12(1):e0170032.
 42. Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res.* 1999; 99(2):191–200. [PubMed: 10512585]
 43. Barker JM, Wright DW, Goldstein FC, Ockerman J, Ratcliff JJ, Laplaca MC. The DETECT system: portable reduced-length neuropsychological testing for mild traumatic brain injury via a novel immersive environment. *J Med Eng Technol.* 2007; 31(3):161–9. [PubMed: 17454403]
 44. Datta S, O'Malley MW. Fear extinction memory consolidation requires potentiation of pontine-wave activity during REM sleep. *The Journal of Neuroscience.* 2013; 33(10):4561–4569. [PubMed: 23467372]
 45. Siwek DF, Knapp CM, Kaur G, Datta S. Dorsal subcoeruleus nucleus (SubCD) involvement in context-associated fear memory consolidation. *Exp Brain Res.* 2014; 232(5):1535–1545. [PubMed: 24525958]
 46. Datta S, O'Malley MW. Fear extinction memory consolidation requires potentiation of pontine-wave activity during REM sleep. *J Neurosci.* 2013; 33(10):4561–9. [PubMed: 23467372]
 47. Siwek DF, Knapp CM, Kaur G, Datta S. Dorsal subcoeruleus nucleus (SubCD) involvement in context-associated fear memory consolidation. *Exp Brain Res.* 2014; 232(5):1535–45. [PubMed: 24525958]
 48. Gururajan A, Hill RA, van den Buuse M. Brain-derived neurotrophic factor heterozygous mutant rats show selective cognitive changes and vulnerability to chronic corticosterone treatment. *Neuroscience.* 2015; 284:297–310. [PubMed: 25445195]
 49. Bettis T, Jacobs LF. Sex differences in object recognition are modulated by object similarity. *Behav Brain Res.* 2012; 233(2):288–92. [PubMed: 22580056]
 50. Gururajan A, Hill R, van den Buuse M. Long-term differential effects of chronic young-adult corticosterone exposure on anxiety and depression-like behaviour in BDNF heterozygous rats depend on the experimental paradigm used. *Neuroscience letters.* 2014; 576:6–10. [PubMed: 24907687]
 51. St Laurent R, Helm SR, Glenn MJ. Reduced cocaine-seeking behavior in heterozygous BDNF knockout rats. *Neuroscience. letters.* 2013; 544:94–9.
 52. Albasser MM, Olarte-Sanchez CM, Amin E, Brown MW, Kinnavane L, Aggleton JP. Perirhinal cortex lesions in rats: Novelty detection and sensitivity to interference. *Behavioral neuroscience.* 2015; 129(3):227–43. [PubMed: 26030425]

53. Kinnavane L, Amin E, Olarte-Sanchez CM, Aggleton JP. Detecting and discriminating novel objects: The impact of perirhinal cortex disconnection on hippocampal activity patterns. *Hippocampus*. 2016; 26(11):1393–1413. [PubMed: 27398938]
54. Olarte-Sanchez CM, Amin E, Warburton EC, Aggleton JP. Perirhinal cortex lesions impair tests of object recognition memory but spare novelty detection. *The European journal of neuroscience*. 2015; 42(12):3117–27. [PubMed: 26474445]
55. Duclot F, Kabbaj M. Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2013; 33(27):11048–60. [PubMed: 23825410]
56. Oztan O, Aydin C, Isgor C. Stressful environmental and social stimulation in adolescence causes antidepressant-like effects associated with epigenetic induction of the hippocampal BDNF and mossy fibre sprouting in the novelty-seeking phenotype. *Neuroscience letters*. 2011; 501(2):107–11. [PubMed: 21767606]
57. Chen Z-Y, Jing D, Bath KG, Ieraci A, Khan T, Siao C-J, Herrera DG, Toth M, Yang C, McEwen BS. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*. 2006; 314(5796):140–143. [PubMed: 17023662]
58. Liu IY, Lyons WE, Mamounas LA, Thompson RF. Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. *Journal of Neuroscience*. 2004; 24(36):7958–7963. [PubMed: 15356210]
59. Endres T, Lessmann V. Age-dependent deficits in fear learning in heterozygous BDNF knock-out mice. *Learning & memory*. 2012; 19(12):561–570. [PubMed: 23154927]
60. Andriankaja A, Boisson-Dernier A, Frances L, Sauviac L, Jauneau A, Barker DG, de Carvalho-Niebel F. AP2-ERF transcription factors mediate Nod factor dependent Mt ENOD11 activation in root hairs via a novel cis-regulatory motif. *Plant Cell*. 2007; 19(9):2866–85. [PubMed: 17827349]
61. Silhol M, Bonnichon V, Rage F, Tapia-Arancibia L. Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. *Neuroscience*. 2005; 132(3):613–624. [PubMed: 15837123]
62. Hall J, Thomas KL, Everitt BJ. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature neuroscience*. 2000; 3(6):533–535. [PubMed: 10816306]
63. Young CK, McNaughton N. Coupling of Theta Oscillations between Anterior and Posterior Midline Cortex and with the Hippocampus in Freely Behaving Rats. *Cereb Cortex*. 2009; 19(1):24–40. [PubMed: 18453538]
64. LeBlanc BW, Lii TR, Silverman AE, Alleyne RT, Saab CY. Cortical theta is increased while thalamocortical coherence is decreased in rat models of acute and chronic pain. *Pain*. 2014; 155(4):773–782. [PubMed: 24457192]
65. Buzsaki G, Draguhn A. Neuronal oscillations in cortical networks. *Science*. 2004; 304(5679):1926–1929. [PubMed: 15218136]
66. Lakatos P, Shah AS, Knuth KH, Ulbert I, Karmos G, Schroeder CE. An oscillatory hierarchy controlling neuronal excitability and stimulus processing in the auditory cortex. *J Neurophysiol*. 2005; 94(3):1904–1911. [PubMed: 15901760]
67. Knyazev GG. EEG delta oscillations as a correlate of basic homeostatic and motivational processes. *Neurosci Biobehav R*. 2012; 36(1):677–695.
68. Colgin LL, Denninger T, Fyhn M, Hafting T, Bonnevie T, Jensen O, Moser MB, Moser EI. Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature*. 2009; 462(7271):353–U119. [PubMed: 19924214]
69. Csicsvari J, Jamieson B, Wise KD, Buzsaki G. Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron*. 2003; 37(2):311–322. [PubMed: 12546825]
70. Senior TJ, Huxter JR, Allen K, O'Neill J, Csicsvari J. Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *Journal of Neuroscience*. 2008; 28(9):2274–2286. [PubMed: 18305260]
71. Winson J. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science*. 1978; 201(4351):160–3. [PubMed: 663646]

72. Tort ABL, Kramer MA, Thorn C, Gibson DJ, Kubota Y, Graybiel AM, Kopell NJ. Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *P Natl Acad Sci USA*. 2008; 105(51):20517–20522.
73. Muzzio IA, Levita L, Kulkarni J, Monaco J, Kentros C, Stead M, Abbott LF, Kandel ER. Attention Enhances the Retrieval and Stability of Visuospatial and Olfactory Representations in the Dorsal Hippocampus. *Plos Biol*. 2009; 7(6)
74. Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. Hippocampal damage exploratory preferences in rats: Memory for objects places and contexts. *Learning & Memory*. 2002; 9(2):49–57. [PubMed: 11992015]
75. Sun MK, Alkon DL. Differential gender-related vulnerability to depression induction and converging antidepressant responses in rats. *J Pharmacol Exp Ther*. 2006; 316(2):926–932. [PubMed: 16227471]
76. Hill RA, van den Buuse M. Sex-dependent and region-specific changes in TrkB signaling in BDNF heterozygous mice. *Brain Res*. 2011; 1384:51–60. [PubMed: 21281620]

Highlights

- BDNF heterozygous rats display sex-specific deficits in novel object recognition.
- BDNF heterozygous rats have impaired fear acquisition.
- EEG power in BDNF heterozygous rats is decreased in delta and increased in beta and gamma.
- BDNF heterozygous rats displayed sex-specific differences in electrophysiological and behavioral tests.

Figure 1.

Experimental setup. A) Screw electrodes attached to the skull bilaterally to record electroencephalogram (EEG) activity from the primary motor cortex. B) A Depth electrode was inserted into the CA1/ dentate gyrus regions of the right hippocampus. Input from the electrodes was transferred to Sirenia Sleep Pro for visualization and offline analysis.

Figure 2.

Sequence of the BDNF gene in the wild-type (A) and heterozygous BDNF knockout animals (B). Chromatogram illustrating a 34 base-pair (bp) DNA segment of the BDNF gene in the wild-type (A) and heterozygous BDNF knockout (KO) animals (B). Notice the double peaks found in the KO sequence, and that the first double peak point has one peak that matches the first base in the deletion, and another peak that matches the first base after the deletion, indicating that the BDNF gene is absent from half of the genetic material sequenced.

Figure 3.

Effects of BDNF heterozygosity on object exploration and novel object recognition. A) Histograms illustrating average object exploration time (sec) for wild-type (WT) and BDNF heterozygous knockout (KO) male and female rats during the sample phase. Notice no differences in exploration were found. B) Object exploration during the test phase, 24 hour later. Note that there were no significant differences in exploratory behavior during the familiarization phase, but during the test phase, the KO Male group showed significantly decreased exploration of the familiar object compared to the WT Male group. C) Histograms

plotting average total object exploration time for WT and KO animals during the sample and test phases, with sexes collapsed. Note that, during the test phase, WT animals increased object exploration above sample phase levels, but KO animals did not. D) Histograms displaying Recognition Indices. Note that the KO Females had significant deficits in NOR, while the all other groups were able to successfully discriminate novel from familiar objects. E) Histograms illustrating average locomotion during the test phase for male and female wildtype (WT) and BDNF heterozygous knockout (KO) rats. Note that KO animals did not significantly differ from WT animals, but KO males showed significantly reduced locomotion compared to KO females. F) Heat maps plotting nose-point exploration during the test phase, from blue (low exploration) to red (high exploration). Notice that the KO animals show much less exploration of objects in general. Asterisk represents comparisons between genotypes, $*p < 0.05$, $**p < 0.01$. represents comparisons between the same animals at different time points, $p < 0.01$.

Figure 4.

BDNF heterozygosity significantly affects fear acquisition and retention. Histograms showing the average (mean $\pm$ SEM) percentages of time spent freezing (% Freezing). Notice that both male and female BDNF KO rats spent significantly less time freezing than their wild-type counterparts during acquisition during CS2 and CS3. Also, female BDNF KO rats spent significantly less time freezing during both cued and contextual fear memory retention testing. On the other hand, male BDNF KO rats only froze significantly less than wild-type males during contextual fear memory retention testing. Note that there were no significant differences in neutral tone (NT) freezing in either sex. Taken together, these data indicate an overall deficit in the acquisition and retention of conditioned fear in both male and female BDNF KO rats, although contextual fear memory is modulated in a sex-dependent manner. Post-hoc Tukey's tests: Asterisk represents comparisons with wild-type animals of the same sex; * $p < 0.05$.

Figure 5.

BDNF heterozygosity significantly affects fear retention. Histograms showing the average (mean $\pm$ SEM) percentages of time spent freezing (% Freezing). Although female BDNF KO rats spent significantly less time freezing compared to their wild-type counterparts during acquisition at CS2 and CS3, they froze equivalently at POST. Male BDNF KO rats also froze significantly less at CS2 but at no other time point during training. Also, female BDNF KO rats spent significantly less time freezing during both cued (CUE) and contextual (CONTEXT) fear memory retention testing. On the other hand, male BDNF KO rats only

froze significantly less than wild-type males during contextual fear memory retention testing. Note that there were no significant differences in neutral tone (NT) freezing in either sexes. Taken together, these data indicate an overall deficit in the retention of conditioned fear in both male and female BDNF KO rats, although contextual fear memory is modulated in a sex-dependent manner. Post-hoc Tukey's tests: Asterisk represents comparisons with wild-type animals of the same sex; * $p < 0.05$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 6.

Effect of BDNF heterozygosity and sex on hippocampal delta and theta power. (A) Histogram presenting freely behaving delta (0.5–4.5 Hz). Bars represent percentage of total power (mean + SEM). Groups consist of wild type females (WT-F, red bar), heterozygous females (KO-F, pink bar), wild type males (WT-M, blue bar), and heterozygous males (KO-M, light blue bar). Data is shown for each hour of the six-hour recording period (one-hour to six-hour). (B) Histogram presenting hippocampal theta (4.6–10 Hz) power (mean + SEM). Notice that in both frequencies, BDNF heterozygous animals showed obvious decreases in

mean total power. In addition, heterozygous males displayed greater deficits compared to the wild types than corresponding females. * $p < 0.05$

Figure 7.

Effect of BDNF heterozygosity and sex on hippocampal alpha, beta, and gamma power. (A) Histogram presenting freely behaving alpha (10.1–14 Hz). Bars represent percentage of total power (mean + SEM). Groups consist of wild type females (WT-F, red bar), heterozygous females (KO-F, pink bar), wild type males (WT-M, blue bar), and heterozygous males (KO-M, light blue bar). Data is shown for each hour of the six-hour recording period (one-hour to six-hour). (B) Histogram presenting hippocampal beta (14.1–35 Hz) power (mean + SEM). (C) Histogram for hippocampal gamma (35.1–100 Hz) power (mean + SEM). In contrast to

delta and theta, alpha, beta and gamma show increases in percentage of total power in BDNF heterozygous animals. Interestingly, hippocampal alpha does not display noticeable sex differences, while hippocampal beta and gamma present a markedly greater effect in heterozygous males than females.* $p < 0.05$

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 8.

Effects of heterozygosity and sex on cortical power. (A) Histogram representing changes between heterozygous and wild type males and females. Bars represent percentage of total power (mean + SEM) for delta, theta, alpha, beta, and gamma frequencies over six hours. One-way analysis of variance did not reveal significant differences other than the decrease in cortical theta for BDNF KO males. As a result, means are being presented as the mean of each group across all six hours. Groups consist of wild type females (WT-F, red bar), heterozygous females (KO-F, pink bar), wild type males (WT-M, blue bar), and heterozygous males (KO-M, light blue bar).