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BDNF haplo-insufficiency impairs high-frequency cortical oscillations in mice

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Abstract

Schizophrenia is a complex psychiatric disorder with a heterogeneous aetiology involving genetic and environmental factors. Deficiencies in both brain-derived neurotrophic factor (BDNF) and NMDA receptor function have been implicated in the disorder, and may play causal and synergistic roles. Perturbations in the regulation of electrophysiological signals, including high-frequency (gamma: 30-80 Hz and beta: 20-30 Hz) neuronal oscillations, are also associated with the disorder. This study investigated the influence of BDNF deficiency and NMDA receptor hypofunction on electrophysiological responses to brief acoustic stimuli. Adult BDNF heterozygote ($BDNF^{+/-}$) and wildtype littermate C57Bl/6J mice were surgically implanted with EEG recording electrodes. All mice underwent EEG recording sessions to measure ongoing and auditory-evoked electrophysiological responses following treatment with MK-801 (0.3mg/kg ip) or vehicle. Western blotting on post-mortem cortical tissue assessed parvalbumin and GAD67 expression – markers of interneurons which are involved in the generation of gamma oscillations. Compared to wildtype controls, $BDNF^{+/-}$ mice exhibited markedly dampened electrophysiological responses to auditory stimuli, including reductions in the amplitude of multiple components of the event-related potential and auditory-evoked oscillations, as well as reduced ongoing cortical gamma oscillations. MK-801 elevated ongoing gamma power but suppressed evoked gamma power, and this was observed equally across genotypes. $BDNF^{+/-}$ mice also displayed reductions in parvalbumin, but not GAD67 expression. We conclude that reduced BDNF expression leads to impairments in the generation of high-frequency neural oscillations, but this is not synergistic with NMDA receptor hypofunction. Reduced parvalbumin expression associated with BDNF haplo-insufficiency may provide a molecular explanation for these electrophysiological deficits.

Keywords: BDNF, electrophysiology, event-related potential (ERP), gamma oscillations, parvalbumin, NMDA receptors, animal model

Introduction

Schizophrenia is a debilitating psychiatric condition characterised by deficits in many aspects of higher order behaviour, including information processing, sensory perception, and cognition. Both genetic predisposition and environmental exposures, and perhaps their interaction, seem to contribute to the pathogenesis of the disease (Oh & Petronis, 2008), although the underlying neural dysfunction remains unclear. Several lines of evidence support the theory that abnormalities in coordinated neuronal activity play a central role in the pathophysiology of schizophrenia (Uhlhaas & Singer, 2010). In particular, disturbances of high-frequency oscillations, including beta (20-30Hz) and gamma (30-80Hz) oscillations, may be indicative of, or cause, the disrupted network signalling and cognitive impairments of this disorder. Elucidating the function of these oscillations, and their regulation in health and disease states, would greatly aid our understanding of their relevance to schizophrenia.

Brain-derived neurotrophic factor (BDNF) has received significant attention as an important molecular factor involved in the pathophysiology of schizophrenia. BDNF plays key roles in brain development and function (Lu *et al.*, 2008), and abnormal BDNF signalling during development could lead to structural perturbations and aberrant neuronal network connectivity. BDNF expression is reduced in the prefrontal cortex (Hashimoto *et al.*, 2005) (Weickert *et al.*, 2003) and hippocampus (Durany *et al.*, 2001; Thompson Ray *et al.*, 2011) of brains of schizophrenia patients, and in serum (Niitsu *et al.*, 2011). In addition, polymorphisms in the BDNF gene are associated with cognitive impairments in patients with schizophrenia (Lu *et al.*, 2012; Notaras *et al.*, 2015). Despite the large literature on BDNF abnormalities in schizophrenia, little has been done to relate this growth factor to electrophysiological responses. One pertinent study deleted exon IV of the BDNF promoter, leading to reduced BDNF expression, and identified reduced power and coherence of beta and gamma oscillations following auditory stimulation (Hill *et al.*, 2016). Also, hippocampal deletion of TrkB – the primary receptor of BDNF - from parvalbumin positive (PV+ve) GABAergic interneurons significantly decreases the power of gamma oscillations in a slice preparation (Zheng *et al.*, 2011), while BDNF also acutely reduces excitability of

hippocampal PV+ve interneurons (Nieto-Gonzalez & Jensen, 2013), an effect which might be expected to result in network disinhibition and increased oscillations. Together, these studies suggest that BDNF signalling may influence the regulation of high-frequency oscillations, but it remains unclear how developmental abnormalities in BDNF itself might be relevant to the generation of neural circuits which underlie these oscillations.

Another prominent theory describing the pathophysiology of schizophrenia revolves around NMDAr hypofunction (Coyle, 2012). This is based on observations that NMDAr antagonists replicate and exacerbate many of the symptoms of schizophrenia (Adler *et al.*, 1998; Newcomer *et al.*, 1999). NMDAr antagonists produce an electrophysiological phenotype in rodents and healthy humans reminiscent of that observed in schizophrenia patients, with increased ongoing, or 'baseline', gamma oscillations diffusely throughout the brain (Hakami *et al.*, 2009; Jones *et al.*, 2012; Anderson *et al.*, 2014; Jones *et al.*, 2014), as well as reduced sensory-evoked gamma responses (Ehrlichman *et al.*, 2009; Hong *et al.*, 2010; Kulikova *et al.*, 2012; Long *et al.*, 2015; Anderson *et al.*, 2016). Beta oscillations (20-30 Hz) may also be affected and relevant to schizophrenia (Roopun *et al.*, 2008; Kulikova *et al.*, 2012), although oscillations of this frequency band have been less studied with respect to NMDAr hypofunction. The electrophysiological effects of NMDAr antagonists appear to be due to direct effects on fast-spiking PV+ve GABAergic interneurons (Carlen *et al.*, 2012).

In addition to the regulation of neural oscillations, other electrophysiological signatures can also be measured to investigate schizophrenia-relevant genes and environmental exposures. For example, event-related potentials (ERP) are a translational measure commonly used to assess sensory processing in a variety of species. A short acoustic stimulus results in a complex pattern of cortical electrophysiological activity which is time-locked to the stimulus onset. These patterns consist of several distinct components, although the specific components may vary depending on species and even in different strains of rodent (Amann *et al.*, 2010), but there seems to be excellent overlap between mouse and human for several of these components. ERP components have been shown to be modified by NMDAr antagonists in mice (Maxwell *et al.*, 2006), and by BDNF genetic variation in humans (Schofield *et al.*, 2009).

Here we investigate the role of BDNF in the regulation of coordinated neural activity, and the interaction with NMDAr hypofunction, by characterising electrophysiological responses in conscious, freely moving BDNF haploinsufficient mice in the presence and absence of the non-competitive NMDAr antagonist, MK-801. **These mice exhibit ~50% BDNF protein, compared to wild-type mice, due to the genetic deletion of one copy of**

the BDNF gene (Hill & van den Buuse, 2011). We studied ERPs, as well as both ongoing neural oscillations and also oscillations evoked by auditory stimuli. Since the generation of high-frequency rhythms is thought to be due to the activity of fast-spiking PV+ve GABAergic interneurons, we also performed post-mortem examination of markers of these cells. We hypothesised that BDNF-deficient animals would exhibit reduced expression of PV, and an electrophysiological phenotype reminiscent of schizophrenia, including dampened sensory-evoked oscillatory activity and increases in ongoing oscillations. We predicted these deficits would be exacerbated in mice following MK-801 treatment.

Experimental Procedures

Animals

The study used 28 male BDNF heterozygote ($BDNF^{+/-}$) or wild-type (WT) littermates obtained from our breeding colony at the Melbourne Brain Centre, Victoria, Australia. The colony was originally derived from breeders obtained from JAX Mice and Services (Bar Harbour, ME, USA) and maintained on a C57B1/6 background. The mice were weaned at 3 weeks of age and housed in groups of 3-5 mice in individually-ventilated cages with access to rodent chow and water *ad libitum*. Once they underwent electrode surgery, they were housed singly to prevent experimental loss. All experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes as set out by the National Health and Medical Research Council of Australia and approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee (Ethics number – 13-073-FNI).

Electrode implantation surgery

To facilitate cortical EEG recordings, recording electrodes were surgically implanted into all animals, as adapted from our previous study (Jones *et al.*, 2008). Briefly, mice were anaesthetised with isoflurane (Abbott Australasia Pty Ltd, Australia) and placed in a stereotaxic frame. A midline incision was made over the head to expose the skull, and small holes were made in the skull using an electric drill. The holes were located at 2mm lateral and 2mm anterior to bregma (above motor cortex - active electrodes), and ±2mm lateral and 2.5mm posterior to bregma (ground and reference). Gold ‘male’ connector electrodes previously soldered onto nickel alloy jeweller’s screws were gently screwed into the holes,

and dental acrylic (Vertex Dental, Netherlands) was applied to secure the electrodes. Animals were left to recover for at least one week before further study.

Electrophysiology studies

These studies were designed to assess the influence of BDNF genotype on EEG activity, and the interaction with the effects of acute MK-801 challenge (MK-801 obtained from Sigma-Aldrich, NSW, Australia). Mice were subjected to a repeated-measures drug treatment paradigm such that all mice received MK-801 (0.3 mg/kg ip) or vehicle (0.9% sterile saline ip) in pseudo-randomised order. These studies began when mice were ~11 weeks old.

EEG equipment involved a Powerlab 4/30 A-D converter (ADInstruments, Bella Vista, NSW, Australia) connected to 2 bioamplifiers and a computer running LabChart 7 software (AD Instruments). The sampling rate was set at 2000Hz and in order to reduce 50Hz electrical interference from the power mains, selective noise eliminators (Humbugs; Digitimer, Letchworth Garden City, UK) were used. At the start of each experimental session, a custom-made cable connected the electrode pins to the bioamplifiers, and continuous EEG data was collected for the left hemisphere (Channel 1) and the right hemisphere (Channel 2). A third channel was connected to the audio amplifier to accurately record the timing of auditory stimuli. The raw EEG data was band pass filtered offline at 0.5-500Hz prior to any subsequent analysis.

For the recording sessions, mice were placed in a Plexiglass cylinder within an automated startle box (SR-LAB, San Diego Instruments, San Diego, CA, USA). Auditory stimuli were delivered using SR-LAB Startle Software (San Diego Instruments). The stimuli were 20ms 85dB delivered every six seconds against a background noise level of 70dB. A 15-minute habituation period was followed by 20 minutes of EEG recording with acoustic stimuli before drug administration. Following injection, a further 40 minutes of pulses were delivered. At least 3 days was allowed between drug/vehicle treatments. The number of animals which completed all electrophysiology sessions was n=8 WT and n=7 $BDNF^{+/-}$ mice.

Electrophysiology analyses

Frontal cortical EEG activity was visualised in LabChart, and signals were inspected carefully in MATLAB software (version 7.10.0, Natick, Massachusetts: The MathWorks Inc., 2010) for noise or movement artefact. These episodes were removed from the final analyses. We performed two analyses: (1) evoked responses caused by, and time-locked to, the acoustic pulses (this incorporated assessment of the individual components of the ERP), as

well as analysis of oscillations triggered by the stimulus within specific frequency bands, and (2) ongoing oscillations across the frequency spectrum.

First, to characterise the electrophysiological responses elicited by the auditory stimuli (ERP), the evoked responses generated post-injection were averaged in the time domain for each session. We focused on 5 distinct components of the ERP which were consistently observed in all WT animals (see Figure 1A). We quantified the average magnitude and average latency of each of these peaks using custom-designed MATLAB scripts. The magnitude values were determined as change from baseline potential.

Subsequently, we filtered the ERP responses into the frequency bands of interest using fourth-order zero-phase Butterworth band-pass filters (Figure 2). This allowed us to quantify the oscillatory responses occurring within different frequency bands. To quantify evoked oscillatory responses, we used wavelet analyses using three wavelet cycles for the lowest frequency (1 Hz), linearly increasing to 10 cycles for highest frequency (200 Hz). This allowed the oscillatory power of the evoked responses to be calculated in the different frequency bands of interest (beta: 20-30Hz; gamma: 30-80Hz). Power responses occurring within 100ms of the auditory stimulus were then calculated by subtracting post-stimulus power from the baseline power occurring from -300ms – 0ms relative to the auditory pulse (baseline-corrected).

For the ongoing oscillatory calculations, Fast-Fourier Transformation was performed on 3s epochs prior to each auditory pulse using a Hanning window with a frequency resolution of 0.2441Hz. The spectra from each epoch occurring after injection was then averaged to determine the overall (ongoing) spectral power for each treatment group. The total power was then broken down into individual frequency bands. EEGLab (v12; SCCN, University of California, San Diego,USA) toolbox was used for all frequency analyses.

Tissue preparation

In separate cohorts of age-matched mice (n=6 WT and n=7 $BDNF^{+/-}$), animals were killed via lethal injection of Phenobarbital (Lethabarb, Virbac, Australia), and the brains rapidly excised on ice. The medial prefrontal cortex region was dissected using a mouse brain mould: the frozen brains were placed inverted on an ice-cold mouse brain mould (-8°C) with 1mm inserts. Three 2mm coronal slices were taken from the forebrain. The second slice (1.18 - 3.08mm from bregma) contained the medial prefrontal cortex (mPFC), which was dissected with a surgical blade. We chose to study this region because of it close associative links with

working memory and other higher order cognitive processes which can be disrupted in schizophrenia (Curtis & D'Esposito, 2003). Samples were snap-frozen in dry ice and stored in -80°C until further use.

Protein extraction

Frozen tissue samples were weighed and appropriate amounts of lysis buffer containing 150mM sodium chloride (ChemSupply, AU), 1.0% Triton-X-100 (Sigma Aldrich), 0.1% sodium dodecyl sulphate (Sigma Aldrich), 50mM Tris pH 8.0 (Trizma Base, Sigma Aldrich), protease inhibitor cocktail set III (dilution 1:200) and phosphatase inhibitor cocktail set IV (dilution 1:50, Merck; Kilsyth, Vic., Australia) were added according to tissue weight (1000µl per 100µg). Tissue samples were homogenized with a hand-held homogenizer and were left on ice for 10 minutes. Samples were then left to rotate for one hour at 4°C, followed by 15 minutes on the centrifuge at 14 000g at 4°C. The supernatant was then extracted and 3µl of the supernatant protein stock was used for a bicinchoninic acid protein assay to determine total protein levels. The remaining stock protein was then stored at -80°C.

Western Blotting

An equal volume of loading buffer (0.4M Tris, pH 6.8, 37.5% glycerol, 10% SDS, 1% 2 mercaptoethanol, 0.5% bromphenol blue, dH_2O) was added to each sample (50µg) and samples were denatured for 10 minutes at 95°C on the heat block before SDS-polyarcylamide gel electrophoresis (15% acrylamide gel, 120V, 1.5h) for separation of the denatured sample proteins. Protein samples were then transferred to a nitrocellulose membrane for 1.5h at 120V, 4°C. To prevent non-specific antibody binding, the membranes were blocked with 5% milk in TBST (TBST; 20mM Tris Base pH 7.5, 150 mM NaCl, 0.01% Tween 20) for one hour at room temperature on a shaker. The membranes were then washed with TBST for 1 minute then incubated overnight with primary antibody (in 5% bovine serum albumin (BSA) in TBST) at 4°C.

The primary antibodies used were mouse anti-PV (dilution 1:1000; 12 kDa; Merk-millipore; MAB1572; Billerica, MA, USA), mouse anti-GAD67 (dilution 1:1000; 67kDa; Sigma-Aldrich; G5419; Castle Hill, NSW, Australia), and mouse anti-β-actin (dilution 1:10,000; 42kDa; Sigma-Aldrich; A5316; Castle Hill, NSW, Australia).

The next day, the membranes were washed twice for 15 minutes in TBST then applied with secondary antibody of anti-mouse (dilution 1:2000; Cell signalling; Danvers, MA, USA). The blots were imaged by Luminescence Image Analyzer (LAS-4000; FujiFilm

Life Sicence, Stamford, CT, USA) and were further analysed using Image Quant software (GE Healthcare, Milwaukee, WI, USA). PV and GAD67 were normalised against the level of the control gene β-actin.

Statistical analyses

Based on results from our previous studies assessing electrophysiological outcomes in mice (eg: Long *et al.*, 2015), we calculated using the t-test that to detect a 30% decrease in evoked power (the primary outcome), with the study parameters set at p=0.05 (two-tailed) and beta=0.20, the estimated minimum number of mice required in each treatment group was 6. For all data, these were first assessed for normality using the Shapiro-Wilk test. All group data passed this assessment (p>0.05), and so we conducted subsequent parametric testing.

Since we acquired data from both hemispheres, we first assessed whether hemispheric asymmetries existed for the outcomes of interest. We found that, in WT mice, evoked beta power was significantly lower (p<0.05) in the right hemisphere than in the left. However, when incorporating data from BDNF$^{+/-}$ mice, there was no significant interaction between hemisphere and genotype, suggesting that this did not represent a disease-relevant hemispheric asymmetry. No other outcomes differed across hemispheres for either WT or BDNF$^{+/-}$ mice. As such, data for all outcomes were averaged across hemispheres.

Electrophysiological outcomes were assessed using two-way ANOVA (independent variables: drug and genotype) with repeated measures, and Bonferroni's post-hoc test used where appropriate. Molecular data were assessed with Student's unpaired t-test. In all cases, statistical significance was defined as $p<0.05$. Analyses were performed using Graphpad Prism 6.0 (La Jolla, CA, USA), and data are plotted as the individual data points, or represent the group mean ± standard error of the mean (S.E.M.).

Results

Effects of BDNF haploinsufficiency and NMDAr antagonism on ERP measures.

We identified five consistent ERP peaks from WT mice. These were: C1 – a sharp peak occurring 10ms from auditory pulse; C2 - a sharp negative trough occurring ~15ms from the stimulus; C3 (also referred to in literature as P1) – a sharp positive deflection occurring at

~20ms; C4 – a large negative deflection at ~40ms (also referred to as N1); and C5 - a slowly developing positive wave peaking at around 120ms (also referred to as P2). These peaks are depicted in Figure 1A. When comparing the individual components of the ERP, we found significant differences between the groups. With respect to the amplitude of the responses, significant effects were found for both genotype and drug, although no interactions were identified. $BDNF^{+/-}$ mice exhibited significantly reduced C2, C3 and C4 components, whereas mice treated with MK-801 showed significantly reduced C2, C4 and C5 components. See Table 1 for statistical details. We also assessed the latency to reach these peaks, and found that MK-801 treatment also reduced the latency of C1, C2, C3 and C5. No effect of genotype was found for these outcomes. Together, these findings indicated distorted ERPs independently resulting from BDNF haploinsufficiency and NMDAr antagonism.

Effects of BDNF haploinsufficiency and NMDAr antagonism on auditory-evoked oscillatory responses

Figure 2 depicts the group average electrophysiological responses to the auditory stimuli in the treatment groups, including the raw EEG (i.e. the ERP), the beta oscillatory response (20-30 Hz), the gamma oscillatory response (30-80 Hz), and the spectral frequency heatmap. When comparing the effects of BDNF genotype on these sensory-evoked oscillatory responses, evoked gamma appeared to be reduced in $BDNF^{+/-}$ mice, but this did not reach statistical significance ($F_{(1,13)}$=2.39, p=0.14; Figure 3A). This may be due to the observation that MK-801 itself significantly reduced evoked gamma power ($F_{(1,13)}$=44.34, p<0.0001); and this appears to reach a floor level – when comparing the data only from Vehicle treatment, we did identify a significant reduction of evoked gamma responses in $BDNF^{+/-}$ mice ($t_{(13)}$=3.19; p=0.006). ANOVA also revealed that evoked beta power was significantly lower in $BDNF^{+/-}$ mice compared to WT mice ($F_{(1,13)}$=5.08, p=0.042; Figure 3B), and that MK-801 significantly reduced evoked beta frequency responses ($F_{(1,13)}$=7.37, p=0.017). No interactions between drug treatment and genotype for either gamma or beta were observed (p>0.05).

Effects of BDNF haploinsufficiency and NMDAr antagonism on spontaneous EEG spectral power

Assessment of the ongoing (spontaneous) electrophysiological spectra generated from the two genotypes showed clear changes as a result of the BDNF deficiency (Figure 4). Overall, there were no significant differences in total power measures ($t_{(13)}$=0.32; p=0.75). However,

when assessing specific frequency bands, BDNF$^{+/-}$ mice showed significantly reduced ongoing gamma power ($F_{(1,13)}$=4.67, p=0.049; Figure 5A). We also observed reduced ongoing beta power in BDNF$^{+/-}$ mice, although this did not reach statistical significance ($F_{(1,13)}$=2.85, p=0.11; Figure 5B). MK-801 produced significant increases in ongoing gamma power ($F_{(1,13)}$=16.9, p=0.0012, Figure 5A), as well as significant reductions in ongoing beta power ($F_{(1,13)}$=30.14, p<0.0001; Figure 5B). No statistical interactions were found between genotype and drug treatment (p>0.05) suggesting genotype and drug treatment effects were independent.

Effects of BDNF genotype on parvalbumin levels.

To assess the influence of BDNF heterozygosity on markers of interneuron function, we probed the prefrontal cortex of mice for protein levels of relevant genes **using western blotting**: parvalbumin (PV) and GAD67 (Figure 6). We found a striking reduction (~50%) in PV **immunoreactivity** in the cortex in BDNF$^{+/-}$ mice compared to WT ($t_{(11)}$=2.5, p=0.031), but no changes in GAD67 **immunoreactivity** between genotypes ($t_{(11)}$=1.1, p=0.30).

Discussion

The most striking observation from this study was impaired generation and regulation of high-frequency oscillations in BDNF$^{+/-}$ mice - specifically reduced ERPs and evoked beta power following auditory stimuli, as well as reduced ongoing gamma power. The impaired ability to generate appropriate responses to brief auditory stimuli may underlie sensorimotor gating and cognitive deficits previously observed in these mice (Manning & van den Buuse, 2013; Wu *et al.*, 2015), since these behaviours may rely on such responses (Jones *et al.*, 2014). Further, given post-mortem findings of reduced BDNF levels in the brains of patients with schizophrenia (Weickert *et al.*, 2003), these findings promote this mouse model as one which could be used to study the relationships between deficits in BDNF signalling and oscillatory abnormalities which are relevant to schizophrenia.

The reduction in **parvalbumin (PV) immunoreactivity in BDNF$^{+/-}$ mice, suggestive of reduced protein abundance,** provides a potential molecular mechanism mediating the observed abnormalities in neural oscillations. PV is a calcium-binding protein expressed in a subset of inhibitory interneurons in the brain, and many studies implicate this protein in the pathology of schizophrenia (Lewis *et al.*, 2012). Signalling of BDNF can regulate the development of GABAergic interneurons, such as those expressing PV (eg: Jones *et al.*, 1994; Huang *et al.*, 1999; Rickman, 1999), and our data demonstrating reduced PV

immunoreactivity agrees with this. Several studies implicate PV cells as key generators of gamma oscillations, (eg: Sohal *et al.*, 2009; Carlen *et al.*, 2012), such that inhibiting PV cells using optogenetic tools reduces ongoing gamma oscillations, and driving these cells stimulates gamma activity. One might suggest, therefore, that loss of PV in $BDNF^{+/-}$ mice contributes to the observed reduction in ongoing gamma oscillations. **However, this is speculative, and there may be alterations to other (inter)neuron populations which contribute to neuronal oscillation disturbances here. Also, these changes may not be consistent in other brain sites. In addition, our studies suggest reduced PV expression, but this may be caused by reduced cell density or reduced expression per cell, and it is unclear what the specific consequences of each of these would be on network activity. Alternatively, we cannot rule out other non-interneuron-based mechanisms caused by reduced BDNF as drivers of electrophysiological dysfunction in these mice (eg: Huang & Morozov, 2011).**

Evidence also exists for aberrant synchronisation of beta oscillations in schizophrenia (eg: Uhlhaas *et al.*, 2006), and this may contribute to regional brain connectivity and cognitive disturbance. While less is known about the neural generators, oscillations in the beta frequency range appear to be generated by distinctly different cellular sources compared with gamma rhythms (see Roopun *et al.*, 2008). This may explain the inconsistent effects of NMDAr hypofunction on spontaneous oscillations: we found clear reductions in gamma power, but pronounced increases in beta power following MK-801 administration, suggesting that these frequency bands are differentially modulated by this intervention. In contrast, oscillations in both frequency bands behaved similarly to BDNF haploinsufficiency, with broad band reductions observed.

One of the challenges for the field is to establish whether the same neuronal cells/circuits drive gamma oscillations associated with different conditions/stimuli/cognitive tasks. Most experimental literature to date focus on ongoing, or spontaneous oscillations, which appear to be driven by PV-positive cells. This is in contrast to the clinical literature, which predominantly explores sensory-evoked responses, and it is unclear to date whether the same cells and associated circuits regulate oscillations in different contexts. We provide associative evidence here to suggest that PV interneurons regulate both evoked and ongoing oscillations, since, in $BDNF^{+/-}$ mice, reductions in PV expression were associated with reduced ongoing, as well as reduced evoked gamma oscillations. Clearly though, many neurotransmitters can differentially influence gamma oscillations – a prime example is the

effect of NMDA receptor antagonists, such as ketamine and MK-801, which increase ongoing cortical oscillations, but concurrently reduce evoked gamma oscillatory responses (Ehrlichman *et al.*, 2009 ; Kulikova *et al.*, 2012; Long *et al.*, 2015; Anderson *et al.*, 2016), so further research is needed to clarify whether PV cells are responsible for the generation of gamma oscillations associated with acoustic stimuli or cognitive processes.

BDNF deficiency might also be associated with NMDAr hypofunction to impact high-frequency oscillations. There are strong facilitatory relationships between BDNF and NMDA receptor signalling. For example, BDNF regulates the expression and trafficking of NMDA receptors to cell membranes (Caldeira *et al.*, 2007), and enhances NMDA receptor phosphorylation (Suen *et al.*, 1997) and activity (Levine *et al.*, 1998). Therefore, BDNF deficiency might be expected to result in NMDA receptor hypofunction. However, we found no evidence of altered electrophysiological effects of MK-801 in BDNF-deficient mice when examining both ongoing and evoked oscillations and ERPs. This agrees with reports of unchanged antidepressant-like activity of ketamine (Lindholm *et al.*, 2012), and unchanged MK-801-induced disruption of prepulse inhibition (Manning & van den Buuse, 2013) in BDNF heterozygous mice. However, some controversy does surround this, with other articles suggesting that the antidepressant effect of ketamine is diminished in mice lacking BDNF (Autry *et al.*, 2011), and in mice possessing the Val66Met mutation within the *bdnf* gene (Liu *et al.*, 2012). Nevertheless, our data suggests that there are developmental mechanisms which compensate for reduced BDNF on NMDA receptor function, at least for the electrophysiological endpoints studied here. Another possibility is that the evoked gamma responses were reduced sufficiently in $BDNF^{+/-}$ mice such that these could not be further lowered following MK-801 treatment, a possible 'floor' effect.

While we have identified several electrophysiological alterations in $BDNF^{+/-}$ mice, including aberrant neural oscillations and also marked reductions in several components of the ERP, it is unclear what the consequences of each of these might be. The most notable disturbance to the ERP was the reduced magnitude of C4, a negative deflection on the EEG occurring ~40ms after the acoustic stimuli, an observation similar to that observed in another genetic mouse model exhibiting reduced BDNF expression (Hill *et al.*, 2016). This may be analogous to the N100 component observed in humans, and reflects activity in primary auditory and associative cortex (Gallinat *et al.*, 2002). Reduced amplitude in this component might be suggestive of abnormal neuronal architecture in this area, but further studies are needed to confirm this, and to understand what the functional consequences might be. Similarly, while we

demonstrate alterations in spontaneous and evoked oscillations, we can only speculate about the consequences of these abnormalities. We have previously identified behavioural phenotypes caused by BDNF heterozygosity (Manning & van den Buuse, 2013) and NMDAr hypofunction (Hakami *et al.*, 2009), but without correlative analyses, it is challenging to directly demonstrate causal relationships between electrophysiological phenotypes (including oscillatory responses and ERP components) to functional consequences. Some of our recent endeavours attempt to achieve this (Jones *et al.*, 2014; Hudson *et al.*, 2016).

While several elements of our mouse model are phenotypically similar to schizophrenia, one point of divergence relates to the reduced spontaneous gamma oscillations observed in BDNF$^{+/-}$ mice. Brain BDNF levels are reduced patients, and reports document elevations in ongoing gamma power in schizophrenia (Baldeweg *et al.*, 1998; Flynn *et al.*, 2008; Spencer, 2011; Andreou *et al.*, 2015). Furthermore, the lack of effect on GAD67 levels in cortex of BDNF$^{+/-}$ mice also does not concord with clinical observations. Therefore, while our study suggests that genetically-driven reduction in BDNF is not sufficient to produce an electrophysiological or molecular phenotype which precisely replicates that observed in patients, some aspects of the clinical phenotype, notably reduced auditory-evoked oscillations, are present in these mice, therefore allowing this relationship to be further examined.

To summarise, here we demonstrate that BDNF deficiency leads to diminished high-frequency electrophysiological responses to auditory evoked stimuli, and dampened ongoing gamma frequency cortical oscillations. These changes were associated with reductions in cortical PV expression. The electrophysiological and molecular similarities linking this model and schizophrenia patients support the idea that impaired interneuron function in this disorder may cause the electrophysiological abnormalities observed, and contribute to the symptomatology.

Disclosure

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design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Author contributions

NJ and MvdB designed the study; JF, MH, RH, and EM performed the experiments; JF, NJ, RH and GR performed the analyses; MH, GR and NJ created the figures; NJ wrote the paper; JF, MH, GR, RH, EM and MvdB edited the paper. All authors approved the final manuscript.

Data Accessibility

All data associated with this work are available upon request. Please send your request to the corresponding author (ncjones@unimelb.edu.au).

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None

Table legend:

Table 1: Statistical analyses of the 5 ERP components identified in Figure 1A.

Figure Legends:

Figure 1: Effects of drug and genotype on ERP components. **(A)** Example of a typical ERP trace from a WT mouse treated with vehicle, illustrating the 5 different components quantified. Note that components C3, C4 and C5 are typically referred to as P1, N1 and P2, respectively, in several literature reports. **(B)** Illustration of the grand ERP averages of the different treatment groups. Note in particular the small ERP in the $BDNF^{+/-}$ mice treated with MK-801. **(C)** The amplitudes of the different components were significantly different between the treatment groups, in particular C2, C3 and C4 were reduced in $BDNF^{+/-}$ mice ($^{\#}p<0.05$). MK-801 treatment also significantly reduced the amplitude of C2, C4 and C5 (**p<0.01 indicates post-hoc significance between drug vs vehicle for indicated genotype). (D) The peak latencies of the ERP components were reduced in MK-801-treated mice compared to vehicle (*p<0.05, **p<0.01 indicates post-hoc significance between drug vs vehicle for indicated genotype). There was no effect of genotype on component latency.

Data in panels C and D represent group mean +/- S.E.M. Sample sizes: n=8 WT; 7 BDNF$^{+/-}$.

Figure 2: Grand averages of raw and filtered EEG traces, as well as evoked heat maps of relative power resulting from the auditory stimuli. The columns from left to right are grand averages generated from saline-treated WT mice, MK-801-treated WT mice, saline-treated BDNF$^{+/-}$ mice, and MK-801-treated BDNF$^{+/-}$ mice. The rows from top to bottom represent grand averages of the event-related potential (ERP) generated from the raw EEG, the ERP filtered between 20-30Hz to isolate beta frequency activity, the ERP filtered between 30-80Hz to isolate gamma frequency activity, and the average power heat maps generated by the ERP. The red vertical line on all images represents the onset of the auditory tone.

Figure 3: Effects of drug and genotype on auditory-evoked cortical oscillations. **(A)** Evoked gamma (γ) power is significantly reduced by MK-801 treatment **(B)** Evoked beta (β) power is significantly reduced in BDNF$^{+/-}$ mice, and also following treatment with MK-801. *$p<0.05$, indicates significant difference between MK-801 and vehicle; #$p<0.05$ indicates significant difference between genotypes. **Sample sizes: n=8 WT; 7 BDNF$^{+/-}$.**

Figure 4: (A) Spectrograms of oscillatory power (0.5 - 100Hz) in BDNF$^{+/-}$ (red trace) and WT (blue) mice shows reduced power in the higher frequencies in the BDNF$^{+/-}$ mice. Shaded areas indicate SEM. **(inset)** Quantification of overall total power (1-100Hz) is not significantly different between genotypes. **(B)** Grand average heat maps illustrating ongoing power in WT (left panel) and BDNF$^{+/-}$ mice (right panel) calculated as relative to the baseline period, and the influence of MK-801 (injected at ~23 mins, indicated by the black vertical line). Note the rise in high frequency power together with reductions in the lower frequencies occurring after injection, which are more pronounced in WT compared to BDNF$^{+/-}$ mice. **(C)** Spectrograms of total power in WT (left panel) and BDNF$^{+/-}$ mice (right), and the influence of MK-801 (red traces) compared to Saline (blue). Shaded areas indicate SEM. **Sample sizes: n=8 WT; 7 BDNF$^{+/-}$.**

Figure 5: Effects of drug and genotype on ongoing cortical oscillations. **(A)** Ongoing gamma (γ) power is significantly reduced in BDNF$^{+/-}$ mice, but enhanced by MK-801 treatment similarly in both genotypes. **(B)** In contrast, ongoing beta (β) power is significantly reduced

by MK-801. *p<0.05 indicates significant difference between MK-801 and vehicle; #p<0.05 indicates significant difference between genotypes. **Sample sizes: n=8 WT; 7 BDNF$^{+/-}$.**

Figure 6: Effects of genotype on cortical parvalbumin and GAD67 **immunoreactivity**. **(A)** Representative image of a Western blot. **(B)** PV **immunoreactivity** is significantly reduced in the medial prefrontal cortex (mPFC) of BDNF$^{+/-}$ mice compared with WT mice, whereas no differences in GAD67 **immunoreactivity** between the two genotypes were observed **(C)**. *p<0.05 indicates significant difference between genotypes. **Group data represent mean ± S.E.M, Sample sizes: n=6 WT; 7 BDNF$^{+/-}$.**

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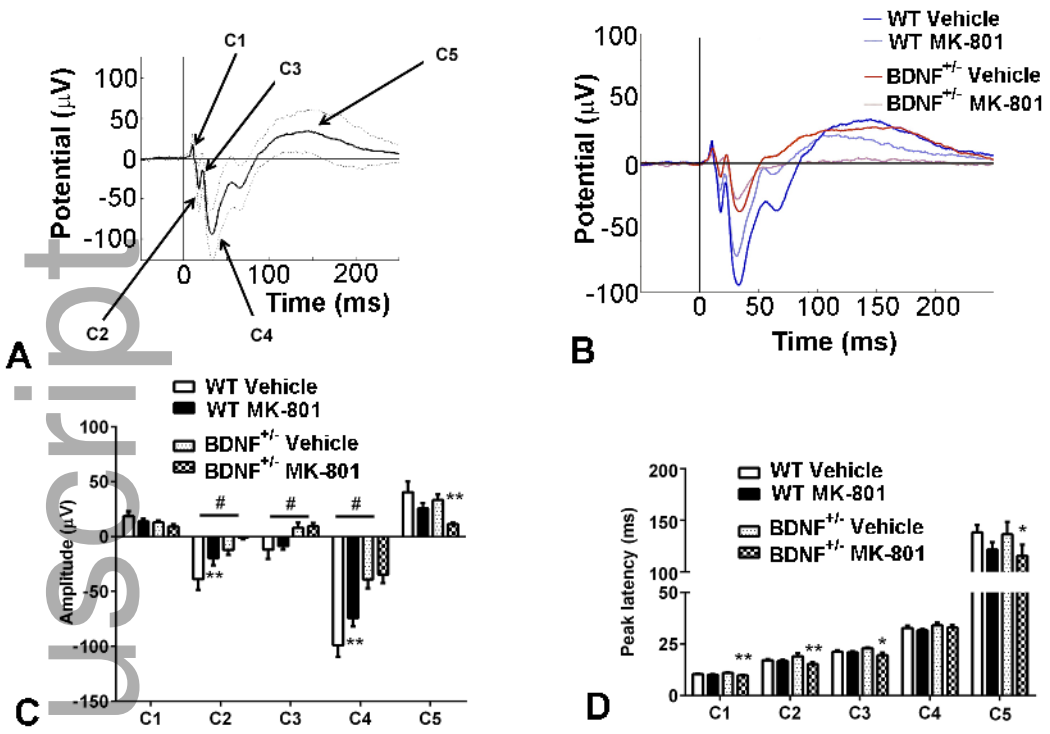
Amplitude

	Component C1		Component C2		Component C3		Component C4		Component C5	
	F	p	F	p	F	p	F	p	F	p
Effect of drug	F (1, 13) = 4.35	P = 0.057	F (1, 13) = 21.16	P = 0.0005	F (1, 13) = 0.33	P = 0.57	F (1, 13) = 10.59	P = 0.006	F (1, 13) = 16.24	P = 0.0005
Effect of genotype	F (1, 13) = 2.54	P = 0.135	F (1, 13) = 7.02	P = 0.020	F (1, 13) = 7.74	P = 0.016	F (1, 13) = 21.70	P = 0.0004	F (1, 13) = 2.19	P = 0.16
Interaction	F (1, 13) = 0	P = 0.995	F (1, 13) = 0.85	P = 0.37	F (1, 13) = 0.09	P = 0.76	F (1, 13) = 2.81	P = 0.117	F (1, 13) = 0.80	P = 0.39

Peak Latency

	Component C1		Component C2		Component C3		Component C4		Component C5	
	F	p	F	p	F	p	F	p	F	p
Effect of drug	F (1, 13) = 26.99	P = 0.0002	F (1, 13) = 8.31	P = 0.013	F (1, 13) = 6.60	P = 0.023	F (1, 13) = 0.28	P = 0.60	F (1, 13) = 11.10	P = 0.0054
Effect of genotype	F (1, 13) = 0.13	P = 0.719	F (1, 13) = 0.41	P = 0.53	F (1, 13) = 0.058	P = 0.81	F (1, 13) = 1.92	P = 0.189	F (1, 13) = 0.41	P = 0.53
Interaction	F (1, 13) = 10.56	P = 0.006	F (1, 13) = 4.75	P = 0.048	F (1, 13) = 4.49	P = 0.054	F (1, 13) = 0.28	P = 0.60	F (1, 13) = 1.58	P = 0.23

Table 1



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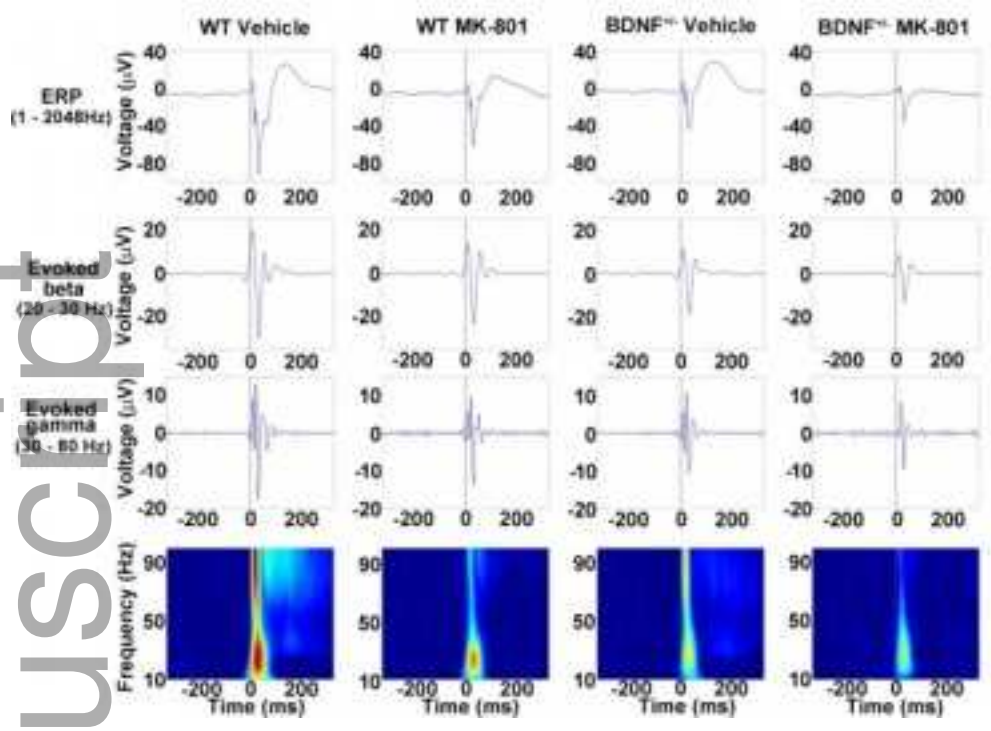

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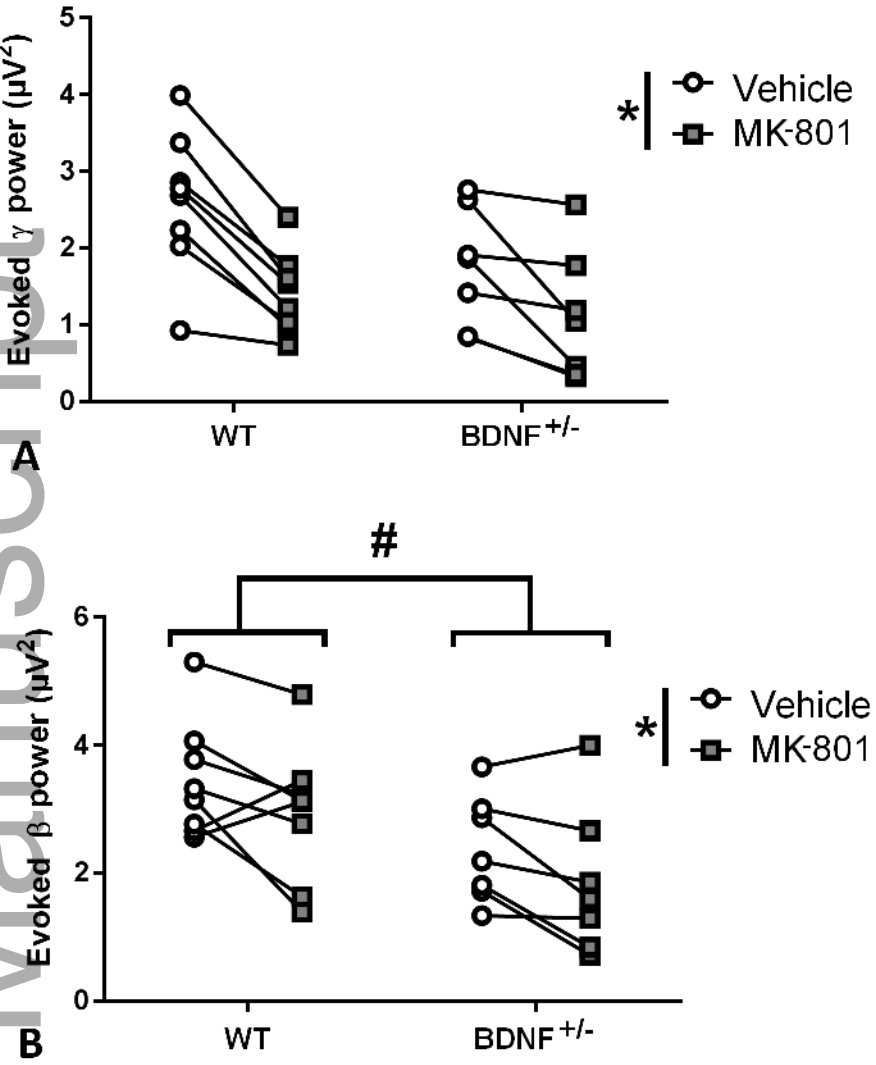

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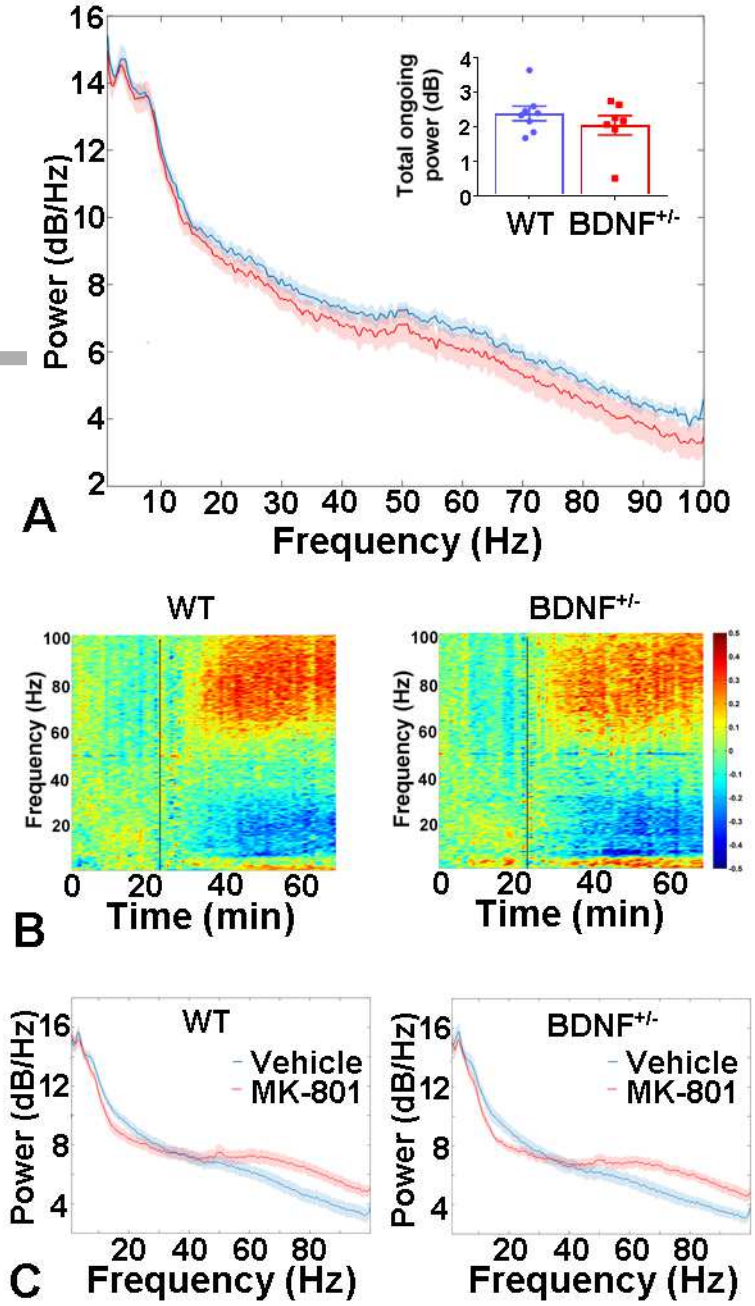

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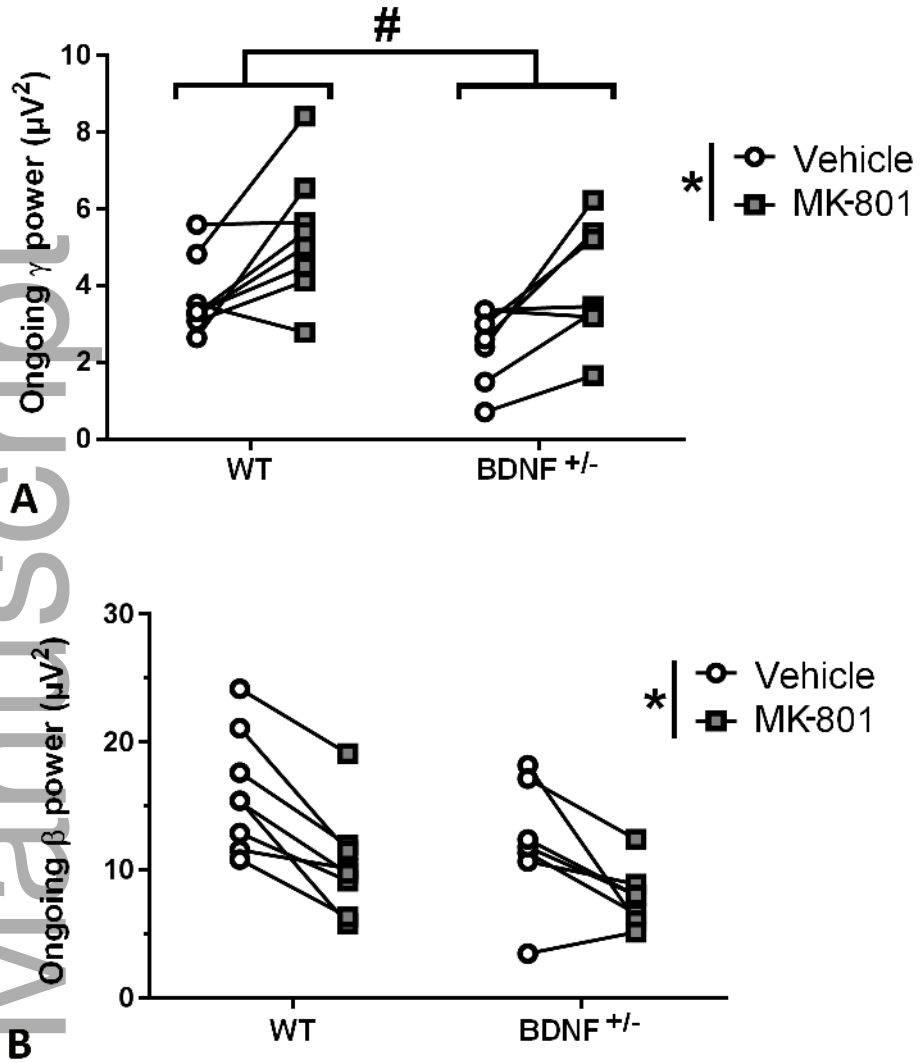

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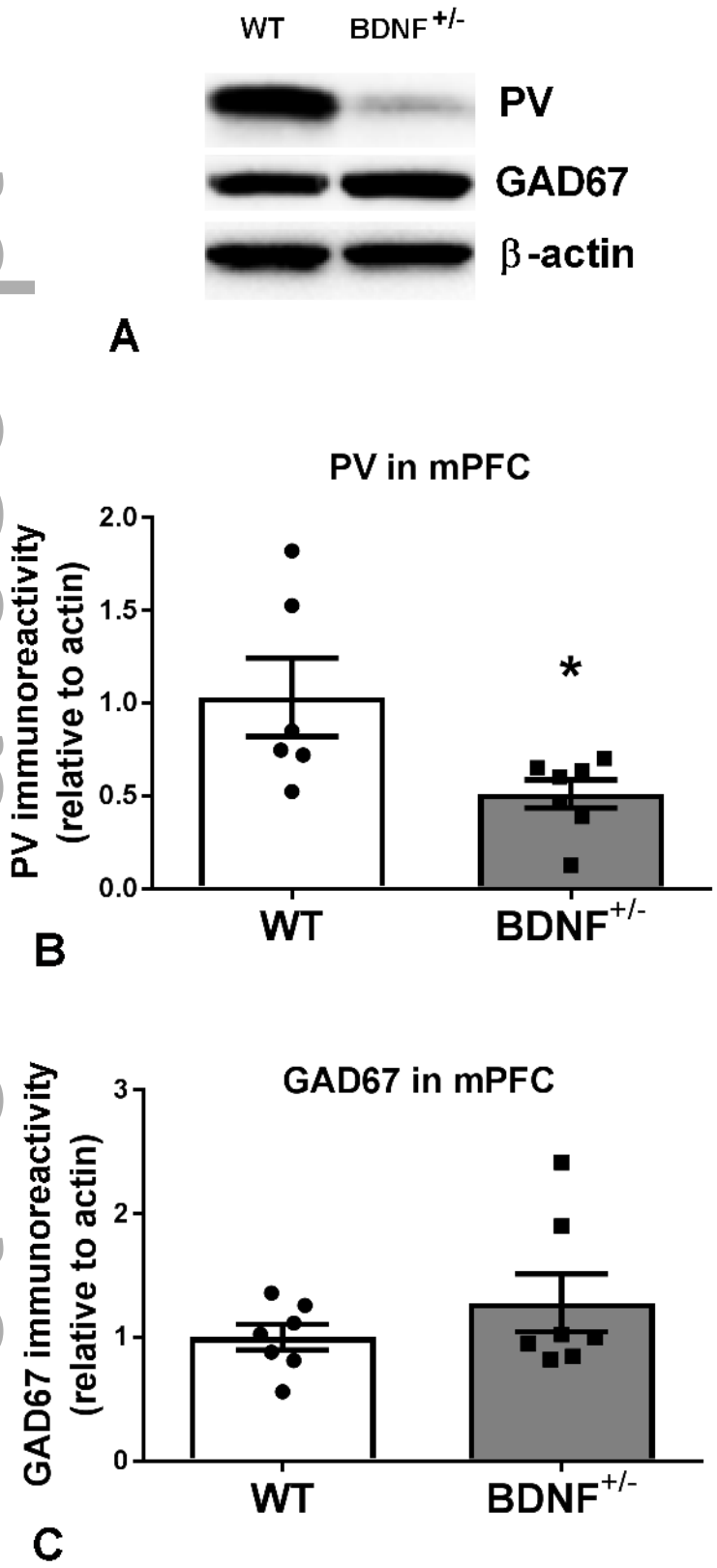

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