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Mice lacking TrkB in parvalbumin-positive cells exhibit sexually dimorphic behavioral phenotypes

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Abstract

Activity-dependent brain-derived neurotrophic factor (BDNF) signaling through receptor tyrosine kinase B (TrkB) is required for cued fear memory consolidation and extinction. Although BDNF is primarily secreted from glutamatergic neurons, TrkB is expressed by other genetically defined cells whose contributions to the behavioral effects of BDNF remain poorly understood.

Parvalbumin (PV)-positive interneurons, which are highly enriched in TrkB, are emerging as key regulators of fear memory expression. We therefore hypothesized that activity-dependent BDNF signaling in PV-interneurons may modulate emotional learning. To test this hypothesis, we utilized the LoxP/Cre system for conditional deletion of TrkB in PV-positive cells to examine the impact of cell-autonomous BDNF signaling on Pavlovian fear conditioning and extinction. However, behavioral abnormalities indicative of vestibular dysfunction precluded the use of homozygous conditional knockouts in tests of higher cognitive functioning. While vestibular dysfunction was apparent in both sexes, female conditional knockouts exhibited an exacerbated phenotype, including extreme motor hyperactivity and circling behavior, compared to their male littermates. Heterozygous conditional knockouts were spared of vestibular dysfunction. While fear memory consolidation was unaffected in heterozygotes of both sexes, males exhibited impaired extinction consolidation compared to their littermate controls. Our findings complement evidence from human and rodent studies suggesting that BDNF signaling promotes consolidation of extinction and point to PV-positive neurons as a discrete population that mediates these effects in a sex-specific manner.

Keywords

Ntrk2; fear conditioning; extinction; vestibular; circling

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1. Introduction

Development, survival, and plasticity of neurons depends in part on brain-derived neurotrophic factor (BDNF) signaling through receptor tyrosine kinase B (TrkB), abnormalities in which have been linked to a range of neurodevelopmental, psychiatric, and neurological disorders [1]. Impaired emotional memory processing is a key feature in many such disorders, and mounting evidence suggests that activity-dependent TrkB signaling is required for both the consolidation of cued fear memories and their extinction [2-7]. While BDNF is primarily synthesized by and secreted from pyramidal neurons, TrkB is expressed by numerous cell types throughout the brain, and the contribution of cell-autonomous TrkB signaling in discrete neuronal populations to emotional memory processes remains largely unexplored.

Parvalbumin (PV) expression delineates a major subpopulation of GABAergic interneurons that are highly enriched in TrkB [8] and mediate synaptic inhibition and neural synchrony through network oscillations in the brain [9]. In preclinical models, neural synchrony has been strongly correlated with the formation, retrieval, and extinction of emotional memories, processes that are disrupted by BDNF loss of function [2, 3, 7, 10, 11] and by optogenetic manipulation of PV-interneurons [12, 13]. Recent evidence indicates that cell-autonomous TrkB signaling in PV-positive cells promotes PV-interneuron maturation and entrainment of local networks [14]. Such effects raise the possibility that activity-dependent BDNF signaling in PV-interneurons may contribute to memory for cued fear and extinction.

To examine this possibility, we crossed mice with LoxP sites flanking critical regions of the TrkB gene ($TrkB^{fl/fl}$) to those expressing Cre recombinase under the control of the PV promoter (PV-Cre). Unexpectedly, homozygous conditional knockout mice exhibited behavioral abnormalities indicative of vestibular dysfunction, a potential consequence of PV-mediated recombination in non-interneuron cell types important for vestibular control [15]. Heterozygous conditional knockouts were spared of vestibular dysfunction and exhibited a selective impairment in the consolidation of auditory fear extinction. Behavioral manifestations in conditional homo- and heterozygous knockouts varied between male and female animals. These results provide new evidence that BDNF signaling within PV-expressing neurons facilitates vestibular development and emotional flexibility and that sex may be critical consideration in the therapeutic potential of TrkB-directed pharmacology.

2. Methods

2.1 Animals

The Institutional Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai approved all experimental protocols in advance. Mice with conditional ablation of TrkB in PV-positive cells were created by crossing mice with loxP sites flanking two transcriptional start sites and the first coding exon region of the *TrkB* gene [16] with mice expressing Cre recombinase driven by the *pvalb* promoter [17; Jackson Laboratories, Bar Harbor, ME, USA). Cellular specificity of recombination and TrkB deletion in this genetic cross has been previously described [14], and we verified that recombination did not grossly differ between male and female offspring by crossing PV-Cre mice to an R26-STOP-eYFP

reporter line (Jackson Laboratories). Animals were maintained on a C57B/6 $\times$ 129B/6 hybrid background. Mice were housed in groups of 2-5 per cage and maintained on a standard light:dark cycle with access to food and water *ad libitum*.

2.2 Behavioral Analyses

All behavioral experiments were performed on Cre-positive littermates obtained from TrkB^{fl/+}:PV-Cre^{+/-} breeding pairs on postnatal day (P) 40-50 during the light cycle.

2.2.1 Open field—Mice were placed in a square apparatus (16.5" $\times$ 16.5" $\times$ 13") equipped with a double axis of 16 infrared beams in a dark room for 30 minutes. Data were collected with Fusion v4 Home Cage software (Omnitech Electronics, Columbus, OH, USA). Open field testing was conducted on behavioral naïve animals at least three days prior to SHIRPA screening or auditory fear conditioning.

2.2.2 Modified SHIRPA Screening—Mice were screened according to the modified SHIRPA (acronym of SmithKline Beecham, Harwell, Imperial College, Royal London Hospital, phenotype assessment) protocol published by the European Mouse Phenotyping Resource of Standardised Screens (<http://empress.har.mrc.ac.uk>). Briefly, mice were placed in a glass jar (14 cm diameter, 18 cm height) and observed for three minutes, during which time the presence of head tossing was coded. The Preyer reflex was assessed by response to presentation of a 90 dB tone elicited by an IHR click-box. Contact righting was assessed by placing mice into a clear plastic tube (3 cm diameter, 20 cm length), quickly turning the tube 180°, and measuring the latency for the mouse to return to its original position (dorsal side up) with a 10 s maximum time. The swim test was performed by placing mice in a clear plastic tank of water (30 cm $\times$ 35 cm $\times$ 15 cm; water height 8 cm, 20-23°C). Normal swimming behavior was scored when the animal maintained a horizontal position with its nose above the surface. Animals exhibiting underwater circling were immediately removed from the tank and scored as non-swimmers.

2.2.3 Auditory fear conditioning and extinction—Mice were handled once per day for three days prior to auditory fear conditioning. On the first day, mice underwent fear conditioning that consisted of 6 pairings of an auditory tone (2 KHz, 80 dB, 20 s) with foot shock (1 mA, 2 s), in which the tone and foot shock co-terminated. An acclimation period of 200 s in training arena preceded the onset of cues, and pairings were separated by an 80 s inter-trial interval. On the second and third days, mice underwent extinction and re-extinction that consisted of 20 tone presentations in an altered context. Retention of extinction was tested by 4 tone presentations in the extinction context 7 days after the last extinction session. For reduced-intensity fear conditioning, training entailed 3 pairings of the auditory tone (2 KHz, 80 dB, 20 s) with a milder footshock (0.7 mA, 2 s). Stimulus delivery and automated analysis of freezing behavior was conducted with VideoFreeze software (MedAssociates, St. Albans, VT, USA).

2.3 Immunofluorescence

On postnatal day 35, male (n = 3) and female (n = 3) PV-Cre^{+/-}:R26-STOP-eYFP^{+/-} mice were deeply anesthetized with a mixture of ketamine (9mg/mL) and xylazine (1mg/mL).

Mice were transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA), and brains were removed and postfixed in 4% PFA for 24 hours. Brains were cryoprotected in 30% sucrose and embedded in OCT (Sekura FineTek, Torrance, CA, USA) before freezing in dry-ice cooled isopentane. Brains were cryostat sectioned at 30 μ M on the coronal plain, mounted onto charged slides, and stored at -80°C . Immunofluorescence was conducted on serial sections throughout the rostral-caudal axis. Primary antibodies included mouse anti-parvalbumin (Millipore, Billerica, MA, USA) and rabbit anti-GFP (Millipore). Brain sections were incubated in 10% normal goat serum in PBS for one hour at room temperature prior to overnight incubation in primary antibody with 5% normal goat serum in PBS over night at 4°C . Slides were washed in PBS and incubated for two hours in secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) with 5% normal goat serum in PBS at room temperature. Slides were washed in PBS, coverslipped with Prolong Antifade Gold (Life Technologies, Grand Island, NY, USA), and stored at 4°C . Images were obtained on an upright Zeiss Confocal microscope with a computer equipped with Zenn software (Carl Zeiss Microscopy, Jena, Germany).

2.4 Statistical analyses

Statistical analyses were performed with SPSS Statistics 22 (IBM, Armonk, NY, USA) or GraphPad Prism 5 (La Jolla, CA, USA). All data were checked for normal distribution before proceeding to parametric tests. Differences among genotypes were detected by repeated-measures (open field, cued fear acquisition, cued fear extinction), two-way ANOVA (open field), two-tailed t-tests (extinction retrieval), or χ^2 test for independence (SHIRPA). When applicable, posthoc planned comparisons were conducted with Holm-Bonferroni with family wise error rate maintained at 0.05.

3. Results

3.1 Vestibular dysfunction in mice with homozygous deletion of TrkB in PV-positive cells

Home cage observations of mice with conditional TrkB knockout in PV-positive cells revealed a striking hyperactive phenotype characterized by circling behavior and stereotypical head tossing. These behaviors were present in TrkB^{fl/fl}:PV-Cre mice within the first two weeks of postnatal life and continued throughout adulthood. To quantify these locomotor behaviors, mice were subjected to a novel open field environment for 30 minutes. Movement was detected with an automated system utilizing infrared beams. Repeated-measures ANOVA revealed that TrkB^{fl/fl}:PV-Cre mice traveled significantly farther over the test period compared to their sex-matched TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre littermates (Figure 1A; males: main effect genotype, $F_{(2,41)} = 3.86$, $p = 0.03$; females: main effect genotype, $F_{(2,32)} = 46.69$, $p < 0.0001$). However, female homozygotes traveled significantly farther over the test period than their male counterparts (Figure 1B; repeated-measures ANOVA, main effect sex, $F_{(1,23)} = 21.39$, $p < 0.0001$).

We next investigated specific behaviors of interest binned over the 30 minute test period. TrkB^{fl/fl}:PV-Cre mice of both sexes engaged in increased stereotypical behavior compared to TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre littermates (Figure 1C; two-way ANOVA, main effect of genotype, $F_{(2,82)} = 21.66$, $p < 0.0001$), likely accounted for by the presence of head

tossing in homozygous mice (Figure 1D, χ^2 test for independence, $p < 0.05$). Most female and a small subset of male $\text{TrkB}^{\text{fl/fl}}\text{:PV-Cre}$ mice engaged in perseverative circling behavior (Figure 1E, χ^2 test for independence, $p < 0.05$), with females exhibiting increased counter-clockwise (two-way ANOVA, sex $\times$ genotype interaction, $F_{(2,82)} = 10.85$, $p < 0.0001$) and total (two-way ANOVA, sex $\times$ genotype interaction, $F_{(2,82)} = 12.88$, $p < 0.0001$) revolutions compared to their male $\text{TrkB}^{\text{fl/fl}}\text{:PV-Cre}$ littermates (Figure 1F). Of circling mice, 92.31% (12/13) exhibited lateralized circling, defined by more than two-thirds of rotations being in the same direction.

As hyperactivity, circling, and head tossing are behaviors indicative of vestibular and/or auditory dysfunction, we conducted a modified SHIRPA screen on a subset of mice. While all mice exhibited the Preyer reflex (data not shown), indicating intact auditory functioning, conditional homozygous mice of both sexes were unable to swim (Figure 1G; χ^2 test for independence, $p < 0.05$) and exhibited increased latency to turn on the contact righting reflex (Figure 1H; two-way ANOVA, main effect of sex, $F_{(2,24)} = 56.32$, $p < 0.0001$). Together, these data suggest that complete loss of TrkB signaling in PV-positive cells results in aberrant development of the vestibular system that manifests in a more pronounced behavioral phenotype in female compared to male animals.

3.2 Males with conditional deletion of TrkB in PV-positive cells exhibit impaired extinction learning

Since vestibular dysfunction in $\text{TrkB}^{\text{fl/fl}}\text{:PV-Cre}$ mice precluded their use in further behavioral testing, emotional learning was examined in $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ mice, which did not perform differently than $\text{TrkB}^{\text{WT}}\text{:PV-Cre}$ littermates on any open field measurement. To determine whether PV-specific TrkB signaling is important for fear acquisition and/or attenuation, we subjected $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ mice to auditory fear conditioning and extinction training. Regardless of sex, $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ mice exhibited no differences in tone-evoked freezing compared to $\text{TrkB}^{\text{WT}}\text{:PV-Cre}$ mice during fear conditioning or fear memory retrieval, which is expressed during the first block of extinction training (Figure 2). Furthermore, repeated-measures ANOVA revealed a significant main effect of block for males and females during extinction (male: $F_{(1,27)} = 69.06$, $p = 6.42 \times 10^{-9}$; female: $F_{(1,23)} = 12.07$, $p = 0.002$) and re-extinction (male: $F_{(1,27)} = 24.38$, $p = 3.60 \times 10^{-5}$; female: $F_{(1,23)} = 22.42$, $p = 9.00 \times 10^{-5}$), indicating that both sexes decreased freezing in response to extinction training. However, there was a significant main effect of genotype ($F_{(1,27)} = 5.72$, $p = 0.02$) as well as a block $\times$ genotype interaction ($F_{(1,27)} = 9.43$, $p = 0.005$) for males during re-extinction. Post-hoc analyses revealed that $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ males exhibited impaired extinction retrieval, as indicated by increased freezing during blocks 1 and 2 of re-extinction (Figure 2A, Holm-Bonferroni, $p < 0.05$). Male $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ also exhibited a trend to an increase in freezing during the extinction retrieval test given seven days after re-extinction (two-tailed t-test, $t_{(26)} = 1.89$, $p = 0.07$), suggesting that increased freezing in male $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ mice persisted even after re-extinction. Meanwhile, female $\text{TrkB}^{\text{fl/+}}\text{:PV-Cre}$ mice did not perform differently than female $\text{TrkB}^{\text{WT}}\text{:PV-Cre}$ littermates on any measure (Figure 2B).

To ensure that ceiling effects did not mask differences in fear memory expression in TrkB mutants, we subjected TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre mice to fear conditioning at reduced training intensity. TrkB haploinsufficiency had no effect on acquisition or retrieval of auditory-cued fear in a new context (Figure 3). Furthermore, mice of both genotypes displayed similar levels of context-evoked fear when re-exposed to the training arena.

3.3 Similar levels of reported recombination in PV-Cre male and female mice

Sex differences in recombination in PV-Cre animals could contribute to the sexually dimorphic behavioral manifestations in conditional TrkB homo- and heterozygous knockout mice. To investigate this possibility, we created crossed PV-Cre mice to R26-STOP-eYFP reporter mice and examined eYFP expression throughout the rostral-caudal axis in male and female mice. We focused on regions with a demonstrated role for PV-positive cells in vestibular/motor function, such as the cerebellum [15], and cued fear extinction, such as the medial prefrontal cortex [13] and basolateral amygdala [12; Figure 4]. eYFP expression was restricted to PV-rich brain regions and to PV-expressing cells throughout the brain. We did not observe any gross anatomical differences in eYFP expression between male and female animals, and similar levels of recombination were present in both sexes.

4. Discussion

While our initial goal was to determine whether PV-neuron-specific BDNF signaling regulates fear memory processes, we show here that mice with PV-specific loss of TrkB exhibit a sexually dimorphic phenotype encompassing both vestibular and emotional behavior. Homozygous knockouts of both sexes exhibit vestibular dysfunction, but the behavioral manifestation of this dysfunction is more pronounced in females. Consolidation of cued extinction memory was selectively impaired in male but not female heterozygous mice. We hypothesize that complete knockdown of TrkB in PV-interneurons with conditional shRNA or miRNA techniques in regions known to mediate extinction learning would result in greater extinction deficits than are observed in TrkB^{fl/+}:PV-Cre mice.

In addition to interneurons, PV is expressed by numerous cell populations involved in vestibular function, including vestibular ganglion cells, all brainstem vestibular nuclei, the ventral posterolateral nucleus, the organ of Corti, inner ear hair cells, and many cell types of the cerebellar cortex and deep nuclei [15]. Although embryonic lethal, mice with homozygous germline deletion of either BDNF or TrkB lack innervation to the semicircular canals [18]. Thus, the vestibular deficits observed in TrkB^{fl/fl}:PV-Cre mice may be due to loss of vestibular nerve innervation. Unlike the forebrain, PV expression in many vestibular regions occurs during gestation. Future studies should examine whether deletion of TrkB from PV-positive cells in adulthood circumvents the vestibular deficits observed in TrkB^{fl/fl}:PV-Cre mice. While it is unclear why female TrkB^{fl/fl}:PV-Cre mice exhibit increased hyperactivity and circling behavior compared to males, one publication reported increased cell number in the medial vestibular nucleus of adult female compared to male rats [19]. Female animals may therefore be more sensitive to TrkB signaling in PV-positive cells in this nucleus to maintain proper vestibular functioning and/or development.

Consistent with a role for PV-neuron-specific BDNF signaling in fear attenuation, we found a selective impairment in extinction consolidation in male but not female TrkB^{fl/+}:PV-Cre mice. Our results complement evidence from mice expressing the BDNF Val66Met mutation in which fear conditioning is preserved but extinction is impaired [20]. Importantly, human Val66Met mutation is also associated with impaired extinction as well as poor response to exposure-based therapy for post-traumatic stress disorder [20, 21]. Our results indicate that BDNF signaling may support extinction in part by modulating the activity of PV-interneurons. These data are particularly intriguing since antidepressant-mediated enhancement of fear extinction is accompanied by increased BDNF levels and altered expression of PV-interneuron markers in the amygdala [22]. While defining the precise mechanisms by which PV-interneurons regulate extinction may help uncover additional constraints on emotional flexibility, our data also indicate that such effects may be highly sex-specific. Future studies should investigate whether estrus cycle stage, which has been shown to modulate extinction learning [23], might interact with genotype to influence emotional flexibility in female animals. However, interestingly, the Val66Met polymorphism has been found to have sexually dimorphic disease predictability with a strong bias towards men in affective disorders and women in neurodegenerative disease [24, 25]. While preclinical studies of behavior are biased towards male subjects, sex-related differences may strongly affect translation of these findings to human populations. This clinical reality warrants an examination of BDNF and TrkB manipulations in female animals, which have been neglected in previous investigations [2-7, 10, 11, 20, 22].

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References

1. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nat Rev Drug Discov.* 2011; 10(3):209–19. [PubMed: 21358740]
2. Rattiner LM, et al. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *J Neurosci.* 2004; 24(20):4796–806. [PubMed: 15152040]
3. Chhatwal JP, et al. Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nat Neurosci.* 2006; 9(7):870–2. [PubMed: 16783370]
4. Peters J, et al. Induction of fear extinction with hippocampal-infralimbic BDNF. *Science.* 2010; 328(5983):1288–90. [PubMed: 20522777]
5. Andero R, et al. Effect of 7,8-dihydroxyflavone, a small-molecule TrkB agonist, on emotional learning. *Am J Psychiatry.* 2011; 168(2):163–72. [PubMed: 21123312]
6. Choi DC, Gourley SL, Ressler KJ. Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Transl Psychiatry.* 2012; 2:e205. [PubMed: 23250006]
7. Rosas-Vidal LE, et al. Hippocampal-Prefrontal BDNF and Memory for Fear Extinction. *Neuropsychopharmacology.* 2014
8. Gorba T, Wahle P. Expression of TrkB and TrkC but not BDNF mRNA in neurochemically identified interneurons in rat visual cortex in vivo and in organotypic cultures. *Eur J Neurosci.* 1999; 11(4):1179–90. [PubMed: 10103114]

9. Bartos M, Vida I, Jonas P. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci.* 2007; 8(1):45–56. [PubMed: 17180162]
10. Endres T, Lessmann V. Age-dependent deficits in fear learning in heterozygous BDNF knock-out mice. *Learn Mem.* 2012; 19(12):561–70. [PubMed: 23154927]
11. Psotta L, Lessmann V, Endres T. Impaired fear extinction learning in adult heterozygous BDNF knock-out mice. *Neurobiol Learn Mem.* 2013; 103:34–8. [PubMed: 23578839]
12. Wolff SB, et al. Amygdala interneuron subtypes control fear learning through disinhibition. *Nature.* 2014; 509(7501):453–8. [PubMed: 24814341]
13. Courtin J, et al. Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. *Nature.* 2014; 505(7481):92–6. [PubMed: 24256726]
14. Zheng K, et al. TrkB signaling in parvalbumin-positive interneurons is critical for gamma-band network synchronization in hippocampus. *Proc Natl Acad Sci U S A.* 2011; 108(41):17201–6. [PubMed: 21949401]
15. Celio MR. Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience.* 1990; 35(2):375–475. [PubMed: 2199841]
16. Luikart BW, et al. TrkB has a cell-autonomous role in the establishment of hippocampal Schaffer collateral synapses. *J Neurosci.* 2005; 25(15):3774–86. [PubMed: 15829629]
17. Hippenmeyer S, et al. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. *PLoS Biol.* 2005; 3(5):e159. [PubMed: 15836427]
18. Fritzsch B, et al. The role of neurotrophic factors in regulating the development of inner ear innervation. *Trends Neurosci.* 1997; 20(4):159–64. [PubMed: 9106356]
19. Ayyildiz M, et al. Sexual dimorphism in the medial vestibular nucleus of adult rats: stereological study. *Anat Sci Int.* 2008; 83(3):131–9. [PubMed: 18956784]
20. Soliman F, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science.* 2010; 327(5967):863–6. [PubMed: 20075215]
21. Felmingham KL, et al. The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. *Biol Psychiatry.* 2013; 73(11):1059–63. [PubMed: 23312562]
22. Karpova NN, et al. Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science.* 2011; 334(6063):1731–4. [PubMed: 22194582]
23. Milad MR, et al. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience.* 2009; 164(3):887–95. [PubMed: 19761818]
24. Verhagen M, et al. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry.* 2010; 15(3):260–71. [PubMed: 18852698]
25. Fukumoto N, et al. Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: New data and meta-analysis. *Am J Med Genet B Neuropsychiatr Genet.* 2010; 153B(1):235–42. [PubMed: 19504537]

Highlights

- The behavioral impact of TrkB signaling within PV-positive neurons is unknown.
- We created conditional knockout of TrkB in PV-neurons.
- TrkB^{fl/fl}:PV-Cre mice manifest in vestibular deficits more pronounced in females.
- Auditory fear extinction was selectively impaired in TrkB^{fl/+}:PV-Cre males.
- Our data highlight the emerging sexual dimorphism in BDNF-related disorders.

Figure 1. Loss of vestibular function in mice with homozygous deletion of TrkB in PV-positive cells

Automated open field analysis over a 30 minute session revealed that male and female TrkB^{fl/fl}:PV-Cre mice travel a significantly longer distance (cm) than their sex-matched TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre littermates (A). Female TrkB^{fl/fl}:PV-Cre mice also travel significantly farther than their male counterparts (B). TrkB^{fl/fl}:PV-Cre mice of both sexes exhibit increased stereotypies compared to TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre littermates (C), likely due to the presence of head tossing (D). Most female and a small subset of male TrkB^{fl/fl}:PV-Cre mice engage in circling behavior (E), which is lateralized in 92.31% (12/13) of mice with a slight preference toward clockwise rotations (8/13; F). TrkB^{fl/fl}:PV-Cre mice of both sexes are unable to swim (G) and exhibit impaired contact righting (H) compared to their TrkB^{WT}:PV-Cre and TrkB^{fl/+}:PV-Cre littermates. Significance was assessed by repeated-measures ANOVA (A, B), two-way ANOVA (C, F, H), or χ^2 test for independence (D, E, G) followed by planned comparisons with Holm-

Bonferroni when appropriate. * $p < 0.05$ for $\text{TrkB}^{\text{fl/fl}}$ versus TrkB^{WT} . # $p < 0.05$ for $\text{TrkB}^{\text{fl/fl}}$ versus $\text{TrkB}^{\text{fl/+}}$. @ $p < 0.05$ for $\text{TrkB}^{\text{fl/fl}}$ male versus $\text{TrkB}^{\text{fl/fl}}$ female. Data displayed as mean $\pm$ SEM.

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Figure 2. Selective impairment of extinction learning in TrkB^{fl/+};PV-Cre males

While auditory fear memory acquisition (Fear Conditioning) or retrieval (Extinction, Block 1) was not affected by TrkB haploinsufficiency in PV-positive cells, extinction memory retrieval (Re-extinction, Block 1-2) was significantly impaired in TrkB^{fl/+};PV-Cre compared to TrkB^{WT};PV-Cre males, and there was a trend to an impairment in extinction retrieval tested seven days after re-extinction. Haploinsufficiency in female animals did not produce deficits in fear conditioning or extinction (B). Blocks represent bins of 4 tone presentations. Significance was assessed by repeated-measures ANOVA followed by planned comparisons with Holm-Bonferroni (fear conditioning, extinction) or two-tailed t-tests (retrieval). * p < 0.05. Data displayed as mean ± SEM.

Figure 3. Unaltered fear memory recall in TrkB^{fl/+}:PV-Cre mice after low intensity fear conditioning

Cued and contextual memory recall were assessed 24 hours after a low threshold auditory fear conditioning paradigm. TrkB haploinsufficiency did not affect fear memory acquisition (Fear Conditioning) or retrieval (Recall) in male (A) or female (B) animals. Significance was assessed by repeated-measures ANOVA (fear conditioning) or two-tailed t-tests (recall). Data displayed as mean $\pm$ SEM.

Figure 4. Patterns of reported recombination in male and female PV-Cre mice

Confocal images of coronal PV-Cre:R26-STOP-eYFP brain sections colabeled with an antibody against PV. Low magnification images of eYFP expression in male (right) and female (left) serial brain sections reveal no obvious differences in recombination (left panel). Higher magnification photos of medial prefrontal cortex (A), basolateral amygdala (B), and cerebellum (C) demonstrate that eYFP (green) expression is confined to cells expressing PV (red; arrowheads) in male and female mice. In both sexes, approximately 50% of PV labeled cells do not exhibit recombination (red only; arrows). Boxed portions in left panel represent the location of higher magnification images. Scale bars = 500 μ M for left panel; 100 μ M for A and B; 20 μ M for C.