Published in final edited form as:

Eur J Neurosci. 2012 March ; 35(6): 932–939. doi:10.1111/j.1460-9568.2012.08021.x.

Enhanced Extinction of Cocaine Seeking in Brain-derived Neurotrophic Factor Val66Met Knock-In Mice

Lisa A. Briand^{1,2}, Francis S. Lee³, Julie A. Blendy¹, and R. Christopher Pierce²

¹Department of Pharmacology, Perelman School of Medicine, The University of Pennsylvania

²Department of Psychiatry, Perelman School of Medicine, The University of Pennsylvania

³Department of Psychiatry, Pharmacology, Weill Medical College of Cornell University

Abstract

The Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene results in alterations in fear extinction behavior in both human populations and mouse models. However, it is not clear whether this polymorphism plays a similar role in extinction of appetitive behaviors. Therefore, we examined operant learning and extinction of both food and cocaine self-administration behavior in an inbred genetic knock-in mouse strain expressing the variant *Bdnf*. These mice provide a unique opportunity to relate alterations in aversive and appetitive extinction learning as well as provide insight into how human genetic variation can lead to differences in behavior. BDNF^{Met/Met} mice exhibited a severe deficit in operant learning as evidenced by an inability to learn the food self-administration task. Therefore, extinction experiments were performed comparing wildtype (BDNF^{Val/Val}) animals to mice heterozygous for the Met allele (BDNF^{Val/Met}), which did not differ in food or cocaine self-administration behavior. In contrast to the deficit in fear extinction previously demonstrated in these mice, we found that BDNF^{Val/Met} mice exhibited more rapid extinction of cocaine responding compared to wildtype mice. No differences were found between the genotypes in the extinction of food self-administration behavior or the reinstatement of cocaine seeking, indicating the effect is specific to extinction of cocaine responding. These results suggest that the molecular mechanisms underlying aversive and appetitive extinction are distinct from one another and BDNF may play opposing roles in the two phenomena.

Keywords

Cocaine; Extinction; BDNF; Single-nucleotide polymorphism; Neurotrophin; Self-Administration

Addiction is characterized by compulsive drug use that persists in the face of negative consequences and desire to stop use (Kalivas & O'Brien, 2008; O'Brien, 2011). Conditioned drug craving elicited by drug-paired cues is among the many factors that contribute to this compulsive use. Addicts report that these conditioned cues are one of the most potent factors contributing to relapse (Heather *et al.*, 1991). Understanding how to extinguish this cue-elicited craving is a major goal in addiction research. Genetically modified mice provide a useful model system to examine molecular mechanisms underlying cocaine extinction behavior and how this might lead to alterations in vulnerability in human populations.

Address correspondence to: Lisa A. Briand, Ph.D. Center for Neurobiology and Behavior, Perelman School of Medicine, University of Pennsylvania, TRL, 125 South 31st Street, Philadelphia, PA 19104, Tel: (215) 573-5204, Fax: (215) 573-7605, lbriand@mail.med.upenn.edu.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of polypeptide growth factors, is widely expressed throughout the brain and is regulated in an activity dependent manner (Goodman *et al.*, 1996; Egan *et al.*, 2003). BDNF has been implicated in synaptic plasticity, learning, and addiction. Manipulations of BDNF levels in the brain have been demonstrated to modulate addictive behaviors, such as cocaine-induced locomotor activity (Pierce & Bari, 2001), cocaine conditioned place preference (Horger *et al.*, 1999; Hall *et al.*, 2003), and cocaine self-administration (Lu *et al.*, 2004). Furthermore, upregulation of BDNF expression is observed in various limbic nuclei during abstinence from cocaine self-administration (Sadri-Vakili *et al.*, 2010), and levels of BDNF expression correlate with measures of cue-elicited craving (Grimm *et al.*, 2003).

Along with this evidence from the preclinical literature, there is also a relationship between substance abuse and BDNF in human populations. At a genetic level, single nucleotide polymorphisms (SNPs) conferring vulnerability for polysubstance abuse were identified flanking the BDNF gene (Uhl *et al.*, 2001). More recently, a common SNP in the BDNF gene that leads to a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met) was linked to substance abuse vulnerability. A higher frequency of the Val allele is found among methamphetamine addicts compared to non-addicted individuals, suggesting the Met allele may confer resistance to addiction (Cheng *et al.*, 2005). However, there is an increase in 66Met allele frequency in smokers and healthy Met carriers consume more alcohol per week than 66Val homozygotes (Lang *et al.*, 2007; Colzato *et al.*, 2011). Parsing the role of this SNP in addictive behavior using an animal model could provide unique insight into the pathophysiology of addiction.

The current study utilized an inbred genetic knock-in mouse strain that expresses the variant BDNF allele to recapitulate the specific phenotypic properties of the human polymorphism *in vivo*. Previous work has demonstrated that the BDNF Val66Met genotype is associated with a resistance to the extinction of fear memories (Soliman *et al.*, 2010). While there is considerable overlap between the neurocircuitry underlying the extinction of aversive and appetitive memories (Ressler *et al.*, 2004; Davis *et al.*, 2006; Peters *et al.*, 2009; Myers & Carlezon, 2010b), it is clear that the mechanisms molecular mechanisms are not identical (Chhatwal *et al.*, 2009; Ward *et al.*, 2009; Bernardi & Lattal, 2010). The objective of this study was to assess the acquisition, stability and extinction of an appetitive behavior (cocaine self-administration) in Vall66Met knock-in mice.

Materials and Methods

Subjects

Mice bred and maintained on a C57Bl/6 background as described previously (Chen *et al.*, 2006), were single-housed with food and water available *ad libitum*. Adult wildtype (BDNF^{Val/Val}), littermate BDNF^{Val/Met}, and littermate BDNF^{Met/Met} male and female mice derived from BDNF^{Val/Met} × BDNF^{Val/Met} crossed parents were used for all experiments. All animals (2-4 months of age) were housed in a temperature and humidity controlled animal care facility with a 12 hr light/dark cycle (lights on a 7:00 A.M.). All procedures were approved by the University of Pennsylvania Animal Care and Use Committee.

Drugs

Cocaine was obtained from the National Institutes of Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

Operant Food Training

Prior to catheterization, mice were trained to perform an operant response for sucrose pellets. Although prior food training can lead to cross-sensitization, mice will not self-administer reliably without this training (Thomsen & Caine, 2007). The mice were placed in operant chambers (Med-Associates) and trained to spin a wheel manipulandum to receive a sucrose pellet. A compound cue stimulus consisting of a cue light above the active lever, a 2,900 Hz tone, and house light off was concurrent with each pellet administration, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Mice were allowed to self-administer a maximum of 50 pellets per 60 min operant session. During the food self-administration phase, mice were food restricted to approximately 90% of their free feeding weight. Mice received 10 training sessions and animals that did not distinguish between the active and inactive wheel (>60% active responding) were excluded from further study.

Jugular Catheterization Surgery

Prior to surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a mesh backmount platform (Strategic Applications, Inc) that secured the placement. Catheters were flushed daily with 0.1 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use.

Cocaine Self-administration

After surgery, mice were allowed 3-4 d to recover before beginning behavioral testing. Mice were tested for cocaine self-administration behavior in 2 hour sessions (6 d per week) in the same chamber used for sucrose pellet self-administration. During testing, a quarter turn of a wheel (FR1) on the same wheel used for sucrose training now delivered an intravenous cocaine injection (0.5 mg/kg/infusion). Each session began with a single infusion of cocaine accompanied by the presentation of the tone and light cue. A compound cue stimulus consisting of a cue light above the active lever, a 2,900 Hz tone, and house light off was concurrent with each injection, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Following 10 days of cocaine self-administration, cocaine-seeking behavior was extinguished by replacing the cocaine with 0.9% saline. Daily 2-h extinction sessions continued for 10 days. Following this 10-day extinction phase, animals that had not reached a criterion of less than 25% of their self-administration responding continued extinction for 5 additional days. Animals underwent cue-induced reinstatement after either meeting the extinction criterion or 15 days of extinction, whichever was shorter. During the cue-induced reinstatement session, the light and tone cues were presented non-contingently for 20 seconds every 2 minutes during the first 10 minutes of the session. After this time period, the cues were presented contingent with operant responding, just as was done during the cocaine self-administration phase. During the reinstatement session, animals received saline infusions following responses on the active wheel.

Sucrose Self-administration

Separate groups of mice were trained to lever press for sucrose as described above, and then received an additional 10 days of 2-hour sucrose self-administration sessions (6 d per week) in the same chamber used for sucrose training. A compound cue stimulus consisting of a cue light above the active lever, a 2,900 Hz tone, and house light off was concurrent with each pellet administration, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Each session began with a

pellet delivery accompanied by the presentation of a tone and light cue. Following 10 days of food self-administration, food-seeking behavior was extinguished by eliminating the food delivery following the operant response. Daily 2-h extinction sessions continued for 10 days.

Data Analysis

Total active operant responses over the course of the ten days of food training were analyzed using a repeated measures two-way ANOVA with genotype (Val/Val, Val/Met, Met/Met) and session as the independent variables and active responses as the dependent variable. Bonferroni's post hoc comparisons were conducted when main effects or interactions were present. Similar analyses were performed for the cocaine self-administration, extinction and reinstatement phases of the experiment.

Results

Acquisition of Sucrose Pellet Self-Administration

The number of pellets earned and active responses during each sucrose self-administration session was measured. BDNF^{Val/Val} and BDNF^{Val/Met} mice acquired stable responding for food, readily responding for 20 or more pellets after 5 days of training. However, BDNF^{Met/Met} mice failed to acquire the operant response for food despite continued training. While no differences were seen between BDNF^{Val/Val} and BDNF^{Val/Met} mice with either variable, BDNF^{Met/Met} mice performed significantly fewer active responses (Fig. 1; two-way repeated measures ANOVA, effect of genotype: $F(2, 441) = 4.41$, $p = .017$; Bonferroni post-hoc BDNF^{Met/Met} v. BDNF^{Val/Val} and BDNF^{Val/Met} $p < .05$) and received significantly fewer pellets [Fig. 1; two-way repeated measures ANOVA, effect of genotype: $F(2, 441) = 11.57$, $p = .0001$; interaction: $F(18, 441) = 1.73$, $p = .032$; Bonferroni post-hoc BDNF^{Met/Met} v. BDNF^{Val/Val} and BDNF^{Val/Met} $p < .05$]. The BDNF^{Met/Met} mice were not included in the cocaine or food extinction experiments because they were unable to acquire the operant response for food during training.

Cocaine Self-Administration Behavior

The number of infusions earned, active responses and percent active responding during each cocaine self-administration session is shown in Fig. 2. Both BDNF^{Val/Val} and BDNF^{Val/Met} mice acquired stable cocaine self-administration behavior after 10 sessions. Although BDNF^{Val/Met} mice showed a trend toward slower acquisition on days 1-4, statistical analyses indicated no differences between the groups in the number of infusions earned or active responses performed over the 10 sessions. Additionally, no differences were seen in the percent active responding with both groups stabilizing at ~70% correct (Fig. 2).

Extinction and Reinstatement of Cocaine Seeking

The number of active responses as well as the percent of responding compared to the average responding on the last two days of cocaine self-administration was measured. BDNF^{Val/Met} mice exhibited significantly faster extinction of cocaine responding compared to BDNF^{Val/Val} mice, as measured by both a decrease in active responding during extinction [Fig. 3; two-way repeated measures ANOVA, effect of genotype: $F(1, 198) = 4.46$, $p = .04$; effect of session: $F(9, 198) = 8.81$, $p = .0001$] and a decrease in the percent responding compared to the cocaine self-administration phase [Fig. 3; two-way repeated measures ANOVA, effect of genotype: $F(1, 198) = 24.56$, $p = .0001$; effect of session: $F(9, 198) = 11.73$, $p = .0001$]. Both BDNF^{Val/Val} and BDNF^{Val/Met} mice exhibited significant reinstatement of responding compared to the prior extinction day, however no differences were seen between the genotypes [two-way repeated measures ANOVA, effect of test: $F(1, 17) = 31.42$, $p = .0001$].

Extinction of Food Seeking

The number of active responses as well as the percent of responding compared to the food self-administration phase was measured. BDNF^{Val/Val} and BDNF^{Val/Met} mice exhibited similar rates of extinction of food responding as measured by equivalent active responding during extinction and equivalent percent responding compared to the food self-administration phase [Fig. 4; two-way repeated measures ANOVA, effect of session: $F(9,99) = 15.04, p = .0006$].

Discussion

Brain-derived neurotrophic factor (BDNF) has been implicated in many facets of addiction in preclinical models, including cocaine conditioned reward (Horger *et al.*, 1999; Lobo *et al.*, 2010), cocaine craving (Grimm *et al.*, 2003), cocaine reinforcement (Sadri-Vakili *et al.*, 2010), and cocaine reinstatement (Berglind *et al.*, 2007). Variants in the BDNF gene have also been implicated in vulnerability to addiction in human addicts (Krebs *et al.*, 2000; Uhl *et al.*, 2001; Tsai, 2007). Using a mouse model system that contains the human SNP in the BDNF gene that leads to a valine (Val) to methionine (Met) substitution at codon 66 (Val66Met), the present study provides several new insights into phenotypes associated with the variant BDNF^{Met}. First, we provide evidence for learning impairment in that BDNF^{Met/Met} mice failed to acquire food self-administration. This learning impairment was specific to the Met homozygotes, as there was no detectable learning deficit in the BDNF^{Val/Met} mice. Second, when examining acquisition and stability of cocaine self-administration behavior, we found no differences between BDNF^{Val/Val} and BDNF^{Val/Met} mice. However, BDNF^{Val/Met} mice exhibited more rapid extinction of cocaine responding compared to BDNF^{Val/Val} controls. This is in direct contrast to the impaired fear extinction behavior seen in both human and mouse BDNF Met carriers (Soliman *et al.*, 2010). Furthermore, there was no difference between the genotypes in extinction of food seeking. Since all mice were food trained prior to cocaine self-administration, it seems unlikely that the cocaine effects resulted from alterations in extinction of food responding during the initial cocaine self-administration sessions. Additionally, this suggests that the molecular mechanisms underlying appetitive and aversive fear extinction are at least somewhat distinct since BDNF appears to play different role in these two forms of learning.

Role of BDNF Val66Met Polymorphism in Operant Learning

The growth factor, BDNF, is the most abundant neurotrophin in the mammalian central nervous system and is involved in mediating synaptic plasticity associated with various forms of learning (Patterson *et al.*, 1996; Mu *et al.*, 1999; Mizuno *et al.*, 2000; Barco *et al.*, 2005). BDNF plays a critical role in hippocampus-dependent spatial learning (Tyler *et al.*, 2002) as well as amygdala-dependent Pavlovian fear conditioning (Rattiner *et al.*, 2004; Ou & Gean, 2006); however, its role in operant learning is less clear. While learning an operant task, BDNF mRNA levels increase in the medial prefrontal cortex (Rapanelli *et al.*, 2010). Despite this, infusions of BDNF into this brain region do not alter established food seeking behavior (Berglind *et al.*, 2007). BDNF function in the nucleus accumbens, while clearly involved in addiction, does not seem to be related to operant learning in that the increases in accumbens BDNF seen following cocaine self-administration are not seen following food self-administration (Grimm *et al.*, 2003). Furthermore, infusions into the nucleus accumbens of either BDNF or an antibody to BDNF had no effect on established operant responding for food (Graham *et al.*, 2007). Additionally, knockdown of TrkB, the primary receptor for BDNF, in the nucleus accumbens had no effect on the acquisition of operant behavior for either sucrose or cocaine (Graham *et al.*, 2009). In contrast, infusions of BDNF into the dorsomedial striatum led to a decrease in established sucrose self-administration (Jeanblanc *et al.*, 2009). However, indirectly decreasing BDNF levels within the striatum, through

knockdown of the transcription factor MeCP2, or directly manipulating BDNF through infusions of a BDNF antibody, leads to a decrease in cocaine intake during extended access cocaine self-administration (Im *et al.*, 2010). Furthermore, viral overexpression of BDNF in the striatum increases cocaine intake during extended access self-administration, but not during limited access paradigms (Im *et al.*, 2010). This suggests that the role of BDNF within operant behavior in the striatum is quite complex and may depend not only on the reinforcer but also on the training regimen.

Although no studies have specifically examined instrumental learning, the BDNF Val66Met polymorphism has been studied clinically in other forms of learning. BDNF Met carriers exhibit deficits in episodic memory (Egan *et al.*, 2003; Dempster *et al.*, 2005), recognition memory (Hariri *et al.*, 2003; Goldberg *et al.*, 2008) and spatial navigation (Banner *et al.*, 2011). Furthermore, these deficits in memory performance are accompanied by a decrease in hippocampal activity in BDNF Met carriers during the performance of memory tasks (Hariri *et al.*, 2003; Hashimoto *et al.*, 2008; Banner *et al.*, 2011). The results of the current study clearly indicate that the deficits in learning seen in BDNF Met carriers may extend beyond tasks dependent upon the hippocampus and demonstrate a need for examining the role of this BDNF gene variant in striatal-dependent learning.

BDNF Val66Met Polymorphism Plays Distinct Roles in Different Forms of Extinction Learning

Recent work demonstrated a role for the BDNF Val66Met polymorphism in aversive extinction learning. Using the same mouse strain utilized in the current studies, the Met allele was associated with both increased anxiety-related behaviors (Chen *et al.*, 2006) and impairment of conditioned fear and conditioned taste aversion extinction (Yu *et al.*, 2009; Soliman *et al.*, 2010). This deficit in extinction learning has also been documented in human BDNF Met carriers (Soliman *et al.*, 2010). In contrast, the current study demonstrated facilitation of drug-related extinction learning in BDNF^{Val/Met} mice compared to wildtype controls. This suggests that the role of BDNF in these two forms of extinction is distinct.

Understanding the role that BDNF may play in these two phenomena may require a better understanding of how the BDNF Val66Met polymorphism affects the levels of BDNF in different brain regions and how BDNF in different brain regions may play diverse roles in appetitive and aversive extinction learning. Knockdown of BDNF within the hippocampus or the amygdala leads to impaired aversive extinction behavior, suggesting that BDNF is necessary in these regions for normal extinction (Chhatwal *et al.*, 2006; Heldt *et al.*, 2007). Furthermore, infusions of BDNF into the infralimbic cortex, following fear conditioning, mimic the effects of extinction training (Peters *et al.*, 2010). These findings are all consistent with the idea that it is a decrease of regulated BDNF that is mediating the impaired aversive extinction behavior seen in the BDNF Met carriers (Soliman *et al.*, 2010).

The effects of BDNF on the extinction of appetitive learning are less clear. Similarly to what is seen with aversive extinction, infusions of BDNF into the prefrontal cortex lead to facilitation of extinction of cocaine seeking (Berglind *et al.*, 2007). However, an infusion of BDNF into the ventral tegmental area blocks extinction of cocaine seeking (Lu *et al.*, 2004). These results suggest that a decrease in BDNF may lead to a decrease in cocaine craving, which is consistent with other preclinical data. During withdrawal from cocaine self-administration, BDNF levels within the ventral tegmental area increase, and this increase has been correlated with levels of cocaine craving (Grimm *et al.*, 2003). Furthermore, knockdown of BDNF within either the VTA or the nucleus accumbens leads a decrease in cocaine-conditioned reward (Graham *et al.*, 2009). Taken together, these data indicate that the decrease in regulated BDNF secretion within the VTA of BDNF Met mice may

predominate over any other alterations in BDNF and thus lead to facilitation of the extinction of cocaine seeking behavior.

Although there is some overlap in the brain regions thought to mediate aversive and appetitive extinction (Peters *et al.*, 2009), it is apparent that the exact mechanisms underlying these forms of learning are not identical. Just as BDNF seems to play opposing roles in aversive and appetitive extinction, other signaling systems also lead to discriminate effects in these two phenomena. For example, while the cannabinoid receptor antagonist, rimonabant, blocks fear extinction (Chhatwal *et al.*, 2009); it facilitates the extinction of operant responding for food or cocaine (Ward *et al.*, 2009). Similarly, the alpha-adrenergic receptor antagonist, prazosin, inhibits fear extinction while having no effect on appetitive extinction (Bernardi & Lattal, 2010). However, the administration of a partial NMDA agonist, a drug that arguably has more widespread effects than either rimonabant or prazosin, facilitates both aversive and appetitive extinction (Ressler *et al.*, 2004; Parnas *et al.*, 2005; Botreau *et al.*, 2006; Davis *et al.*, 2006; Shaw *et al.*, 2009; Myers & Carlezon, 2010a; Thanos *et al.*, 2011a; Thanos *et al.*, 2011b). These studies, along with inactivation studies (Morgan *et al.*, 1993; Morgan & LeDoux, 1995; Peters *et al.*, 2008), suggest that there is some overlap between the circuits mediating these two types of extinction learning but the underlying molecular mechanisms differ.

Furthermore, the mechanisms underlying different types of appetitive extinction may also be distinct. The facilitation of drug-related extinction behavior seen in the BDNF^{Val/Met} mice in the current study did not extend to all forms of appetitive learning, as no effect of the BDNF SNP was seen in the extinction of food seeking behavior. This is consistent with the literature demonstrating a clear role for BDNF in cocaine seeking that is distinct from food seeking. For example, increases in BDNF within the ventral tegmental area, nucleus accumbens and amygdala are seen following withdrawal from cocaine seeking but not from sucrose self-administration (Grimm *et al.*, 2003). Furthermore, manipulations of BDNF that alter cocaine seeking do not have any effect on food seeking behavior (Berglind *et al.*, 2007).

Implications for Clinical Populations

In recent years, BDNF gene variants, most commonly the Val66Met SNP examined in the current study, have been implicated in a wide range of psychiatric disorders. BDNF Met carriers are at a higher risk for depression (Schumacher *et al.*, 2005; Hwang *et al.*, 2006; Verhagen *et al.*, 2010), aggressive forms of schizophrenia (Spalletta *et al.*, 2010), severe depressive episodes in bipolar depression (Hosang *et al.*, 2010) and anxiety-related traits (Montag *et al.*, 2010). Furthermore, BDNF Met carriers have a higher susceptibility to stress, which may contribute to the effect of this genotype on depression and anxiety (Shalev *et al.*, 2009; Alexander *et al.*, 2010). In contrast, the current study found that BDNF^{Val/Met} mice exhibit an increased rate of extinction of cocaine responding. Despite this increased rate of extinction, BDNF^{Val/Met} mice exhibited equivalent levels of cue-induced reinstatement. As extinction is believed to be a new form of learning that is dependent upon the ventral prefrontal cortex, whereas cue-induced reinstatement depends on projections from the amygdala to the dorsal prefrontal cortex. Therefore, our data suggest that BDNF Met carriers may have an increase in the ability to form new associations. In support of this hypothesis, human studies of BDNF Met carriers have demonstrated enhanced behavioral flexibility in memory based switching task as well as enhanced response inhibition (Beste *et al.*, 2010; Gajewski *et al.*, 2011). Although BDNF Met carriers clearly exhibit some learning deficits, our study, as well as these human studies, suggest that this SNP can also confer an advantage in some cognitive domains.

Conclusion

Through the use of a mouse model system, we determined that the variant BDNF^{Met/Met} leads to impaired instrumental learning. This abnormality is not seen in BDNF^{Met/Val} heterozygotes suggesting that there may be a threshold level of activity-dependent BDNF release that is needed for learning in this task and only the BDNF^{Met/Met} mice are below this threshold. Furthermore, we demonstrate that BDNF^{Met/Val} heterozygotes exhibit a facilitation of extinction of cocaine seeking. This is in contrast to previous findings demonstrating impairment in extinction of aversive learning (Chen *et al.*, 2006; Soliman *et al.*, 2010). To our knowledge, this is the first evidence that a functionally relevant polymorphism can lead to opposing behavioral phenotypes in these two types of extinction learning. These findings provide the basis for further studies to determine whether human BDNF Met carriers exhibit a similar disparity in behavioral extinction phenotypes.

Acknowledgments

We thank Ted Huang, Blake Kimmey and Jennifer Xue for technical assistance. Research supported by NIDA F32 DA026660 (LB), R01DA15214 (RCP), K02 DA18678 (RCP), R01 DA-011649 (JAB) and NINDS R01 NS052819 (FSL). The authors report no biomedical financial interests or potential conflicts of interest.

References

- Alexander N, Osinsky R, Schmitz A, Mueller E, Kuepper Y, Hennig J. The BDNF Val66Met polymorphism affects HPA-axis reactivity to acute stress. *Psychoneuroendocrinology*. 2010; 35:949–953. [PubMed: 20079575]
- Banner H, Bhat V, Etchamendy N, Joobor R, Bohbot VD. The brain-derived neurotrophic factor Val66Met polymorphism is associated with reduced functional magnetic resonance imaging activity in the hippocampus and increased use of caudate nucleus-dependent strategies in a human virtual navigation task. *Eur J Neurosci*. 2011; 33:968–977. [PubMed: 21255124]
- Barco A, Patterson SL, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER. Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. *Neuron*. 2005; 48:123–137. [PubMed: 16202713]
- Berglind WJ, See RE, Fuchs RA, Ghee SM, Whitfield TW Jr, Miller SW, McGinty JF. A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. *Eur J Neurosci*. 2007; 26:757–766. [PubMed: 17651427]
- Bernardi RE, Lattal KM. A role for alpha-adrenergic receptors in extinction of conditioned fear and cocaine conditioned place preference. *Behav Neurosci*. 2010; 124:204–210. [PubMed: 20364880]
- Beste C, Baune BT, Domschke K, Falkenstein M, Konrad C. Paradoxical association of the brain-derived-neurotrophic-factor val66met genotype with response inhibition. *Neuroscience*. 2010; 166:178–184. [PubMed: 20034542]
- Botreau F, Paolone G, Stewart J. d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference. *Behav Brain Res*. 2006; 172:173–178. [PubMed: 16769132]
- Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*. 2006; 314:140–143. [PubMed: 17023662]
- Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, Tsai SJ. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. *Brain Res Mol Brain Res*. 2005; 140:86–90. [PubMed: 16109452]
- Chhatwal JP, Gutman AR, Maguschak KA, Bowser ME, Yang Y, Davis M, Ressler KJ. Functional interactions between endocannabinoid and CCK neurotransmitter systems may be critical for extinction learning. *Neuropsychopharmacology*. 2009; 34:509–521. [PubMed: 18580872]
- Chhatwal JP, Stanek-Rattiner L, Davis M, Ressler KJ. Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nat Neurosci*. 2006; 9:870–872. [PubMed: 16783370]

- Colzato LS, Van der Does AJ, Kouwenhoven C, Elzinga BM, Hommel B. BDNF Val(66)Met polymorphism is associated with higher anticipatory cortisol stress response, anxiety, and alcohol consumption in healthy adults. *Psychoneuroendocrinology*. 2011
- Davis M, Ressler K, Rothbaum BO, Richardson R. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. *Biol Psychiatry*. 2006; 60:369–375. [PubMed: 16919524]
- Dempster E, Touloupoulou T, McDonald C, Bramon E, Walshe M, Filbey F, Wickham H, Sham PC, Murray RM, Collier DA. Association between BDNF val66 met genotype and episodic memory. *Am J Med Genet B Neuropsychiatr Genet*. 2005; 134B:73–75. [PubMed: 15719396]
- Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*. 2003; 112:257–269. [PubMed: 12553913]
- Gajewski PD, Hengstler JG, Golka K, Falkenstein M, Beste C. The Met allele of the BDNF Val66Met polymorphism enhances task switching in elderly. *Neurobiol Aging*. 2011; 32:2327, e2327–2319. [PubMed: 21803453]
- Goldberg TE, Iudicello J, Russo C, Elvevag B, Straub R, Egan MF, Weinberger DR. BDNF Val66Met polymorphism significantly affects d' in verbal recognition memory at short and long delays. *Biol Psychol*. 2008; 77:20–24. [PubMed: 17988784]
- Goodman LJ, Valverde J, Lim F, Geschwind MD, Federoff HJ, Geller AI, Hefti F. Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Mol Cell Neurosci*. 1996; 7:222–238. [PubMed: 8726105]
- Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci*. 2007; 10:1029–1037. [PubMed: 17618281]
- Graham DL, Krishnan V, Larson EB, Graham A, Edwards S, Bachtell RK, Simmons D, Gent LM, Berton O, Bolanos CA, DiLeone RJ, Parada LF, Nestler EJ, Self DW. Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. *Biol Psychiatry*. 2009; 65:696–701. [PubMed: 18990365]
- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci*. 2003; 23:742–747. [PubMed: 12574402]
- Haile CN, Kosten TR, Kosten TA. Pharmacogenetic treatments for drug addiction: cocaine, amphetamine and methamphetamine. *Am J Drug Alcohol Abuse*. 2009; 35:161–177. [PubMed: 19462300]
- Hall FS, Drongova J, Goeb M, Uhl GR. Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. *Neuropsychopharmacology*. 2003; 28:1485–1490. [PubMed: 12784114]
- Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci*. 2003; 23:6690–6694. [PubMed: 12890761]
- Hashimoto R, Moriguchi Y, Yamashita F, Mori T, Nemoto K, Okada T, Hori H, Noguchi H, Kunugi H, Ohnishi T. Dose-dependent effect of the Val66Met polymorphism of the brain-derived neurotrophic factor gene on memory-related hippocampal activity. *Neurosci Res*. 2008; 61:360–367. [PubMed: 18501457]
- Heather N, Stallard A, Tebbutt J. Importance of substance cues in relapse among heroin users: comparison of two methods of investigation. *Addict Behav*. 1991; 16:41–49. [PubMed: 2048457]
- Heldt SA, Stanek L, Chhatwal JP, Ressler KJ. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol Psychiatry*. 2007; 12:656–670. [PubMed: 17264839]
- Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, Taylor JR. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci*. 1999; 19:4110–4122. [PubMed: 10234039]

- Hosang GM, Uher R, Keers R, Cohen-Woods S, Craig I, Korszun A, Perry J, Tozzi F, Muglia P, McGuffin P, Farmer AE. Stressful life events and the brain-derived neurotrophic factor gene in bipolar disorder. *J Affect Disord.* 2010; 125:345–349. [PubMed: 20172611]
- Hwang JP, Tsai SJ, Hong CJ, Yang CH, Lirng JF, Yang YM. The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. *Neurobiol Aging.* 2006; 27:1834–1837. [PubMed: 16343697]
- Im HI, Hollander JA, Bali P, Kenny PJ. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat Neurosci.* 2010; 13:1120–1127. [PubMed: 20711185]
- Jeanblanc J, He DY, Carnicella S, Kharazia V, Janak PH, Ron D. Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. *J Neurosci.* 2009; 29:13494–13502. [PubMed: 19864562]
- Kalivas PW, O'Brien C. Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology.* 2008; 33:166–180. [PubMed: 17805308]
- Krebs MO, Guillin O, Bourdell MC, Schwartz JC, Olie JP, Poirier MF, Sokoloff P. Brain derived neurotrophic factor (BDNF) gene variants association with age at onset and therapeutic response in schizophrenia. *Mol Psychiatry.* 2000; 5:558–562. [PubMed: 11032392]
- Lang UE, Sander T, Lohoff FW, Hellweg R, Bajbouj M, Winterer G, Gallinat J. Association of the met66 allele of brain-derived neurotrophic factor (BDNF) with smoking. *Psychopharmacology (Berl).* 2007; 190:433–439. [PubMed: 17186223]
- Liu QR, Walther D, Drgon T, Polesskaya O, Lesnick TG, Strain KJ, de Andrade M, Bower JH, Maraganore DM, Uhl GR. Human brain derived neurotrophic factor (BDNF) genes, splicing patterns, and assessments of associations with substance abuse and Parkinson's Disease. *Am J Med Genet B Neuropsychiatr Genet.* 2005; 134B:93–103. [PubMed: 15666411]
- Lobo MK, Covington HE 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ. Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science.* 2010; 330:385–390. [PubMed: 20947769]
- Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. *J Neurosci.* 2004; 24:1604–1611. [PubMed: 14973246]
- 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. *J Neurosci.* 2000; 20:7116–7121. [PubMed: 10995859]
- Montag C, Basten U, Stelzel C, Fiebach CJ, Reuter M. The BDNF Val66Met polymorphism and smoking. *Neurosci Lett.* 2008; 442:30–33. [PubMed: 18602452]
- Montag C, Basten U, Stelzel C, Fiebach CJ, Reuter M. The BDNF Val66Met polymorphism and anxiety: support for animal knock-in studies from a genetic association study in humans. *Psychiatry Res.* 2010; 179:86–90. [PubMed: 20478625]
- Morgan MA, LeDoux JE. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav Neurosci.* 1995; 109:681–688. [PubMed: 7576212]
- Morgan MA, Romanski LM, LeDoux JE. Extinction of emotional learning: contribution of medial prefrontal cortex. *Neurosci Lett.* 1993; 163:109–113. [PubMed: 8295722]
- Mu JS, Li WP, Yao ZB, Zhou XF. Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res.* 1999; 835:259–265. [PubMed: 10415381]
- Myers KM, Carlezon WA Jr. D-cycloserine facilitates extinction of naloxone-induced conditioned place aversion in morphine-dependent rats. *Biol Psychiatry.* 2010a; 67:85–87. [PubMed: 19782965]
- Myers KM, Carlezon WA Jr. Extinction of drug- and withdrawal-paired cues in animal models: relevance to the treatment of addiction. *Neurosci Biobehav Rev.* 2010b; 35:285–302. [PubMed: 20109490]

- O'Brien C. Addiction and dependence in DSM-V. *Addiction*. 2011; 106:866–867. [PubMed: 21477226]
- Ou LC, Gean PW. Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology*. 2006; 31:287–296. [PubMed: 16034442]
- Parnas AS, Weber M, Richardson R. Effects of multiple exposures to D-cycloserine on extinction of conditioned fear in rats. *Neurobiol Learn Mem*. 2005; 83:224–231. [PubMed: 15820858]
- 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; 16:1137–1145. [PubMed: 8663990]
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of fear extinction with hippocampal-infralimbic BDNF. *Science*. 2010; 328:1288–1290. [PubMed: 20522777]
- Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem*. 2009; 16:279–288. [PubMed: 19380710]
- Peters J, LaLumiere RT, Kalivas PW. Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *J Neurosci*. 2008; 28:6046–6053. [PubMed: 18524910]
- Pierce RC, Bari AA. The role of neurotrophic factors in psychostimulant-induced behavioral and neuronal plasticity. *Rev Neurosci*. 2001; 12:95–110. [PubMed: 11392459]
- Rapanelli M, Lew SE, Frick LR, Zanutto BS. Plasticity in the rat prefrontal cortex: linking gene expression and an operant learning with a computational theory. *PLoS One*. 2010; 5:e8656. [PubMed: 20111591]
- Rattiner LM, Davis M, French CT, Ressler KJ. Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *J Neurosci*. 2004; 24:4796–4806. [PubMed: 15152040]
- Reichel CM, Moussawi K, Do PH, Kalivas PW, See RE. Chronic N-acetylcysteine during abstinence or extinction after cocaine self-administration produces enduring reductions in drug seeking. *J Pharmacol Exp Ther*. 2011; 337:487–493. [PubMed: 21303920]
- Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, Davis M. Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry*. 2004; 61:1136–1144. [PubMed: 15520361]
- Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM, Overland RP, Xia E, Bass CE, Terwilliger EF, Pierce RC, Cha JH. Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. *J Neurosci*. 2010; 30:11735–11744. [PubMed: 20810894]
- Schumacher J, Jamra RA, Becker T, Ohlraun S, Klopp N, Binder EB, Schulze TG, Deschner M, Schmal C, Hofels S, Zobel A, Illig T, Propping P, Holsboer F, Rietschel M, Nothen MM, Cichon S. Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biol Psychiatry*. 2005; 58:307–314. [PubMed: 16005437]
- Shalev I, Lerer E, Israel S, Uzefovsky F, Gritsenko I, Mankuta D, Ebstein RP, Kaitz M. BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. *Psychoneuroendocrinology*. 2009; 34:382–388. [PubMed: 18990498]
- Shaw D, Norwood K, Sharp K, Quigley L, McGovern SF, Leslie JC. Facilitation of extinction of operant behaviour in mice by D-cycloserine. *Psychopharmacology (Berl)*. 2009; 202:397–402. [PubMed: 18781295]
- Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, Jing D, Tottenham N, Amso D, Somerville LH, Voss HU, Glover G, Ballon DJ, Liston C, Teslovich T, Van Kempen T, Lee FS, Casey BJ. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science*. 2010; 327:863–866. [PubMed: 20075215]
- Spalletta G, Morris DW, Angelucci F, Rubino IA, Spoletini I, Bria P, Martinotti G, Siracusano A, Bonaviri G, Bernardini S, Caltagirone C, Bossu P, Donohoe G, Gill M, Corvin AP. BDNF Val66Met polymorphism is associated with aggressive behavior in schizophrenia. *Eur Psychiatry*. 2010; 25:311–313. [PubMed: 20430595]

- Thanos PK, Bermeo C, Wang GJ, Volkow ND. D-cycloserine facilitates extinction of cocaine self-administration in rats. *Synapse*. 2011a
- Thanos PK, Subrizi M, Lui W, Puca Z, Ananth M, Michaelides M, Wang GJ, Volkow ND. D-cycloserine facilitates extinction of cocaine self-administration in c57 mice. *Synapse*. 2011b
- Thomsen M, Caine SB. Intravenous drug self-administration in mice: practical considerations. *Behav Genet*. 2007; 37:101–118. [PubMed: 17226102]
- Tsai SJ. Increased central brain-derived neurotrophic factor activity could be a risk factor for substance abuse: Implications for treatment. *Med Hypotheses*. 2007; 68:410–414. [PubMed: 16824691]
- Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem*. 2002; 9:224–237. [PubMed: 12359832]
- Uhl GR, Liu QR, Walther D, Hess J, Naiman D. Polysubstance abuse-vulnerability genes: genome scans for association, using 1,004 subjects and 1,494 single-nucleotide polymorphisms. *Am J Hum Genet*. 2001; 69:1290–1300. [PubMed: 11704927]
- Verhagen M, van der Meij A, van Deurzen PA, Janzing JG, Arias-Vasquez A, Buitelaar JK, Franke B. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry*. 2010; 15:260–271. [PubMed: 18852698]
- Ward SJ, Rosenberg M, Dykstra LA, Walker EA. The CB1 antagonist rimonabant (SR141716) blocks cue-induced reinstatement of cocaine seeking and other context and extinction phenomena predictive of relapse. *Drug Alcohol Depend*. 2009; 105:248–255. [PubMed: 19679410]
- Wojnar M, Brower KJ, Strobbe S, Ilgen M, Matsumoto H, Nowosad I, Sliwerska E, Burmeister M. Association between Val66Met brain-derived neurotrophic factor (BDNF) gene polymorphism and post-treatment relapse in alcohol dependence. *Alcohol Clin Exp Res*. 2009; 33:693–702. [PubMed: 19170664]
- Yu H, Wang Y, Pattwell S, Jing D, Liu T, Zhang Y, Bath KG, Lee FS, Chen ZY. Variant BDNF Val66Met polymorphism affects extinction of conditioned aversive memory. *J Neurosci*. 2009; 29:4056–4064. [PubMed: 19339601]

Figure 1. BDNF^{Met/Met} mice exhibit impaired instrumental learning

While wildtype (BDNF^{Val/Val}) and BDNF^{Val/Met} mice gradually learn to spin the wheel to acquire sucrose pellets, the BDNF^{Met/Met} mice do not. A. BDNF^{Met/Met} mice performed significantly fewer active responses (ANOVA; genotype: $F(2, 441) = 4.41$, $*p = .017$; post-hoc BDNF^{Met/Met} v. BDNF^{Val/Val} and BDNF^{Val/Met} $p < .05$) compared to the wildtype and heterozygous mice. B. BDNF^{Met/Met} mice received significantly fewer pellets than the wildtype and heterozygous mice [ANOVA, genotype: $F(2, 441) = 11.57$, $***p = .0001$; interaction: $F(18, 441) = 1.73$, $p = .032$; post-hoc BDNF^{Met/Met} v. BDNF^{Val/Val} and BDNF^{Val/Met} $p < .05$]. No differences were seen between BDNF^{Val/Val} and BDNF^{Val/Met} mice in either variable.

Figure 2. BDNF^{Val/Val} and BDNF^{Val/Met} mice exhibit equivalent levels of cocaine self-administration

Both wildtype and heterozygous mice acquire cocaine self-administration behavior and no differences are seen between the two genotypes in their active responses (A), infusions earned (B), or percent active responding (C).

Figure 3. BDNF^{Val/Met} mice exhibit a decrease in cocaine seeking under extinction conditions
 A. Although both wildtype (BDNF^{Val/Val}) and BDNF^{Val/Met} mice gradually decrease their active responding during the extinction phase [session: $F(9, 198) = 4.03$, $p < .001$], the BDNF^{Val/Met} mice decrease their responding more rapidly than BDNF^{Val/Val} mice [ANOVA, genotype: $F(1, 198) = 4.46$, $*p = .04$]. B. This is particularly evident when examining the percent responding during extinction compared to the average active responses during the last two days of cocaine self-administration. After ten extinction sessions the BDNF^{Val/Met} mice are only responding at ~20% of their cocaine SA levels, whereas the BDNF^{Val/Val} mice are still responding at around 100% [ANOVA, genotype: $F(1, 198) = 24.56$, $***p = .0001$].

Figure 4. $BDNF^{Val/Val}$ and $BDNF^{Val/Met}$ mice exhibit similar reinstatement of cocaine seeking
Both wildtype and heterozygous mice exhibit significant reinstatement of responding during a cue-induced reinstatement session and are responding at over 125% of their cocaine self-administration levels [ANOVA, test: $F(1,17)=31.42$, $***p=.0001$]. No differences are seen between the two genotypes in their percent responding during reinstatement.

Figure 5. BDNF^{Val/Val} and BDNF^{Val/Met} mice exhibit similar extinction of food seeking
 Both wildtype and heterozygous mice gradually decrease their active responding to the same extent during the extinction phase and are responding at less than 20% of their food self-administration levels and no differences are seen between the two genotypes in their active responses during extinction (A) or their percent responding during extinction compared to the average active responses during the last two days of food self-administration (B).