

Reduced Behavioral Effects of Cocaine in Heterozygous Brain-Derived Neurotrophic Factor (BDNF) Knockout Mice

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Brain-derived neurotrophic factor (BDNF) affects the development of brain neurotransmitter systems, including dopamine and serotonin systems that are important for cocaine's rewarding and locomotor stimulatory properties. Human genomic markers within or near the BDNF locus have been linked to or associated with substance abuse. Post-mortem human brain specimens reveal individual differences in the levels of BDNF mRNA and in mRNA splicing patterns. To assess the effects of lifelong alterations in the levels of BDNF expression on a measure of psychostimulant reward, we have compared locomotor stimulant and rewarding effects of cocaine in heterozygous BDNF knockout mice with effects in their wild-type littermates. Heterozygous BDNF knockout mice displayed less locomotion during habituation and less locomotion after cocaine injections. Cocaine-conditioned place preferences were reduced in the BDNF heterozygotes. These mice displayed no significant difference from saline control values at a dose of 10 mg/kg s.c. cocaine, although they exhibited cocaine-induced preference at a 20 mg/kg dose. These data confirm important roles for BDNF in psychostimulant actions, presumably via neurotrophic effects on dopamine and serotonin systems. Furthermore, these data support suggestions that differences in human BDNF expression may underlie associations between markers near the human BDNF gene locus and drug addiction.

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INTRODUCTION

The psychostimulant cocaine inhibits the reuptake of dopamine, serotonin, and norepinephrine by blocking their respective transporters (DAT, SERT, and NET). The resulting potentiation of neurotransmission, particularly dopaminergic neurotransmission, has been believed to mediate cocaine's rewarding (Kuhar *et al*, 1991) and locomotor stimulant effects (Giros *et al*, 1996). However, dopamine transporter knockout mice exhibit normal or near-normal cocaine reward (Rocha *et al*, 1998; Sora *et al*, 1998); double knockout mice that lack both DAT and SERT lack cocaine reward and cocaine locomotor stimulation (Sora *et al*, 2001) and mice that lack both DAT and one copy of the 5HT1B serotonin receptor gene display normal cocaine-stimulated locomotion (Hall *et al*, submitted), which suggests a much more complicated picture of the substrates of cocaine-induced behavior; both serotonin and dopamine systems appear to be important determinants of

both cocaine reward and cocaine-stimulated locomotion. Genes that influence the development of both of these brain systems are thus especially attractive candidates for variation that might predispose to individual differences in locomotor and addictive processes, especially those triggered by psychostimulants.

Competition for limited quantities of neurotrophic factors, including brain-derived neurotrophic factor (BDNF) help (Oppenheim, 1991) determine the adult complements of specific neuronal populations (see Barde, 1994; Price and Willshaw, 2000 for a review). BDNF can also participate in activity-dependent synaptic plasticity (for a review, see Korte and Bonhoeffer, 1997) likely to contribute to specific neuroprotective and neurotrophic effects that include effects on survival of dopaminergic neurons *in vitro* (Hyman *et al*, 1991; Hyman *et al*, 1994; Son *et al*, 1999).

Human variation at the BDNF locus is therefore a strong candidate to contribute to individual differences in substance abuse vulnerability. Genomic markers within the human BDNF locus have been associated with substance abuse comorbidity in a population of French schizophrenic patients (Krebs *et al*, 2000). Genomic markers that flank the human BDNF locus display different frequencies in each of the two populations of polysubstance abusers compared to ethnically matched controls (Uhl *et al*, 2001). Initial results from RT-PCR amplification of BDNF transcripts from human brain samples indicate that there are substantial human individual quantitative and qualitative differences in

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the levels of BDNF mRNA, in use of alternative transcriptional start sites, and in the patterns of splicing the BDNF primary transcripts produced from these transcriptional start sites (Q-R Liu and GRU, in preparation).

Gene knockout studies of the BDNF locus might be highly informative about the potential influences of BDNF expression on responses to cocaine. Homozygous knockout mice that lack BDNF display substantial abnormalities, morbidity, and mortality. Homozygous BDNF knockouts display profound neuronal loss in regions including cerebellum (Carter *et al*, 2002), olfactory bulb (Linnarsson *et al*, 2000), visual cortex (Huang *et al*, 1999), and cochlea (Wiechers *et al*, 1999) and often die prior to their third postnatal week (Ernfors *et al*, 1994; for a review see Conover and Yancopoulos, 1997). By contrast, heterozygous BDNF knockout mice are viable, display roughly half of wild-type BDNF levels, and thus provide a potentially less confounded model of influences of lifelong variation at this locus for behavioral characterizations. Heterozygote mice display results equivalent to wild-type animals in tests of locomotor activity (Dluzen *et al*, 2001; MacQueen *et al*, 2001), exploration (MacQueen *et al*, 2001), sucrose ingestion (MacQueen *et al*, 2001), anxiety (Montkowski and Holsboer, 1997), forced swimming (MacQueen *et al*, 2001), and spatial learning (Korte *et al*, 1995). Heterozygous BDNF knockout mice do display altered hippocampal long-term potentiation (Korte *et al*, 1995; Montkowski and Holsboer, 1997), subtle alterations in sensory and nociceptive systems (Korte *et al*, 1995; MacQueen *et al*, 2001), modest impairments in beam walking (Dluzen *et al*, 2001), altered learned helplessness responses (MacQueen *et al*, 2001), and reduced cocaine-induced locomotor sensitization (Horger *et al*, 1999). Given these relatively specific and subtle deficits heterozygous BDNF knockout mice provide a reasonable experimental model for behavioral studies.

Monoaminergic systems are subtly altered by reduced BDNF expression in BDNF knockout mice, just as monoamines and monoamine-associated behaviors are influenced by experimental BDNF administration (Siuciak *et al*, 1996; Siuciak *et al*, 1998; Horger *et al*, 1999). While dopamine neuronal counts are similar to wild-type values in developing BDNF homozygous knockouts (Ernfors *et al*, 1994), postnatal differences could well emerge as BDNF mRNA and protein levels increase over the first few weeks of life (Timmusk *et al*, 1994). Some evidence does support this idea of emerging alterations in BDNF heterozygous knockout mice as they age, including alterations in the striatonigral pathway (Jones *et al*, 1994), small increases in striatal tissue dopamine levels, and reduced potassium depolarization-induced release of dopamine from striatal slices prepared from heterozygote brains (Dluzen *et al*, 2001). BDNF modulation of dopaminergic function in knockouts is also supported by the findings that central BDNF infusions enhance cocaine's acute locomotor stimulation, locomotor sensitization that can be observed after repeated cocaine treatments, rates of responding for conditioned reinforcers, and the enhancement of conditioned reinforcement produced by cocaine administration (Horger *et al*, 1999).

In addition to changes in dopaminergic systems in BDNF knockout mice, there are also changes in serotonergic systems. Forebrain 5-HT levels are reduced in older BDNF

heterozygous knockouts (Lyons *et al*, 1999). Microdialysis studies reveal increased extracellular striatal 5-HT levels and evidence of reduced serotonin uptake (Mathews *et al*, 1991). Serotonin receptor levels are affected in BDNF heterozygotes in a region- and subtype-dependent manner (Lyons *et al*, 1999): 5-HT 1B and 5-HT 2A receptors are increased in the cortex and hypothalamus, while 5-HT 2C receptors are increased in the hypothalamus but reduced in the hippocampus. The increase in c-FOS expression induced in wild-type mice by dexfenfluramine is also reduced by heterozygous BDNF knockout (Lyons *et al*, 1999).

We now report the extension of these ideas with the study of baseline and cocaine-stimulated locomotion and reward function in heterozygous BDNF knockout mice. The striking effects that we report here, in contrast to the modest sizes of other subtle sensory and motor alterations noted for these animals, suggest the importance of BDNF for these processes. These results also provide a reference point for the sorts of behavioral differences that human allelic variation at the BDNF locus might confer.

MATERIALS AND METHODS

Animals

Heterozygous and wild-type BDNF mutant mice (Jackson Laboratories, Bar Harbor, ME) were generated by wild-type \times heterozygote crosses. These mice, in which Exon V of the BDNF gene is deleted, resulting in half normal expression of BDNF mRNA, were originally described by Ernfors (Ernfors *et al*, 1994). In these mice the homozygous condition is lethal; therefore, the wild-type \times heterozygote breeding strategy was adopted. Mice were genotyped by PCR using the following primers: BDNF132 GGGAAGCTTCTGACTAGGGG, BDNF133 ATGAAAGAAGTAAACGTCCAC, and BDNF134 CCAGCAGAAAGAGTAGGAG (as described in Dluzen *et al*, 1999). The mice were housed at 24°C with a 12/12-h light-dark cycle and *ad libitum* access to food and water. All experiments were conducted on mice between 12 and 20 weeks of age. Mice were weighed prior to the beginning of the experiments and tested for motor ability in the rotorod test. Subsequently, mice were tested for the behavioral effects of cocaine on reward, in the conditioned place preference paradigm, and then for basal and cocaine-stimulated locomotion.

Conditioned Place Preference

Reward was assessed by conditioned place preference testing using a two compartment Plexiglas chamber (Sora *et al*, 2001). Briefly, one compartment (18 \times 18 \times 18 cm³) had a wire mesh floor (1.3 cm grids) mounted over Plexiglas, and the other compartment (18 \times 18 \times 18 cm) had corncob bedding on a smooth Plexiglas floor. A removable Plexiglas wall separated the two sides. For pre- and postconditioning test sessions, a 5 cm opening in the center wall allowed access to both compartments. During the conditioning sessions the opening was eliminated to restrict animals to a single compartment. Locomotion and time spent in each compartment was recorded using an Optovarimax animal activity monitoring apparatus (Co-

lumbus Instruments, Columbus, OH). Conditioned place preferences were assessed by determination of compartment preference in three phases. Initial preference was determined as the side in which a mouse spent more than 600 s out of a 20 min trial. Conditioning was conducted over a 2-day period in which cocaine hydrochloride (10 mg/kg s.c.) was administered to the animal when it was confined for 20 min in the initially nonpreferred compartment and saline administered to the animal when restricted for 20 min to the initially preferred compartment. Animals received two conditioning sessions per day, counterbalanced between saline and cocaine for a total of two saline pairings and two cocaine pairings. Conditioned place preference assessment followed the last conditioning session and the last injection by 24 h. Time spent in the drug-paired compartment was compared to the preconditioning values obtained on initial assessments.

Locomotor Testing

Locomotor activity was assessed as the total distance traveled. Distance was calculated from measurement of infrared beam breaks by mice placed individually in $46 \times 25 \times 19 \text{ cm}^3$ clear plastic cages in Optovarimax activity monitors (Columbus Instruments, Columbus, OH) under dim light, sound-attenuated conditions. An initial 3 h activity test was conducted under novel conditions. Subsequently mice were tested for cocaine-stimulated activity. After habituation to the apparatus for 1 h mice were injected with cocaine hydrochloride (0, 5, 10, or 20 mg/kg s.c.), and the distance traveled was monitored for an additional 2 h. Doses were tested using a within-subjects design, doses counterbalanced across testing days, and at least 48 h separating each testing day.

Drugs

Cocaine HCl (NIDA IRP Drug Supply Program) was dissolved in saline and administered s.c. in a volume of 1.0 ml/kg.

Statistical Analyses

Weight data were subjected to ANOVA with the between-subjects factors of GENOTYPE (+/+ vs +/-) and SEX (male vs female). Rotorod data were subjected to ANOVA with the between-subjects factor of GENOTYPE and the within-subjects factor of TRIAL.

Conditioned place preference was defined by the difference between the time spent on the initially nonpreferred side postconditioning and the time spent on that side preconditioning. These data were then subjected to ANOVA with the between-subjects factors of GENOTYPE and DOSE.

Baseline locomotor activity data were analyzed by ANOVA with the between-subjects factor of GENOTYPE and the within-subjects factor of TIME, with the data divided into 10-min bins. For cocaine-stimulated locomotion the distance traveled was summed over each test session and analyzed by ANOVA with the between-subjects factor of GENOTYPE and the within-subjects factor of cocaine DOSE. In addition, each individual dose was analyzed in a separate ANOVA with the between-subjects

factor of GENOTYPE and the within-subjects factor of TIME, with the data divided into 10-min bins.

RESULTS

Baseline Weight and Gross Motor Behavior

Mice were weighed prior to the beginning of behavioral experiments. Male BDNF +/+ mice weighed $32.1 \pm 1.5 \text{ g}$, while male BDNF +/- mice weighed $41.9 \pm 1.6 \text{ g}$. A similar increase in weight in females was observed where the female BDNF +/+ mice weighed $26.0 \pm 0.8 \text{ g}$ and the female BDNF +/- mice weighed $31.9 \pm 1.1 \text{ g}$. Thus, in addition to the significant effect of SEX in the ANOVA ($F(1,63) = 34.5$, $p < 0.0001$), there was also a significant effect of GENOTYPE ($F(1,63) = 32.9$, $p < 0.0001$).

In tests of the ability of mice to remain on an accelerating rotorod, there was no effect of GENOTYPE (data not presented). There was no gross ataxia or other marked motor impairment of these mice on observation (data not shown).

Baseline Locomotion

BDNF +/- mice were less active than littermate wild-type BDNF +/+ mice in the first hour of a 3-h locomotor habituation test (Figure 1: $F(17,493) = 1.7$, $p < 0.04$). The difference in locomotion between genotypes was eliminated at time periods following the first hour of within-session habituation.

Cocaine-Induced Locomotion

The reduced locomotion observed in the first hour of baseline locomotor assessments was also observed in the 1-h habituation periods prior to drug injections, but again had been eliminated by the end of the hour of habituation (time-course data not shown). This difference was not attenuated with repeated testing; it was present in each of the four preinjection habituation sessions (Figure 2).

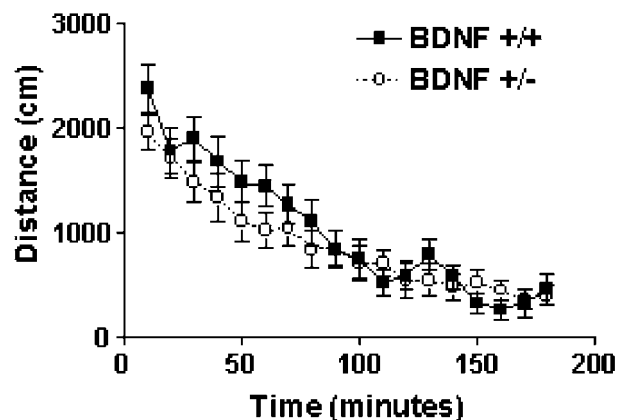


Figure 1 Time course of locomotor activity and habituation to a novel environment in BDNF +/+ and +/- mice. Time course of locomotor activity in BDNF +/+ and +/- expressed in terms of total distance traveled over the 3-h session. Data expressed as mean \pm standard error of the mean.

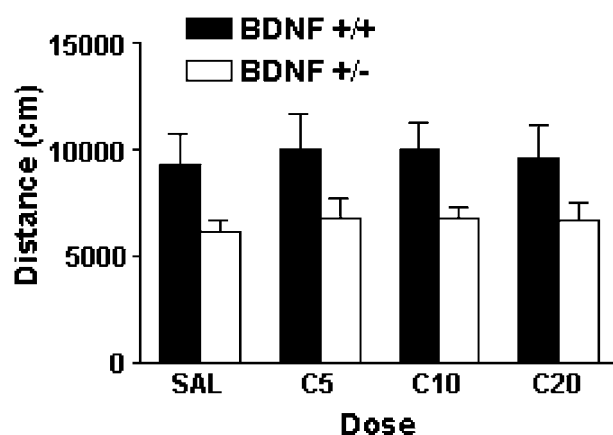


Figure 2 Total baseline locomotor activity in BDNF +/+ and +/- mice for habituation testing prior to cocaine injection. Total locomotor activity in BDNF +/+ and +/- mice expressed in terms of total distance traveled for the four habituation sessions prior to cocaine injection (5, 10, and 20 mg/kg i.p. cocaine; C5, C10, and C20 respectively) or saline (SAL) injection. Data are presented in dose order, which was counterbalanced, not the order of testing. Data expressed as mean \pm standard error of the mean.

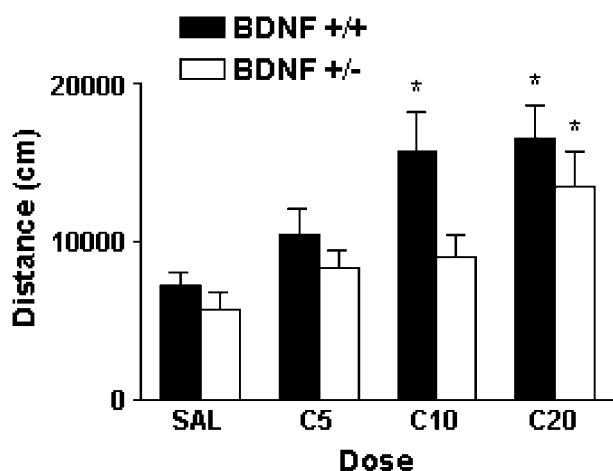


Figure 3 Cocaine-induced locomotor activity in BDNF +/+ and +/- mice. Cocaine-induced locomotor activity in BDNF +/+ and +/- mice expressed as the total distance traveled over 2 h of testing after the injection. Data expressed as mean \pm standard error of the mean. *Significant difference from saline based on Scheffe's *post hoc* comparison ($p < 0.05$).

ANOVA comparing GENOTYPE, HABITUATION, and TIME revealed a significant effect of GENOTYPE ($F(1,29) = 5.1$, $p < 0.04$). By the end of the habituation period there was no difference in activity between genotypes, so that after saline administration there was no statistically significant difference in activity between genotypes (Figure 3).

Both genotypes demonstrated significant dose-dependent effects of cocaine on locomotion (Figure 3; DOSE effect $F(3,87) = 11.3$, $p < 0.0001$). However, cocaine effects were reduced in BDNF +/- mice producing a statistically significant effect of GENOTYPE ($F(1,29) = 4.6$, $p < 0.05$). A 10 mg/kg dose of cocaine was sufficient to elevate locomotion in BDNF +/+ mice, while 20 mg/kg was necessary to increase locomotion in BDNF +/- mice.

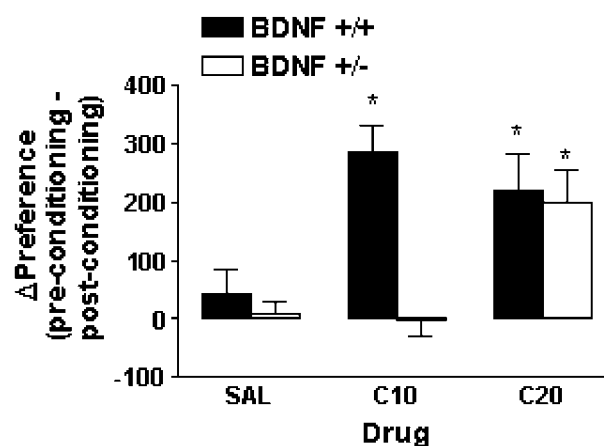


Figure 4 The conditioned place preference for cocaine is reduced in the BDNF +/- mice. Conditioned place preference induced by cocaine (10 and 20 mg/kg, s.c.) in BDNF +/+ and BDNF +/- mice expressed as the difference between the preconditioning preference and the postconditioning preference. BDNF +/+, but not BDNF +/-, mice displayed a significant effect of cocaine relative to saline treatment at the 10 mg/kg dose, while both genotypes displayed a significant difference at the 20 mg/kg dose (* $p < 0.01$ Scheffe's *post hoc* comparison).

Cocaine-Induced Conditioned Place Preference

Cocaine produced a significant place preference (Figure 4; DRUG $F(2,89) = 9.7$, $p < 0.01$). There was an overall significant effect of GENOTYPE on cocaine conditioning ($F(1,89) = 9.4$, $p < 0.01$). The BDNF +/- mice exhibited less preference for an environment previously paired with cocaine. This effect of GENOTYPE interacted with DRUG treatment ($F(2,89) = 6.3$, $p = 0.01$). Wild-type BDNF +/+ mice displayed substantial preferences for cocaine-paired environments at both the 10 and 20 mg/kg doses of cocaine. Strikingly, however, heterozygous BDNF +/- mice failed to show any significant preference for cocaine-paired environment at the 10 mg/kg dose of cocaine and required 20 mg/kg cocaine to produce a place preference equivalent to wild-type levels (Figure 4; *post hoc* Scheffe's comparisons, $p < 0.01$).

DISCUSSION

These striking data can be discussed in relation to their implications for important BDNF roles in cocaine reward and locomotion, the relation between effects on reward and locomotor measures, and the way in which the current results might influence thinking about the role of BDNF variation in human addiction.

The rewarding effects of cocaine assessed in the conditioned place preference paradigm are attenuated in BDNF +/- mice. Cocaine-conditioned place preference was eliminated at the 10 mg/kg dose of cocaine. The re-emergence of near-normal place preference at 20 mg/kg doses in the heterozygotes is consistent with this knockout producing a rightward shift in the dose-response curve for cocaine. The re-emergence of cocaine reward at 20 mg/kg doses suggests that it is unlikely that nonspecific learning deficits account for the findings at the lower cocaine doses, although some behavioral deficits have been documented in BDNF +/- mice (see Introduction).

These changes in the conditioned place preference paradigm were strikingly similar to the effects of BDNF knockout on cocaine-induced locomotion. Locomotor-stimulating effects of cocaine were reduced but not eliminated in BDNF +/– mice with a similar dose–effect profile. Decreases in cocaine efficacy with reduced BDNF expression are consistent with observations that cocaine sensitization is delayed in the BDNF +/– mice and with observations that BDNF administration enhances cocaine sensitization and conditioned reinforcement (Horger *et al*, 1999). These data add to initial suggestions that human allelic variation at a locus in or near the BDNF gene could contribute to individual differences in human addiction vulnerability (Krebs *et al*, 2000; Uhl *et al*, 2001).

The BDNF +/– mice studied here exhibited greater weights than wild-type littermates, as previously reported (Horger *et al*, 1999; Lyons *et al*, 1999; Kernie *et al*, 2000). These mice also exhibited reduced locomotion that has not been found in all locomotor assessments that have been previously reported (Horger *et al*, 1999; MacQueen *et al*, 2001). Previous authors have reported combined data from 12-h test periods (MacQueen *et al*, 2001) without time-course analyses (Horger *et al*, 1999; Kernie *et al*, 2000). Since the locomotor differences observed in our studies were most pronounced at the beginning of each locomotor test, these differences would not be prominent without time-course analyses. In addition, the lack of any motor deficit in the rotorod test indicates that the small locomotor decreases observed here do not represent a gross, generalized motor incapacity.

BDNF affects many brain systems. Despite the plethora of effects that BDNF reduction can have, particularly in homozygous knockouts (Conover and Yancopoulos, 1997), the cumulative behavioral profiles that emerge from the studies of heterozygous BDNF knockout mice reveal relative specificity for the behavioral consequences of reduced BDNF expression. Since combined knockouts of DAT and SERT genes are required to eliminate cocaine reward (Sora *et al*, 2001) and since BDNF effects on both systems have been identified (Hyman *et al*, 1991; Hyman *et al*, 1994; Eaton *et al*, 1995; Mamounas *et al*, 1995; Son *et al*, 1999; Galter and Unsicker, 2000; Zhou and Iacovitti, 2000), the large effects of partial BDNF deletion on cocaine reward mechanisms might also be largely because of actions on both dopaminergic and serotonergic systems. The present experiments thus provide some indirect support for the idea that dopamine and serotonin functions are critical determinants of cocaine reward (Uhl *et al*, 2002). The current results thus identify the BDNF gene, because of its individual variations and its regulatory effects on systems that include both of these monoamine systems, as an important gene for cocaine reward and cocaine addiction.

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