Partial deletion of glial cell line-derived neurotrophic factor (GDNF) in mice: Effects on sucrose reward and striatal GDNF concentrations

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ABSTRACT

Glia cell line derived neurotrophic factor (GDNF) has been reported to alter the reward value of abused substances such as alcohol and cocaine as well as neural circuitry underlying reward. The role of GDNF in reward was further characterized in the present study using operant procedures to determine the value of a natural reward, sucrose, in GDNF heterozygous (GDNF+/−) mice versus wild-type (WT) mice. Female mice were tested for 2 h daily for 10 days in operant chambers with 2 levers. Responses on the correct lever allowed 5-s access to a dipper cup containing 15% sucrose. GDNF+/− mice emitted more responses than WT mice for sucrose, suggesting enhanced reward value of sucrose in these mice. In a separate experiment, concentrations of GDNF protein in striatal tissue were determined at 4, 8, and 12 months of age and found to be 38%–68% lower in GDNF+/− than WT mice at all three ages. Together, the results are consistent with an emerging literature indicating that reduced GDNF levels augment reward and increased GDNF levels attenuate reward, suggesting that GDNF plays an important role in neural systems mediating reward.

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Glia cell line derived neurotrophic factor (GDNF) plays an important role in the development and maintenance of several neurotransmitter systems, including the noradrenergic (Quintero et al., 2004; Zaman et al., 2003), GABAergic (Pozas and Ibanez, 2005), and dopaminergic (Airavaara et al., 2004; Granholm et al., 2000; Oo et al., 2005; Zheng et al., 2005) systems. Dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) are recognized as a critical neural substrate in motivation and reward (Koob, 1992a,b; Robinson and Berridge, 1993, 2001), and additional reports implicate the nigrostriatal dopaminergic projections in reward-related processes (Kantak et al., 2002; O’Doherty et al., 2004; Porrino et al., 2004). Consistent with involvement of GDNF in the development and maintenance of the dopaminergic system, administration of GDNF into the VTA of rats was reported to block cocaine-induced increases in tyrosine hydroxylase immunoreactivity and increases in NMDA receptor expression typically associated with repeated exposure (Messer et al., 2000). In addition, although a single 10 mg/kg dose of cocaine increased...
Fig. 1 – Lever pressing behavior for sucrose reinforcement in female mice heterozygous for GDNF deletion (GDNF+/−; n = 11) and their wild-type littermates (WT; n = 14). GDNF+/− mice exhibited greater lever pressing rates than WT mice (*P < 0.05).

locomotor activity to the same extent in wild-type (WT) and mice heterozygous for GDNF deletion (GDNF+/−) (Airavaara et al., 2004; Messer et al., 2000), repeated exposure to 10 mg/kg cocaine produced greater locomotor sensitization in GDNF−/− mice (Messer et al., 2000). These reports suggest that GDNF influences neuroadaptations in dopaminergic systems in response to abused drugs.

The effects of GDNF on the reward system appear to be reflected in drug-seeking behavior. Central administration of GDNF, either by cell graft (Green-Sadan et al., 2003), osmotic minipump (Green-Sadan et al., 2003), or nanoparticles (Green-Sadan et al., 2005) impeded the acquisition of cocaine administration in rats. In addition, GDNF infused into the VTA reduced lever pressing for ethanol reinforcement in rats (He et al., 2005). Moreover, GDNF+− mice exhibited cocaine place conditioning at a lower dose than their WT counterparts (Messer et al., 2000), indicating that reduced GDNF expression enhances the rewarding effects of cocaine. Thus, it appears that GDNF level is inversely related to the rewarding properties of drugs with elevated and reduced levels of GDNF, respectively related to attenuated and augmented rewarding properties of drugs.

The primary objective of this study was to determine if GDNF partial deletion would augment the rewarding properties of a natural reward as indicated by operant responding for sucrose reinforcement. We hypothesized that the reduction in endogenous GDNF would facilitate the acquisition and the rate of responding for sucrose. A second objective was to determine GDNF protein levels in striatum of GDNF+− and WT mice over a range of ages to establish if previously reported reduction in striatal GDNF of the heterozygotes (Airavaara et al., 2004) was long-lasting and included the age of mice used in the behavioral experiment. We hypothesized that GDNF−/− mice would have less GDNF protein than WT mice at the ages tested.

Animals. Female GDNF+/− and WT littermates born and raised in the AAALAC accredited animal facility at the Medical University of South Carolina were used. Heterozygous mice were used because homozygous GDNF deletion is lethal (Granholm et al., 1997). Genotype was verified at 2 weeks of age (Granholm et al., 1997; Pichel et al., 1996). Mice were maintained on a 12-h light cycle (lights on 0600 h) with free access to water and food, except as noted below. For the behavioral study, mice were 6 months of age and individually housed. For the GDNF concentration measurements, mice were 4, 8, and 12 months of age and were group housed. Behavioral testing and tissue extraction occurred during the light phase of their circadian cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were consistent with NIH guidelines (NIH Publication No. 80-23, revised 1996).

Sucrose self-administration: apparatus and procedure. Mice were tested 2 h daily over a 10-day period in chambers with 2 levers (Griffin and Middaugh, 2003). Each response on the active lever activated a dipper arm (model ENV-302W) for 5 s providing the mouse access to 0.01 ml of 15% sucrose at a port located between the 2 levers. Lever presses produced no reward-related cues except for the sound of the activated dipper arm. Sucrose reward was delivered for each response (Fixed Ratio 1; FR1) throughout the experiment. No shaping procedures were used for lever responses. Acquisition was defined as 3 consecutive days with ≥10 reinforcement deliveries. To encourage lever responding, mice were given a 2.5 g ration of standard laboratory rodent chow as their only food beginning 24 h prior to their first session and after each of the first four sessions. After the 5th session, ad libitum feeding resumed. After the experiment was completed, genotype was confirmed (Granholm et al., 1997; Pichel et al., 1996).

Lever responses for 15% sucrose were greater for GDNF+/− than WT. Fig. 1 indicates that lever responses increased across sessions in both groups and, during later sessions, was greater in the GDNF+− mice. This interpretation is supported by a 2 (Genotype) × 10 (Session) ANOVA with Session as a repeated factor which indicated a significant interaction of the main factors (F(9,207) = 2.6, P = 0.0078). Post hoc analysis using Tukey’s Protected t test confirmed that lever presses were greater for GDNF+− mice than WT mice. This elevated response rate by GDNF+− compared with WT mice for sucrose was not accompanied by genotype-related differences in either the acquisition or the accuracy of responses. The

Fig. 2 – GDNF concentrations in the striatum of WT and GDNF+/− mice at three different ages (n = 8–10). The WT mice had increased GDNF content compared to the GDNF+/− mice (*P < 0.05).
in GDNF+/− concentrations (pg/mg tissue) in striatal tissue were reduced (was not statistically supported by an interaction of the factors across age (68% lower at 4 versus 38% lower at 12 months), this enhanced responding for sucrose by the female GDNF+/− mice in the absence of attenuated accuracy or accuracy of responding suggests that partial deletion of GDNF might be selective for reward processes.

Brain concentrations of GDNF: methods and procedure. Mice were decapitated and the brain rapidly removed. Striatal tissue was block dissected, weighed, and homogenized on ice. GDNF levels were determined, in duplicate, using a commercially available assay kit from Promega® (GDNF Emax Immunoassay Systems) according to our standard protocol (Albeck et al., 2003). Briefly, GDNF is bound by a primary antibody with <3% cross-reactivity with other neurotrophic factors (e.g., NGF, BDNF). The GDNF:primary antibody complex is bound by a secondary antibody conjugated to horseradish peroxidase which utilizes tetramethylbenzidine as a chromogenic substrate. Standard curves, using known amounts of GDNF, are generated on each plate and curve fitting to determine sample concentrations accomplished using (Softmax Pro®, Molecular Devices). Using these kits, GDNF can be quantified in the range of 0.15–1000 pg/ml. In this study, GDNF was expressed as pg/mg tissue.

GDNF concentration in the striatum of GDNF+/− mice is reduced at 4, 8, and 12 months of age. Fig. 2 shows that GDNF protein concentrations (pg/mg tissue) in striatal tissue were reduced in GDNF+/− female mice over the range of ages examined. This observation is supported by a 2 (Genotype) × 3 (Age) ANOVA which indicated a main effect of Genotype (F(1,2) = 13.9, P < 0.0005), but not of Age (F(1,2) = 1.99). Although the extent of the GDNF reduction for GDNF+/− mice appeared to decline across age (68% lower at 4 versus 38% lower at 12 months), this was not statistically supported by an interaction of the factors (F(2,49) < 1). Thus, female GDNF+/− mice, over a 4- to 12-month age range had lower GDNF protein concentrations in the striatum, a result consistent with the reduced GDNF protein in the striatum reported for 10- to 14-week-old heterozygous male mice (Airavaara et al., 2004). Furthermore, the reduction in GDNF across age indicates that potential compensatory changes in GDNF gene product regulation, which could restore GDNF protein to WT levels, do not occur in GDNF+/− mice during the first year of life.

The confirmation that GDNF−/− mice have reduced GDNF content in the dorsal striatum, which has been implicated in reward-related processes (Kantak et al., 2002; O’Doherty et al., 2004; Forrino et al., 2004), together with enhanced responding for a natural reward, provides further evidence for GDNF’s role in brain mechanisms related to reward. The elevated lever pressing for sucrose by GDNF−/− mice in comparison to WT controls in our study is consistent with a report that GDNF+/− mice exhibited cocaine place conditioning (Messer et al., 2000), suggesting that reduced GDNF enhances rewarding properties of drug and natural reinforcers. These results are also consistent with reports that exogenous application of GDNF in wild-type mice reduced cocaine (Green-Sadan et al., 2003, 2005) and ethanol (He et al., 2005) self-administration. Thus, it appears that GDNF protein levels are inversely related to motivation for both drug and natural reinforcers, at least within the levels thus far examined.

The mechanism by which the reduced GDNF protein concentration noted for the GDNF−/− mice exerts its effects on reward processes is unknown; however, evidence suggests the involvement of dopaminergic systems. For example, extracellular dopamine levels in the nucleus accumbens and dorsal striatum of GDNF−/− mice were reported to be 1.5 to 2 times greater than those for WT mice (Airavaara et al., 2004). Consistent with elevated dopamine levels, GDNF−/− mice had elevated levels of DeltaFosB (Airavaara et al., 2004), a transcription factor known to enhance acquisition and maintenance of cocaine self-administration (Colby et al., 2003; McClung and Nestler, 2003; Zhang et al., 2002). Because prior reports indicate that GDNF influences the effects of abused drugs and the present report extends those findings to natural reinforcers, these studies implicate GDNF in neuroadaptations of the dopaminergic system important in reward processes. Furthermore, these findings suggest a role for GDNF in regulating satiety for drug and natural reinforcers.

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