The Effects of Norepinephrine Transporter Inactivation on Locomotor Activity in Mice

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Background: *Acute administration of different classes of antidepressants can enhance or reduce spontaneous locomotor activity in a novel environment, but the effects of chronic antidepressant treatment on spontaneous locomotor activity in novel and familiar environments are less well characterized. Because norepinephrine is an important regulator of spontaneous locomotor activity, we speculated that norepinephrine transporter blockade contributes to the effects of some antidepressants on spontaneous locomotor activity.*

Methods: *Antidepressant drugs (reboxetine, desipramine, imipramine, venlafaxine, bupropion) were administered acutely (intraperitoneal) or chronically (via osmotic minipump) to control and norepinephrine transporter knockout mice, and spontaneous locomotor activity in novel or familiar environments was recorded.*

Results: *Acute treatment with most norepinephrine transporter–blocking antidepressants decreased spontaneous locomotor activity in a novel environment, whereas chronic treatment decreased spontaneous locomotor activity in both novel and familiar environments. The exception was bupropion, a dual norepinephrine transporter/dopamine transporter blocker, which tended to increase spontaneous locomotor activity. Coadministration of reboxetine and the dopamine transporter blocker GBR 12909 also increased spontaneous locomotor activity. Norepinephrine transporter knockout mice had low basal spontaneous locomotor activity, which was increased by bupropion, whereas reboxetine had no effect in norepinephrine transporter knockout mice.*

Conclusions: *Acute or chronic inactivation of the norepinephrine transporter decreases spontaneous locomotor activity in novel and familiar environments unless coupled with dopamine transporter blockade.*

Key Words: Norepinephrine transporter, antidepressant, mice, locomotor activity, chronic, knockout

The monoamines dopamine (DA), serotonin, norepinephrine (NE), and epinephrine (EPI) are important modulators of both psychomotor activity and mood (Brown and Gershon 1993; Fishman et al 1983; Geyer 1996; Ressler and Nemeroff 1999; Stone et al 2003). Alterations of the monoamine systems likely contribute to depression (Brocco et al 2002; Brunello et al 2002; Frazer 2000; Stone et al 2003), which interestingly is often marked by abnormal, usually retarded, psychomotor activity (Caligiuri and Ellwanger 2000; Sachdev and Aniss 1994; Sobin and Sackeim 1997). In addition, some forms of depression in humans and animal models show psychomotor hyperactivity or agitation, which is thought to reflect increased responsiveness to stressful stimuli (Marks et al 1971; Sobin and Sackeim 1997).

The standard pharmacologic treatments for depression block one or more monoamine transporters, increasing extracellular monoamine availability. Although these drugs alleviate such symptoms as anhedonia and despair in both humans and animals, one of their most common, and perhaps debilitating, side effects is sedation and decreased psychomotor activity (Brocco et al 2002; Tucker and File 1986). Currently there is no generally accepted explanation for the phenomenon, and surprisingly few studies have characterized the effects of antidepressants on motor activity. Furthermore, previous animal work has three main limitations: First, many studies have used antidepressants with targets other than monoamine transporters (e.g., desipramine; Rommelspacher et al 1989; Vogel et al 1986). Second, none has used a paradigm that approaches therapeutic conditions (i.e., chronic therapeutic serum drug levels). Third, none has assessed long-term (e.g., 24-hour) locomotor activity during chronic treatment.

Our study focused on NE and norepinephrine transporter (NET) inhibitor antidepressants and was designed to address all three limitations. Using the mouse model, we systematically tested the effects of chronic antidepressant administration on short-term spontaneous locomotor activity (SLA) in a novel environment and long-term (24-hour) in a familiar environment. To address drug specificity, we used the selective NET inhibitor reboxetine, which does not interact with other transporters or receptors (Wong et al 2000), and NET knockout (NET KO) mice, which have a specific deletion of the gene encoding NET. To mimic human antidepressant treatment, we administered reboxetine (RBX) via an osmotic minipump for 18–20 days at a dose that produced therapeutic serum levels of drug. Finally, we systematically tested the chronic effects of four other antidepressant NET inhibitor classes on SLA at therapeutically relevant serum levels: desipramine (DMI; tricyclic NET inhibitor), imipramine (IMI; tricyclic NET and serotonin transporter [SERT] inhibitor), venlafaxine (VEN; selective NET and SERT inhibitor) and bupropion (BUP; selective NET and dopamine transporter [DAT] inhibitor).

Methods and Materials

Animals

For the first round of experiments, dopamine β-hydroxylase heterozygote (*Dbh* +/−) control mice, maintained on a C57BL6/J and 129SvEv background, were bred and used from our (*Dbh* −/−) knockout colony. *Dbh* −/− males were crossed to *Dbh* +/− females, with half the progeny being *Dbh* +/− and the other half being *Dbh* −/−. Originally, we wished to include the analysis of the *Dbh* −/− mice that completely lacked NE, but they did not tolerate the minipump surgeries well and showed

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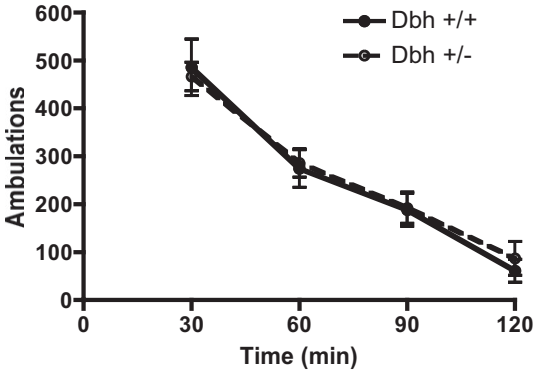

Figure 1. Comparison of SLA in *Dbh* +/+ and *Dbh* +/− mice in a novel environment. Mice were placed in activity chambers, and SLA was recorded for 2 hours. Shown are mean ± SEM ambulations (consecutive beam breaks). SLA, spontaneous locomotor activity.

signs of general malaise, so they were not included. *Dbh* +/− mice have normal NE levels and are indistinguishable from wild-type littermates for all previously tested phenotypes (Thomas et al 1995; Thomas et al 1998; Thomas and Palmiter 1997a; Szot et al 1999), including SLA in a novel (Figure 1) and familiar (Thomas et al 1997b) environment. For the second set of experiments, NET KO and wild-type (WT) control mice, maintained on a pure C57BL6/J background, were generated from NET +/− heterozygote breeders obtained from Marc Caron (Duke University, Durham, North Carolina). Adult male and female mice, 3–7 months old at testing, were used, and all experimental subjects had age-matched control subjects. No sex or age differences were observed and results were combined. The colony room was maintained at 22°C with lights on from 7:00 AM to 7:00 PM throughout the experiment. Food and water were available ad libitum, with animals maintained according to the *Guide for Care and Use of Laboratory Animals* (National Academy of Sciences, Washington, DC). All experiments were approved by the Emory International Animal Care and Use Committee.

Drugs

Drugs used in this study were reboxetine (Pfizer, Groton, Connecticut), desipramine (Sigma-Aldrich, St. Louis, Missouri), imipramine (Sigma-Aldrich), bupropion (Sigma-Aldrich), GBR 12909 (Sigma-Aldrich), and venlafaxine (Wyeth, Monmouth Junction, New Jersey).

Antidepressant Administration

Drugs were administered acutely via intraperitoneal (IP) injection or chronically via Alzet osmotic minipumps (Model #2004, .25 μL/hour, 28 days; Durect, Cupertino, California). For chronic administration, antidepressants were dissolved in either .9% NaCl (reboxetine, 20 mg/kg/day; imipramine, 120 mg/kg/day; venlafaxine, 20 mg/kg/day; bupropion, 40 mg/kg/day) or an aqueous solution containing 50% ethanol and .9% NaCl (desipramine, 20 mg/kg/day), and loaded into pumps. Doses were chosen to achieve serum levels that fell within or very close to the human therapeutic range (Ahern et al 2006). Minipumps containing .9% NaCl or a 50% ethanol/.9% NaCl aqueous solution were used as control vehicles. All pumps were placed in a sterile 37°C saline bath for 1 day before implantation. Mice were anesthetized with isoflurane, and minipumps were implanted in the IP cavity. Buprenorphine (2.5 mg/kg subcutaneous) was

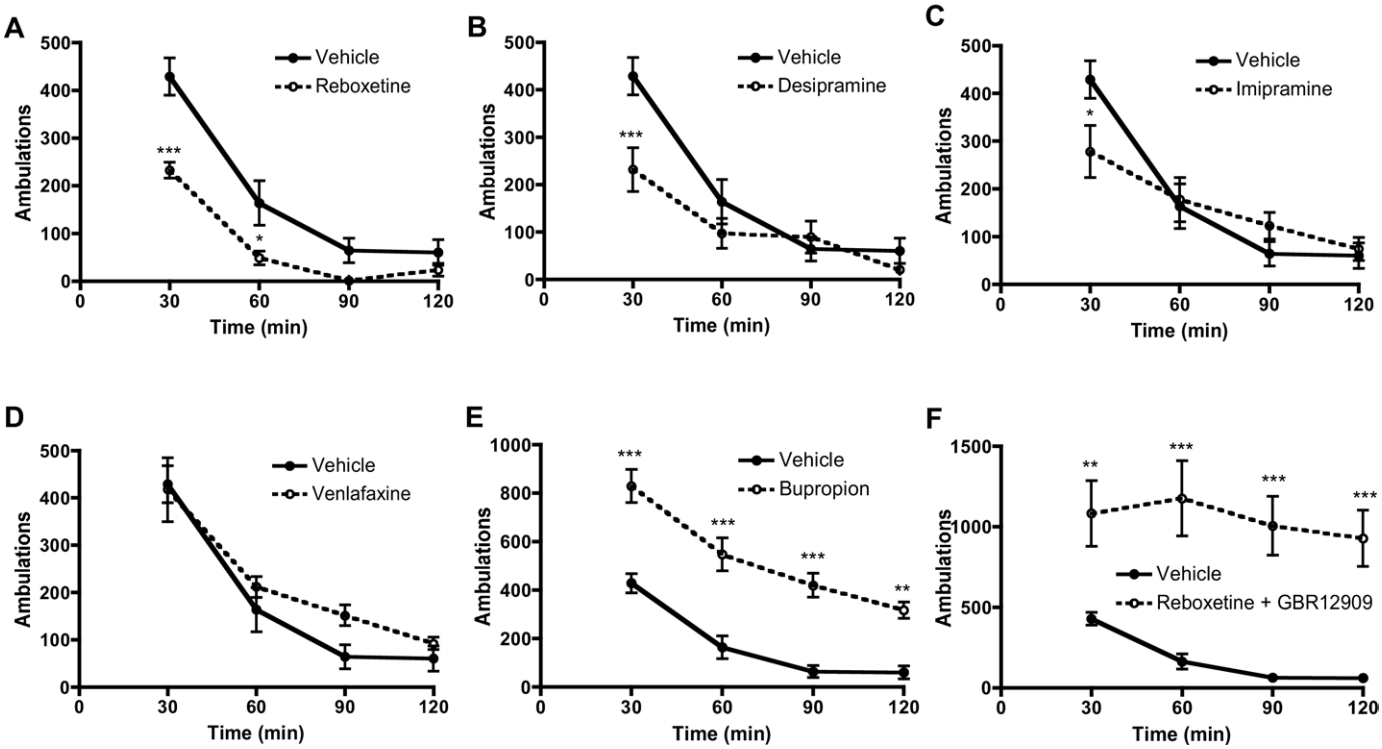

Figure 2. Effects of acute antidepressant drug treatment on SLA in a novel environment. Shown are mean ± SEM ambulations (consecutive beam breaks) of *Dbh* +/− mice administered **(A)** reboxetine (20 mg/kg), **(B)** desipramine (20 mg/kg), **(C)** imipramine (20 mg/kg), **(D)** venlafaxine (20 mg/kg), **(E)** bupropion (40 mg/kg), or **(F)** reboxetine (20 mg/kg) + GBR 12909 (20 mg/kg), compared with vehicle (.9% NaCl), via intraperitoneal injection 30 min before test ($n = 8$ per group; *** $p < .001$, ** $p < .01$, * $p < .05$ compared with vehicle). SLA, spontaneous locomotor activity.