Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors

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Mice unable to synthesize norepinephrine (NE) and epinephrine due to targeted disruption of the dopamine β-hydroxylase gene, Dbh, were used to critically test roles for NE in mediating acute behavioral changes elicited by different classes of antidepressants. To this end, we used the tail suspension test, one of the most widely used paradigms for assessing antidepressant activity and depression-related behaviors in normal and genetically modified mice. Dbh–/– mice failed to respond to the behavioral effects of various antidepressants, including the NE reuptake inhibitors desipramine and reboxetine, the monoamine oxidase inhibitor paroxetine, and the atypical antidepressant bupropion, even though they did not differ in baseline immobility from Dbh+/+ mice, which have normal levels of NE. Surprisingly, the effects of the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, sertraline, and paroxetine were also absent or severely attenuated in the Dbh–/– mice. In contrast, citalopram (the most selective SSRi) was equally effective at reducing immobility in mice with and without NE. Restoration of NE by using L-threo-3,4-dihydroxyphenylserine reinstated the behavioral effects of both desipramine and paroxetine in Dbh–/– mice, thus demonstrating that the reduced sensitivity to antidepressants is related to NE function, as opposed to developmental abnormalities resulting from chronic NE deficiency. Microdialysis studies demonstrated that the ability of fluoxetine to increase hippocampal serotonin was blocked in Dbh–/– mice, whereas citalopram’s effect was only partially attenuated. These data show that NE plays an important role in mediating acute behavioral and neurochemical actions of many antidepressants, including most SSRIs.

Antidepressant medications are thought to elicit their therapeutic effects by increasing synaptic concentrations of the monoamines serotonin (5-HT) and/or norepinephrine (NE) (1). Most tricyclic antidepressants enhance NE and 5-HT transmission because they inhibit presynaptic reuptake, although most of these drugs have affinity for other neurotransmitter receptors that contribute to their side effect profile. More recently, selective 5-HT reuptake inhibitors (SSRIs) have become widely prescribed because of their improved safety profile from limited interaction with other neurotransmitter receptors (1). However, some investigators have recently challenged whether SSRIs produce effects selectively on the serotonergic system over the noradrenergic and dopaminergic systems (2, 3). Indeed, a number of in vivo microdialysis studies have shown that acute (4–6) and chronic (7–9) treatment with certain SSRIs can increase extracellular concentrations of NE in addition to that of 5-HT.

Attempts to delineate the role of NE in the behavioral effects of antidepressants have used inhibition of synthetic enzymes to deplete the neurotransmitter or chemical lesion techniques to destroy NE terminals. However, many depletion agents, such as the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine or the vesicle disruptor reserpine, affect other neurotransmitters as well. Further, incomplete lesions and spraying and increased turnover at surviving terminals can make negative results with depletion studies difficult to interpret (10, 11). For these reasons, attempts to resolve the role of NE in the behavioral effects of antidepressants by using neurotoxin-induced lesions has remained largely controversial and unresolved (12–16).

The generation of mice with a targeted disruption of the gene for dopamine (DA) β-hydroxylase (DBH) provides a genetic model for examining the role of NE in complex physiological and behavioral functions (17–21). NE can also be replaced transiently by administration of L-threo-3,4-dihydroxyphenylserine (L-DOPS), a synthetic precursor of NE which bypasses DBH by means of metabolism by aromatic L-amino acid decarboxylase (22). L-DOPS has been used to reverse behavioral, neurochemical, and physiological phenotypes in the Dbh–/– mice (17–22). Thus, Dbh–/– mice offer a more complete, specific, and readily reversible model for examining the role of adrenergic signaling in the mechanism of antidepressant action. For these reasons, we examined the behavioral effects of antidepressants from several classes in Dbh–/– and littermate control mice. Behavioral effects of antidepressants were studied by using the tail suspension test (TST), a well characterized test sensitive to antidepressants from different pharmacological classes in the mouse (23). This test is among the most widely used in studying antidepressant-related phenotypes in genetically modified mice (24, 25). The results of these studies demonstrate an important role for NE in the acute response to antidepressants from different pharmacological classes, including the SSRIs.

Materials and Methods

Animals. Mice were derived from a hybrid line (129/SvCPJ and C57BL/6J). Dbh–/– and heterozygote (Dbh+/–) mice were bred as described (18) and maintained on a 12-h light/dark cycle (lights on at 06:00 hours) in a specific pathogen-free facility. Food and water were freely available, and mice were maintained according to National Institutes of Health guidelines. All experimental procedures were carried out in accordance with protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Genotype was deduced from phenotype (Dbh–/– mice exhibit delayed growth during adolescence and ptosis), and a subset of mice was confirmed by PCR (17). Dbh–/– mice are indistinguishable from wild-type (Dbh+/+)

Abbreviations: NE, norepinephrine; NET, NE transporter; 5-HT, serotonin; SERT, 5-HT transporter; DA, dopamine; DBH, DA β-hydroxylase; SSRi, selective 5-HT reuptake inhibitor; L-DOPS, L-threo-3,4-dihydroxyphenylserine; TST, tail suspension test; FST, forced swim test.

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mice as to NE and epinephrine levels, as determined in various brain regions ex vivo (22). Correspondingly, in all behavioral tests carried out to date, no difference emerged between Dbh+/− mice and wild-type mice; therefore, Dbh+/− mice were used as controls in all experiments. Adult (3–7 months) male and female littermates of each genotype were evenly distributed to experimental and control groups for each treatment. All behavioral experimental sessions were conducted between 12:00 and 19:00 hours.

The TST. The TST was carried out essentially as described (23). Thirty min after injection (24 h and 30 min in the case of pargyline), mice were individually suspended by the tail to a horizontal ring stand bar (distance from floor = 30 cm) using adhesive tape (distance from tip of tail = 2 cm). Typically, mice demonstrated several escape-oriented behaviors interspersed with temporally increasing bouts of immobility. A 6-min test session was used, which was videotaped. Videotapes were subsequently scored by a trained observer who was unaware of the treatment and genotype. The parameter recorded was the number of seconds spent immobile. A subset of the mice in studies 1 and 2 (i.e., for the catecholaminergic agents, desipramine, reboxetine, pargyline, and bupropion) had undergone the forced swim test (FST) 1 week earlier (20). Parallel studies were undertaken to demonstrate that there was no difference in baseline or in response to these compounds in the TST 1 week after FST when compared with mice that have not undergone FST (data not shown).

Drugs. All drugs were freshly prepared before use and injected i.p. by using a volume of 10 ml/kg. Desipramine HCl (M, 266, Sigma); reboxetine HBr (M, 319, Pharmacia and Upjohn, Kalamazoo, MI); pargyline HCl (M, 159, Sigma) bupropion HCl (M, 240, Research Biochemicals, Natick, MA); fluoxetine HCl (M, 309, Eli Lilly, Indianapolis); citalopram HBr (M, 324, Lundbeck, Copenhagen); and paroxetine HCl (M, 329, Smith-Kline Beecham) were dissolved in deionized H2O and were sonicated mildly. All drug doses were calculated as the base weight and expressed as mg/kg, although molecular masses (M)) are provided for conversion of doses to μmol/kg. Control mice received saline in a corresponding volume of 10 ml/kg except for sertraline-treated mice, which were given saline with a drop of Tween-80. L-DOPS was dissolved at 20 mg/ml in 0.2 M HCl containing 2 mg/ml ascorbic acid. The solution was neutralized with 10 M NaOH just before s.c. injection, which was given 4–6 h before behavioral testing.

Microdialysis. Mice were implanted with custom-constructed microdialysis probes (26) in the ventral hippocampus at ~2.8 mm AP, ± 3.2 mm ML, and −5.0 DV relative to bregma. After surgery, the mice were placed in clear polycarbonate cylinders (21.5 cm in height × 17.5 cm in diameter) and allowed to recover overnight. The probes were continuously perfused at 1.5 μl/min with artificial cerebrospinal fluid (147 mM NaCl/1.7 mM CaCl2/0.9 mM MgCl2/4 mM KCl). Only data from mice with confirmed probes in the ventral hippocampus were used. Mice received an injection of fluoxetine or citalopram (at 20 mg/kg i.p.) after 2 h of baseline sampling. In reverse dialysis experiments, the probes were perfused with artificial cerebrospinal fluid for 2 h of baseline sampling and then the perfusion solution was switched to artificial cerebrospinal fluid containing 1 μM fluoxetine by using a liquid switch selector (UniSwitch Biotechnical Systems, West Lafayette, IN). The amount of 5-HT in the dialysate was determined by HPLC coupled to electrochemical detection (26).

Statistical Analysis. A two-way ANOVA, with drug treatment and genotype as factors, was carried out on absolute immobility in studies examining the effects of various antidepressants on behavior in the TST. A three-way ANOVA was carried out on the data from the L-DOPS experiment by using pretreatment with L-DOPS, drug treatment, and genotype as factors. Any factors that demonstrated overall significant differences were analyzed further by using Fisher’s test of least significant differences. The effects of sertraline on behavior were analyzed alone. The behavioral effects of fluoxetine, paroxetine, and citalopram were investigated in groups of mice treated simultaneously with drug or vehicle, and the data were collated and analyzed together. The effects of drug treatments on extracellular 5-HT levels were determined by mixed two-factor ANOVA, with genotype a between-subjects factor and time analyzed by using repeated measures.

Results

The Effects of Various Antidepressants in the TST. Baseline behavior in the TST was examined after injections of physiological saline (0.9%) into Dbh+/− and littermate control mice 30 min before testing. There were no significant differences of immobility between Dbh+/− and Dbh−/− mice given saline (Fig. 1). Furthermore, there are no differences in spontaneous activity in the home cage between genotypes that would mask a differential response in the TST (S.A.T., unpublished observations).

Desipramine dose-dependently decreased immobility of the control mice in the TST (Fig. 1A). In contrast, there was no significant effect in the Dbh−/− mice. Similarly, the higher dose of reboxetine (20 mg/kg) reduced immobility in the control mice but not in the Dbh−/− mice (Fig. 1B). The atypical antidepressant bupropion (20 mg/kg) and the monoamine oxidase inhibitor pargyline (75 mg/kg × 2) were both effective at reducing immobility in the TST only in Dbh+/− mice (Fig. 1C). Pargyline- and bupropion-treated Dbh−/− mice had significantly greater immobility than did their Dbh+/− counterparts.

The Effects of the SSRIs in the TST. The consequences of the absence of NE on the behavioral effects of a series of different SSRIs were examined. The effects of sertraline (5–20 mg/kg) were tested first (Fig. 1D). Sertraline decreased immobility in control mice in a dose-related fashion, but this effect was completely abolished in the Dbh−/− mice. The behavioral effects of fluoxetine (20–40 mg/kg), paroxetine (5–20 mg/kg), and citalopram (5–20 mg/kg) were studied in different squads of mice run simultaneously, and data from these groups were collated (Fig. 1E). Fluoxetine reduced immobility at both doses tested (20 and 40 mg/kg) in the Dbh−/− mice, whereas it was ineffective at the lower dose and increased immobility at the higher dose in the Dbh+/− mice. Although paroxetine reduced immobility at both doses tested (5 and 20 mg/kg) in the Dbh+/− mice, it was ineffective at the lower dose and reduced immobility only partially at the higher dose in the Dbh−/− mice. In contrast, citalopram reduced immobility with equal effect at both doses (5 and 20 mg/kg) in Dbh+/− and Dbh−/− mice (Fig. 1E). In a separate study, 1 mg/kg citalopram produced a nonsignificant reduction in immobility of 24 and 27 s in the Dbh+/− and Dbh−/− mice, respectively, when compared with saline treatment, and the reductions did not differ significantly between genotypes (data not shown).

The Effects of Dual Reuptake Inhibition in the TST. The greatly diminished behavioral effects in the TST of fluoxetine, sertraline, and paroxetine as compared with citalopram correlate with the selectivity of these compounds for inhibition of 5-HT versus NE reuptake in vitro, with citalopram being the most selective (27). One explanation for the differential effects of these SSRIs could be related to a unique aspect of the Dbh−/− mice. Evidence...
suggests that these mice store and release the neurotransmitter DA from their adrenergic terminals (22). This result is expected because DA is the endogenous precursor of NE, and DA is a substrate for both the NE transporter (NET) and the vesicular monoamine transporters (28). In normal animals, the SSRIs fluoxetine, sertraline, and paroxetine augment extracellular levels of NE (4–7). As a result, these compounds would be expected to augment extracellular levels of DA near adrenergic terminals in Dbh^-/- mice. It is possible that an ectopic rise in extracellular DA could counteract the behavioral effects of an increase in extracellular 5-HT. To test this hypothesis, the effects of the two most selective reuptake inhibitors, citalopram for 5-HT and reboxetine for NE, were examined in the TST when given alone and in combination to control and Dbh^-/- mice (Fig. 2). Post hoc analysis revealed that citalopram, reboxetine, and both drugs in combination reduced immobility in the TST (Fig. 2). The effects of the selective NE reuptake inhibitor reboxetine (10 mg/kg i.p.) and the SSRI citalopram (5 mg/kg i.p.) given individually and in combination on immobility in the TST. An ANOVA revealed a significant interaction between drug treatment and genotype \(F(3, 72) = 4.22, P < 0.01\). n = 9–10 mice per group. * Groups that differed significantly from saline-treated mice of the corresponding genotype tested at the same dose \(P < 0.05\); #, groups differed significantly from the opposite genotype tested at the same dose \(P < 0.05\), according to Fisher’s probable least-squares difference test.

**Reinstatement of Behavioral Effects in the TST.** To further test the idea that the behavioral effects of antidepressants may depend on adrenergic signaling, NE was restored acutely in the Dbh^-/- mice by using L-DOPS. Importantly, acute administration of L-DOPS does not reduce the elevated levels of DA found in the Dbh^-/- mice (22). Restoration of NE allowed the Dbh^-/- mice to respond to both desipramine and paroxetine in the TST (Fig. 3). In an independent replication of effects shown in Fig. 1, desipramine and paroxetine reduced immobility in the Dbh^-/- mice only, with no effect in the Dbh^-/- mice pretreated with vehicle. However, in mice pretreated with L-DOPS, desipramine and paroxetine significantly reduced immobility in Dbh^+/+ and Dbh^-/- mice, and the extent of this reduction was similar between genotypes. Interestingly, Dbh^-/- mice (but not Dbh^+/+ mice) pretreated with L-DOPS had significantly lower baseline immobility values as compared with their vehicle-pretreated counterpart. This observation suggests that the Dbh^-/- mice may have enhanced sensitivity to changes in NE levels as compared with control mice. The ability of L-DOPS to restore sensitivity to desipramine and paroxetine in the Dbh^-/- mice indicates that the effects of these antidepressants are mediated in part by an acute physiological requirement for adrenergic signaling. It also indicates that the lack of an effect of these antidepressants in untreated Dbh^-/- mice is not due to abnormal development or ectopic DA.

**SSRIs and Extracellular Levels of 5-HT.** The role of adrenergic signaling in regulating the effects of SSRIs could be upstream or downstream of the elevation in extracellular 5-HT levels elicited by these compounds. Microdialysis was used to measure the ability of the SSRIs to increase extracellular 5-HT levels in the hippocampus of Dbh^+/+ and Dbh^-/- mice. Acute systemic administration of fluoxetine (20 mg/kg) produced a 3-fold increase in extracellular 5-HT in control mice but caused no increase in the Dbh^-/- mice (Fig. 4A). On the other hand,
The lack of effect of both desipramine and reboxetine on the TST in Dbh<sup>−/−</sup> mice is consistent with the hypotheses that both compounds elicit their effects by increasing synaptic NE concentrations after selective blockade of NET (32). Repletion of NE with the precursor L-DOPS restored the behavioral effects of desipramine. Immobility was also reduced by L-DOPS pretreatment alone in Dbh<sup>−/−</sup> mice, suggesting that mechanisms mediating the effect of L-DOPS in the TST become sensitized with the chronic loss of NE. Furthermore, the lack of any difference in NET messenger RNA, immunoreactivity, and radioligand binding between Dbh<sup>−/−</sup> and Dbh<sup>+/+</sup> mice (20, 33), supports the premise that the inability of noradrenergic antidepressants to produce behavioral effects in Dbh<sup>−/−</sup> mice is not due to a reduction in NET, but rather a loss of the endogenous ligand itself. These findings are consistent with our previous report (20) that Dbh<sup>−/−</sup> mice fail to demonstrate behavioral effects to NE antidepressants in the FST, and are one of the few demonstrations that the selective depletion of NE prevents the behavioral effects of noradrenergic antidepressants (12, 15). This outcome could be because adrenergic lesions are often incomplete (10, 11), or there was a failure to target the adrenergic neurons, such as the ventral NE bundle, that are critically involved (16).

The antidepressant bupropion could mediate its effects through potentiation of DA transmission because it has a higher affinity for the DA transporter than either the NET or 5-HT transporter (SERT) (34). However, bupropion and its metabolite 360U73 inhibit the firing of locus coeruleus noradrenergic neurons at doses much lower than that needed to inhibit DA cell firing (35), and bupropion produces effects on both noradrenergic and serotonergic systems (36). Likewise, the inability of the monoamine oxidase inhibitor pargyline to produce antidepressant-like effects in Dbh<sup>−/−</sup> mice suggests that although pargyline would be expected to increase extracellular levels of NE, 5-HT, and DA, its effects on adrenergic transmission are necessary for the mediation of its behavioral effects in the TST and FST (20). Furthermore, we have previously shown that the lack of behavioral effects of pargyline in Dbh<sup>−/−</sup> mice are not due to any potential adaptive changes in monoamine oxidase activity secondary to chronic absence of NE (20). The results from the studies employing bupropion and pargyline indicate that enhancing dopaminergic and/or serotonergic signaling may not be sufficient to observe behavioral effects in the FST and TST in the absence of NE.

The most surprising finding of the current study is the dramatically blunted behavioral responses to the SSRIs fluoxetine, sertraline, and paroxetine in the Dbh<sup>−/−</sup> mice. Intact 5-HT transmission is required for SSRIs to produce their behavioral effects in the TST and other behavioral models because the behavioral effects are prevented after the depletion of 5-HT with the tryptophan hydroxylase inhibitor p-chlorophenylalanine (refs. 13 and 31 and O.F.O. and I.L., unpublished observations). Although two previous studies (37, 38) in rats reported that lesions of the locus coeruleus did not alter the behavioral effects of fluoxetine or sertraline, the substantial noradrenergic innervation spared by these lesions could have sustained the effects of SSRIs. Further, Soubrie et al. (39) have shown that noradrenergic lesions of the hippocampus, induced by 6-hydroxydopamine, significantly delays the reversal of escape deficits by several antidepressants, including the 5-HT reuptake inhibitor clomipramine, in the learned-helplessness model of depression. Interestingly, the lesion does not affect baseline acquisition of learned helplessness. The present data challenge the assumption that 5-HT regulates the effects of all SSRIs exclusively and demonstrate that these structurally heterogeneous compounds have various requirements for NE to manifest behavioral effects. Previous investigators have questioned the selectivity of a number of

![Fig. 3.](image-url) Restoring NE in Dbh<sup>−/−</sup> mice by means of pretreatment with L-DOPS reinstates behavioral changes induced by desipramine and paroxetine in the TST. There was a significant three-way genotype × drug treatment × L-DOPS pretreatment interaction \( [F(1, 160) = 24.22, P < 0.001] \), according to ANOVA. \( n = 9–20 \) mice per group. * Groups that differed significantly from saline-treated mice of the corresponding genotype \( (P < 0.05); \# \) groups that differed significantly from the opposite genotype tested at the same dose \( (P < 0.05) \); $ groups that differed from relevant drug-treated control \( (P < 0.05) \), according to Fisher’s probable least-squares difference test.

delivering fluoxetine (1 μM) directly to the hippocampus by using reverse microdialysis increased extracellular 5-HT levels by 3-fold in Dbh<sup>+/+</sup> and Dbh<sup>−/−</sup> mice (Fig. 4B). In contrast to fluoxetine, acute systemic administration of citalopram (20 mg/kg) caused a 4-fold increase in extracellular 5-HT in control mice and a 3-fold increase in extracellular 5-HT in Dbh<sup>−/−</sup> mice (Fig. 4C). Thus, the refractory behavioral effects of fluoxetine in Dbh<sup>−/−</sup> mice were recapitulated in the effects of systemic fluoxetine on extracellular 5-HT levels and are probably due to upstream alterations in adrenergic circuitry that impact 5-HT transmission. Although citalopram’s effects were also somewhat diminished in Dbh<sup>−/−</sup> mice, 5-HT levels may be increased sufficiently by this SSRI to produce behavioral effects.

**Discussion**

The identification of specific neurochemical substrates that mediate the behavioral effects of antidepressants is important because it provides insight into neurochemical mechanisms determining their therapeutic actions and strategies for developing more effective treatments. The results of the present studies provide striking evidence that NE is essential for the manifestation of the acute behavioral effects of a variety of antidepressants from different classes.

Interestingly, baseline behavior between mice with and without NE was unaltered in the TST. This finding may be surprising, given the large body of evidence supporting a role for this amine in both the pathology of depression and in the integration of the stress response (29, 30). However, it agrees with our previous data that showed that both genotypes have similar baseline behavior in the FST (20), and with studies using various noradrenergic neurotoxins, which show no difference in baseline performance in tests of acquired immobility (13, 15, 16). It is also of interest that depletion of 5-HT by p-chlorophenylalanine or 5,7-dihydroxytryptamine fails to alter baseline immobility in both the FST and the TST (refs. 13, 16, and 31 and O.F.O. and I.L., unpublished observations). These observations suggest that NE and 5-HT are not necessary for the tonic regulation of baseline performance in these tasks, although it is possible that compensatory mechanisms from other neurotransmitters stabilize the behavioral response. In contrast, NE and 5-HT appear to be necessary for reductions of immobility in these tasks when their levels are augmented by antidepressants.
SSRIs for the serotonergic over the adrenergic system (2–4, 40), especially fluoxetine, sertraline, and paroxetine (1). In addition, a number of in vivo microdialysis studies have shown that acute and/or chronic treatment with the SSRIs fluoxetine, sertraline, and paroxetine results in increased NE levels in various brain regions (4–9). Repletion of NE levels with the NE precursor L-DOPS restored the behavioral effects of paroxetine. The reduced sensitivity to SSRIs in Dbh<sup>+/−</sup> mice is not due to an alteration in SERT binding or kinetics or any other detectable changes in 5-HT or metabolite concentrations measured ex vivo (see supporting information, which is published on the PNAS web site). Therefore, an impact on NE transmission may participate or even be required for the behavioral effects of these SSRIs to be fully manifested.

Citalopram is unique among the clinically available SSRIs in having the greatest selectivity for SERT over NET in vitro (27, 41) and does not, after systemic application, increase NE concentrations in vivo (4) [it has been reported to increase NE after reverse dialysis in the hippocampus (42)]. In accordance with this finding, the present data show that citalopram is the only antidepressant tested that is equally effective in manifesting its behavioral effects in mice with and without NE. This result suggests that whereas the other antidepressants must recruit adrenergic mechanisms to elicit their behavioral effects, citalopram reduces immobility independent of NE and therefore may be the only truly selective SSRI of those tested. Microdialysis studies examined the impact of the absence of NE on 5-HT efflux directly. Fluoxetine produced no increase of 5-HT levels in Dbh<sup>/−</sup> mice even though 5-HT levels increased 3-fold in control mice. Although the effects of citalopram were also attenuated in Dbh<sup>−</sup>/− mice when compared with the 4-fold rise in control mice, the 3-fold elevation observed in the mutant mice was likely to be sufficient to sustain the behavioral response in the TST. Importantly, local application of fluoxetine increased hippocampal 5-HT in both genotypes, indicating that fluoxetine is active at SERT in the Dbh<sup>−</sup>/− mice, as expected. Serotonergic cells in the dorsal raphe can be activated by means of α<sub>1</sub>-adrenergic receptor stimulation by afferent noradrenergic input (43, 44). This point of interaction between NE and 5-HT could account for the absence of increased 5-HT efflux and behavioral effects in Dbh<sup>−</sup>/− mice. It appears that the ability of SSRIs to increase 5-HT efflux depends on increased noradrenergic input to the 5-HT neurons. Further studies are required to determine whether this inability of fluoxetine to increase 5-HT in Dbh<sup>−</sup>/− mice can be reversed by pretreatment with L-DOPS. Citalopram seems to have unique properties among SSRIs, perhaps a high selectivity for SERT over NET or other unknown effects, that allow it to bypass the normal requirement for adrenergic input so that it is least affected by the absence of NE. Taken together, these data support the idea that the effects of most SSRIs, both behavioral and neurochemical, are diminished by the absence of NE transmission.

A key concept underlying modern antidepressant drug development is whether NE and 5-HT systems are independent mechanisms for treating depression or whether they are interdependent (45). Much evidence favoring either independent or interdependent outlooks has been discussed in the literature (46, 47). The lack of an increase in extracellular 5-HT after fluoxetine administration to the Dbh<sup>−</sup>/− mice suggests that an interdependence between 5-HT and NE can exist. However, it is also likely that these systems can act independently. The actions of citalopram are mostly unaffected in the Dbh<sup>−</sup>/− mice, and depletion of 5-HT blocks the effects of serotonergic but not noradrenergic antidepressants in mice (ref. 16 and O.F.O. and I.L., unpublished observations).

In conclusion, these data confirm that mice lacking endogenous NE are a useful tool for dissecting the roles of NE in modulating stress and pharmacologically induced behavioral and neurochemical changes of antidepressants. These results establish a critical role for NE in the acute behavioral effects of several classes of antidepressant compounds, including the SSRIs. However, because Dbh<sup>−</sup>/− mice also lack epinephrine, we cannot exclude a role of this catecholamine in the antidepressant-induced responses. The value of the TST is supported by the ability to predict drugs used clinically as antidepressants (predictive validity) and because the tests may capture some
aspect of the subjective experience of depression associated with evolutionary components of thwarted escape persistence or entrapment (25, 48). The positive findings in Dbh−/− mice encourage additional research to evaluate the role of NE in other behavioral tests sensitive to antidepressants to ensure the effects are not idiosyncratic to the TST; although our previous data with catecholaminergic antidepressants in the FST (20) strongly suggest that this is not the case. However, antidepressant drugs must be given chronically before they produce their full clinical effects, and alterations downstream of elevations in neurotransmitter levels likely contribute to their efficacy in treating depression. Therefore, future studies must evaluate the role of NE in the manifestation of the effects of chronic antidepressant drug treatments.

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