Antidepressant drugs have typically been classified into sets of compounds with actions targeted at serotonin (selective serotonin reuptake inhibitors [SSRIs]), norepinephrine (norepinephrine reuptake inhibitors [NRIs]), or both neurotransmitters (serotonin-norepinephrine reuptake inhibitors). Their classification has been based predominantly on their acute pharmacologic effects, usually determined by in vitro radioligand binding assays. The pharmacologic selectivity of antidepressants can be altered after their systemic administration, however, by dose, drug metabolism, physiologic interactions between neurotransmitters, and adaptive effects that emerge after chronic administration. This review examines whether pharmacologic selectivity is maintained by different types of antidepressants in vivo and whether pharmacologic selectivity matters for the production of their behavioral effects. Antidepressants increase extracellular levels of neurotransmitters according to their ability to inhibit presynaptic transporters, although physiologic interactions among neurotransmitters can influence antidepressants’ selectivity in certain brain regions. Chronic administration of many antidepressants also causes down-regulation of postsynaptic and presynaptic receptors. The pattern of responses of presynaptic markers suggests that pharmacologic selectivity is maintained after chronic administration of many antidepressants. Behavioral tests indicate that depletion of serotonin (5-HT) is capable of preventing the effects produced by SSRIs but not NRIs. The depletion of catecholamines also inhibits the effects of NRIs, although test results can be complicated by inhibition of motor activity. Depletion of norepinephrine may also inhibit the effects of some SSRIs, but not highly selective SSRIs like citalopram. Although the pattern of results from in vivo tests supports the concept that parallel neurotransmitter mechanisms lead to antidepressant activity, norepinephrine may participate in the effects of some SSRIs. It is also possible that compounds with dual actions at 5-HT and norepinephrine systems may be effective under circumstances in which selective antidepressants are ineffective.

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Dr. Lucki has received grant/research support from Wyeth and has received honoraria from Forest.

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Most antidepressant drugs currently approved for the treatment of depression produce acute changes in reuptake of 2 neurotransmitter systems, serotonin (5-HT) and norepinephrine, by interfering with the function of extracellular transporter proteins. These transporters are the primary mechanisms for inactivating synaptic biogenic amines by presynaptic reuptake, and increased extracellular neurotransmitter levels result when their function is inhibited. Most classifications categorize antidepressant drugs according to their ability to primarily affect the neurotransmitter norepinephrine, 5-HT, or both.

First-generation antidepressants consisted of tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs). Most TCAs (e.g., desipramine, maprotiline) are potent and selective inhibitors of norepinephrine reuptake (i.e., norepinephrine reuptake inhibitors [NRIs]), although some (e.g., clomipramine, imipramine) are known to inhibit 5-HT transmission as well. Most TCAs also exhibit affinity for α1, H1, and muscarinic neurotransmitter receptors, resulting in serious adverse side effects and toxicity. These clinical disadvantages encouraged the development of newer antidepressants. Discovery efforts resulted in the emergence of different classes of antidepressants (Table 1) that were classified according to their target neurotransmitter(s) based, in large part, on their affinity for 5-HT and norepinephrine transporters as determined by in vitro radioligand binding techniques.14 These classes are the selective serotonin reuptake inhibitors (SSRIs), NRIs, and serotonin-norepinephrine reuptake inhibitors (SNRIs). Unlike TCAs, most of these drugs lack affinity for other

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neurotransmitter receptors (α₁, H₁, and muscarinic receptors), and their introduction dramatically improved the safety and side effect profile of antidepressants. The other member of the group of first-generation antidepressants, the MAOIs, enhance both norepinephrine and 5-HT neurotransmission simultaneously by interfering with their intracellular metabolism. These compounds have not been used widely because patients typically must restrict their diet to avoid developing serious side effects.

Although most classifications of antidepressant drugs are based on their main acute pharmacologic effects of inhibiting the reuptake of biogenic amines, there is substantial debate about how long these effects last for antidepressants. For instance, the acute effects of 5-HT reuptake inhibitors may be less toxic than irreversible inhibitors. Reversible monoamine oxidase inhibitors may be less toxic than irreversible monoamine oxidase inhibitors.

administration for some period of time, although estimates of the length of the lag time between the onset of clinical treatment and behavior changes in depressed patients who respond vary between 2 to 3 weeks and a few days. For many patients, however, long-term treatment for months or years may be required to ensure optimal clinical response and prophylaxis against recurrent episodes. Animal studies have identified a number of neuroadaptive changes on neuronal receptors and intracellular signaling mechanisms that require chronic treatment with antidepressants to appear. The late development of these changes is associated with the onset of clinical symptoms and prophylaxis against recurrent episodes. Animal studies have identified a number of neuroadaptive changes on neuronal receptors and intracellular signaling mechanisms that require chronic treatment with antidepressants.

For all of the above reasons, it is important to consider studies that have attempted to identify specific neural substrates underlying the neurochemical and behavioral effects of antidepressant drugs in vivo. Such efforts have inspired a number of major questions that organize this review. First, do antidepressants that differ in pharmacologic selectivity between the neurotransmitters 5-HT and norepinephrine produce selective neurochemical effects in vivo? Second, can neurochemical selectivity be maintained by antidepressant drugs after chronic administration? Third, depletion of the relevant monoamine neurotransmitters would be expected to prevent the behavioral effects of antidepressant drugs with corresponding phar-

### Table 1. Antidepressant Drug Classes Based on Selectivity for Monoamine Neurotransmitters

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism</th>
<th>Drugs</th>
<th>Advantages/Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine reuptake inhibitors</td>
<td>Inhibit NE reuptake by blocking NET and increase extracellular NE levels</td>
<td>Tricyclic (desipramine, nortriptyline, maprotiline) and non-tricyclic (reboxetine) antidepressants</td>
<td>High affinity for α₁, H₁, and muscarinic receptors of tricyclic drugs contributes to side effects. Reboxetine may improve social functioning. Little affinity for other neurotransmitter receptors reduces toxic side effects. Relative selectivity for 5-HT/NE varies between compounds. Metabolites of some of these drugs may also be active.</td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors</td>
<td>Inhibit 5-HT reuptake by blocking SERT and increase extracellular 5-HT levels</td>
<td>Fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, and escitalopram</td>
<td>blocker NET and SERT</td>
</tr>
<tr>
<td>Serotonin-norepinephrine reuptake inhibitors</td>
<td>Exhibit dual effects of inhibiting NE and 5-HT reuptake by blocking both NET and SERT, increasing both NE and 5-HT transmission</td>
<td>Venlafaxine, duloxetine, and maprotiline</td>
<td>Venlafaxine, duloxetine, and milnacipran block NET and SERT</td>
</tr>
<tr>
<td>Monoamine oxidase inhibitors</td>
<td>Prevent metabolism and increase concentrations of biogenic amine neurotransmitters</td>
<td>Tranlycypromine, phenelzine, and moclobemide</td>
<td>Dietary restrictions necessary to prevent hypertensive side effects limit use. Reversible monoamine oxidase inhibitors may be less toxic than irreversible monoamine oxidase inhibitors.</td>
</tr>
</tbody>
</table>

Abbreviations: 5-HT = serotonin, NE = norepinephrine, NET = norepinephrine transporter, SERT = serotonin transporter.
macologic selectivity. What effect does selective depletion of brain 5-HT exert on behavioral responses mediated by SSRIs and NRIs? Does depletion of catecholamines evoke corresponding blockade of the behavioral effects only of NRIs, or of SSRIs too?

EFFECTS OF PHARMACOLOGICALLY SELECTIVE ANTIDEPRESSANTS ON SEROTONIN AND NORADRENALINE TRANSMISSION

Techniques for estimating the effect of antidepressants on monoamine neurotransmission in vivo formerly required animals to be killed and neurotransmitter contents extracted from brain tissue. Newer and more sensitive techniques have evolved during the past decade for monitoring of brain neurochemicals. Occupation of neurotransmitter receptors in brain can now be determined directly via neuroimaging techniques involving highly sensitive radioligands. Microdialysis and voltammetry allow changes in extracellular levels of neurotransmitters in specific brain regions to be measured directly in conscious animals continuously over time. These techniques provide the best methods for assessing the effects of antidepressant treatments on biogenic amine systems in intact animals.

Microdialysis studies have confirmed that pharmacologically selective antidepressant drugs increase extracellular levels of their targeted neurotransmitters. Acute administration of all SSRIs produces dose-dependent increases in 5-HT in a number of brain regions. The maximal effects caused by the acute systemic administration of SSRIs are generally restricted to only a 2- to 4-fold elevation above baseline values, although 5-HT levels could be increased much higher if SSRIs were applied locally to a brain region through reverse microdialysis. This restraining effect is caused by the activation of autoreceptors that reduce neuronal impulse flow and inhibit neurotransmitter synthesis and release. Pharmacologic blockade or genetic disruption of inhibitory somatodendritic 5-HT1B autoreceptors, for example, augments the magnitude of 5-HT increase produced by acute administration of fluoxetine or paroxetine. By comparison, systemic administration of other drugs, such as the 5-HT releaser fenfluramine and 5-HT precursors, can produce greater increases in 5-HT levels than antidepressants because the actions of these substances are not regulated by neuronal activity, as are those of the SSRIs. Similarly, systemic administration of antidepressants with high affinity for norepinephrine transporters, such as desipramine and reboxetine, has been shown to increase extracellular norepinephrine levels in a number of brain regions.

The pharmacologic selectivity of SSRIs for increasing 5-HT transmission has been evaluated in some microdialysis studies by the simultaneous monitoring of extracellular levels of norepinephrine or dopamine. Fluoxetine increased norepinephrine levels in the frontal cortex and hypothalamus in a number of studies, suggesting that the effects of this SSRI are not completely selective for 5-HT. Bymaster et al. described the nonselective effects on norepinephrine levels as particular to fluoxetine because other SSRIs (i.e., sertraline, paroxetine, and citalopram) failed to increase norepinephrine levels. Acute fluvoxamine (12 mg/kg, IP) was reported to increase norepinephrine levels in the rat frontal cortex. Inconsistent results have been reported for the effect of 10 mg/kg of sertraline on norepinephrine levels in the rat cortex and hippocampus. A lower dose of paroxetine (5 mg/kg, SC) that increased 5-HT levels did not increase norepinephrine levels in the hippocampus, although higher doses (10 or 30 mg/kg, SC) of paroxetine increased norepinephrine levels in the frontal cortex. Citalopram, the most selective SSRI, did not increase cortical norepinephrine levels in rats. In mice, citalopram and paroxetine produced marginal increases in cortical levels of norepinephrine, and these effects were generally smaller than corresponding changes in 5-HT levels. Several mechanisms might explain the ability of some SSRIs to increase norepinephrine levels. First, SSRIs could interfere directly with the function of norepinephrine transporters, even though most SSRIs display lower or marginal affinity for those sites. This effect could be determined by measuring binding directly at norepinephrine transporters. However, pharmacologic effects of some SSRIs or 5-HT at 5-HT receptors, such as the activation of 5-HT1A receptors or blockade of 5-HT1B receptors, also could lead to activation of the locus ceruleus and increase norepinephrine transmission. These effects might be identified by measuring changes in adrenergic transmission in the absence of 5-HT.

Functional interactions between norepinephrine and 5-HT neurons may also contribute to the targeted effects of SSRIs on 5-HT transmission. A principal source of tonic activation of 5-HT neurons in the dorsal and median raphe nuclei is provided by the noradrenergic α1 receptors. Thus, the increase of norepinephrine levels produced by many SSRIs could contribute to the ability of the drugs to increase 5-HT levels through activation of these receptors. SSRIs also may lead to accumulation of 5-HT in catecholaminergic neurons, where 5-HT might be released by stimuli that increase norepinephrine release. In contrast, activation of noradrenergic α1 receptors on 5-HT terminals in the hippocampus has been shown to reduce extracellular 5-HT levels. It may be surprising, then, that extracellular 5-HT levels are not increased by acute systemic administration of NRIs except that dorsal raphe α1 receptors may be maximally stimulated during resting conditions.

One intriguing demonstration of convergent effects between antidepressants of different classes using microdialysis is their ability to increase extracellular dopamine levels in the rat medial prefrontal cortex. This effect is produced by selective NRIs (e.g., desipramine, reboxe-
tine) and a number of SSRIs (e.g., fluoxetine, sertraline, paroxetine), even though these drugs do not block dopamine transporters directly.\textsuperscript{20,31} The most likely reason for this common effect may be that norepinephrine transporters heterologously take up dopamine as well as nor-
epinephrine in cortical areas,\textsuperscript{20,32} and common effects of SSRIs and NRIs on cortical norepinephrine could mediate an increase of dopamine levels. Similar effects of antide-
pressants on dopamine levels are not produced in brain areas with less significant norepinephrine innervation, such as the nucleus accumbens and striatum.\textsuperscript{13} Because citalopram, the most selective SSRI, does not produce
substantial effects on dopamine or norepinephrine lev-
els,\textsuperscript{13,20} dopamine levels do not appear to be increased from 5-HT transporter blockade.

Chronoamperometric methods that measure the re-
moval of locally applied 5-HT from extracellular fluid in
discrete brain regions have also been used to demonstrate
fine spatial differences in the interaction between 5-HT
and norepinephrine neurons within a brain structure.\textsuperscript{33} Local application of the SSRIs fluvoxamine and citalo-
pram prolonged the clearance of 5-HT in the CA3 and
dentate gyrus regions of the hippocampus. In contrast, the
NRIs desipramine and protriptyline did not alter the 5-HT
signal in the CA3 region but did prolong 5-HT clearance
in the dentate gyrus. Thus, the 5-HT transporter may gov-
ern 5-HT clearance in the CA3 region solely, whereas
both the 5-HT and norepinephrine transporters contribute
to the active clearance of exogenously applied 5-HT in
other regions of the hippocampus.\textsuperscript{33}

In summary, microdialysis and voltammetry studies
have shown that acute administration of pharmaco-
ologically selective antidepressants increases extracellular lev-
els of the targeted neurotransmitters: SSRIs increase 5-HT
and NRIs increase norepinephrine. SSRIs also increase
extracellular levels of the catecholaminergic neurotrans-
mitters norepinephrine and dopamine. These “nonselective”
effects of SSRIs may vary according to brain region,
dose, and relative selectivity of the drug. In contrast,
microdialysis studies have not shown that NRIs produce
increases in 5-HT transmission. A number of physiologic
interactions between norepinephrine and 5-HT neurons
are known to exist, and techniques with superior spatial
resolution such as voltammetry may be required to dem-
onstrate them.

\textbf{MAINTENANCE OF SELECTIVITY AFTER CHRONIC
ADMINISTRATION OF ANTIDEPRESSANTS}

Repeated administration of antidepressant drugs pro-
duces a number of adaptive effects on central noradren-
ergic and serotonergic neurons. Some of these effects have
been suggested to underlie the therapeutic effects of anti-
depressants because they emerge following repeated but
not acute treatments and because they are produced com-
monly by antidepressants from different classes. Examina-
tion of the pattern of these chronic effects between classes of
antidepressants that are selective for 5-HT or norepi-
nephrine transporters (Table 2) provides information on
the ability of antidepressants to maintain their pharmaco-
logic selectivity during chronic treatment.\textsuperscript{7}

One effect produced by long-term treatment with many
antidepressant drugs is down-regulation of postsynaptic noradrenergic $\beta_1$-adrenergic receptors in the frontal cor-
 tex, amygdala, and other structures.\textsuperscript{34,35} Because so many antide-
presant treatments (e.g., TCAs, MAOIs, electro-
convulsive shock, sleep deprivation) produce this effect,
down-regulation of $\beta$-adrenergic receptors may be asso-
ciated with their common effects. Down-regulation of $\beta_1$
-adrenergic receptors by desipramine results from a persis-
tent overexposure to higher levels of norepinephrine,\textsuperscript{36} and
only antidepressant drugs that acutely enhance noradren-
ergic function seem to produce this effect. Chronic admin-
istration of SSRIs does not down-regulate $\beta$-adrenergic recep-
tors.\textsuperscript{34} Chronic treatment with antidepressants that
enhance noradrenergic function also produces adaptive ef-
fects in presynaptic receptors located on norepinephrine
neurons, such as down-regulation of $\alpha_2$-adrenoceptors and
norepinephrine transporters.\textsuperscript{37,38} Again, these effects are
not generally produced by chronic treatment with SSRIs,
suggesting that substantial neurotransmitter selectivity is
maintained by pharmacologically selective drugs follow-
ing chronic treatment.\textsuperscript{7} Although down-regulation of pre-
synaptic or postsynaptic adrenergic receptors may be im-
portant to the antidepressant efficacy of the drugs that
cause these effects, the proven efficacy of other agents
suggests that such mechanisms are not solely responsible
for antidepressant clinical response.

Similarly, repeated administration of different types of
antidepressants can affect serotonergic receptors. Antide-

\begin{table}
\caption{Regulation of Monoamine Receptors by Chronic
Administration of Antidepressants}
\label{tab:regulation}
\begin{tabular}{|l|c|c|c|}
\hline
Marker & SNRIs & SSRIs & MAOIs \\
\hline
Noradrenergic markers & & & \\
$\beta_1$-adrenoceptor & ↓ & ↔ & ↓ \\
$\alpha_2$-adrenoceptor & ↓ & ↔ & ↓ \\
NET & ↓ & ↔ & ↓ \\
\hline
Sero
tonergic markers & & & \\
5-HT$_{1A}$ receptor & ↔ & ↓ & ↓ \\
5-HT$_{1B}$ receptor & ↔ & ↓ & ↓ \\
5-HT$_{1D}$ receptor & ↓ & ↓ & ↓ \\
5-HTT & ↔ & ↓ & ND \\
\hline
Cellular or neurotrophic markers & & & \\
CREB & ↑ & ↑ & ↑ \\
BDNF & ↑ & ↑ & ↑ \\
Neurogenesis & ↑ & ↑ & ↑ \\
\hline
\end{tabular}
\end{table}

Abbreviations: 5-HTT = 5-HT transporter, BDNF = brain-derived
neurotrophic factor, CREB = cyclic AMP response element binding
protein, MAOI = monoamine oxidase inhibitor, ND = not
determined, NET = norepinephrine transporter, SNRI = serotonin-
norepinephrine reuptake inhibitor, SSRI = selective serotonin
reuptake inhibitor.

Symbols: ↑ = increase, ↓ = reduction, ↔ = no change.
pressants that cause a persistent enhancement of serotoninergic transmission also cause diminished responsiveness of 5-HT autoreceptors, either somatodendritic 5-HT₁₄ receptors or 5-HT₁₉ autoreceptors located at nerve terminals. In addition, chronic administration of SSRIs causes a down-regulation of serotonin transporters and a prolongation of 5-HT clearance in the CA3 region of the hippocampus. In contrast, chronic treatment with antidepressants that are NRIs does not cause these effects. This pattern of effects suggests that these effects are not solely responsible for clinical response but part of a pattern of neuroadaptation to persistently stimulated 5-HT transmission. The point is that many antidepressants with different acute effects appear to continue to affect different neurotransmitter systems even after chronic administration, which suggests that the initial pharmacologic selectivity among them is substantial and maintained throughout long-term treatment.

More recently, common effects of different types of antidepressant drugs have been described for intracellular markers of physiologic activity and transcription factors that mediate gene regulation. Chronic treatment with different types of antidepressants increased the expression of cyclic AMP response element binding protein (CREB), which may lead to regulation of brain-derived neurotrophic factor (BDNF). BDNF guides the growth of new neurons to their appropriate targets, provides protective effects against neuronal damage, and facilitates cellular signaling mechanisms. Brain levels of BDNF mRNA have been shown to be increased by chronic administration of a variety of different types of antidepressant drugs and electroconvulsive shock. Furthermore, neurogenesis in the hippocampus of adult animals has been shown to be increased following the chronic administration of different types of antidepressant drugs.

The common effects of divergent types of antidepressant treatments on the regulation of CREB, BDNF, and neurogenesis support the suggestion that these markers may evoke common cellular mechanisms leading to physiologically adaptive responses that underlie the therapeutic effects of chronic antidepressant treatments. A recent study suggests that some behavioral effects of antidepressant drugs are absent in animals unable to increase neurogenesis or to activate TrkB receptors, one of the receptors activated by BDNF. However, the relationship between these emergent chronic effects and the acute effects of antidepressant drugs has hardly been explored. It is not known whether these regulatory effects produced by NRIs and SSRIs occur in identical regions or in identical cell types.

One method that has dissected contributions of norepinephrine and 5-HT to the behavioral effects of antidepressant drugs is the use of selective pharmacologic depletions or lesions of individual neurotransmitter systems. If it is true that the common cellular or neurogenic effects of antidepressants are triggered by persistent overexposure to norepinephrine or 5-HT, then the selective depletions of neurotransmitters will help us to understand the association between the divergent acute effects produced by different antidepressant classes and the common effects that emerge following chronic administration.

**BEHAVIORAL EFFECTS OF SELECTIVE SEROTONERGIC AND NORADRENERGIC ANTIDEPRESSANTS**

Another way to establish the significance of the pharmacologic selectivity of antidepressants is to examine whether different classes of antidepressant drugs produce distinct behavioral effects. The forced swimming test (FST) is a behavioral screen for antidepressant drugs that has been used by many laboratories. When rodents are placed in a cylinder of deep water, they eventually develop passive, immobile behavior after initially exploring and attempting to escape. Antidepressant drug treatments cause rodents to persist in escape-directed behaviors for longer periods of time and delay the onset of immobility. The ability of antidepressants to facilitate active coping responses to stress may have parallels to subjective feelings of “entrapment” or passive coping styles shown by depressed patients. The FST remains an attractive and useful tool because it is one of the few behavioral tests that are sensitive to the effects of all major classes of antidepressant treatments, including TCAs, MAOIs, diverse atypical antidepressants, electroconvulsive shock, and somatic interventions. Different versions of the same test have been conducted in rats, mice, and other species.

In the rat FST, distinct behavioral effects of different classes of antidepressants can be distinguished. NRIs that predominantly affect norepinephrine transmission do not produce the same behaviors as SSRIs. By measuring the active behaviors when immobility is reduced, it has been shown repeatedly that noradrenergic antidepressants decreased immobility with a corresponding increase of climbing behavior. In contrast, SSRIs decreased immobility but increased swimming behavior. Additional studies have shown that 5-HT₁₄ and 5-HT₂C receptor agonists produce effects similar to those of SSRIs and appear to play an instrumental role in mediating the behavioral effects of fluoxetine.

One legitimate criticism of this approach is that most studies examine responses in the rat FST after short-term treatment with antidepressants because the test was developed initially as a screen for antidepressant discovery. Weeks of treatment may be required for a clinical response to antidepressants, and drugs may lose their selectivity after chronic administration. However, procedural adjustments to the rat FST reveal that low doses that are initially inactive will produce antidepressant-like responses after chronic administration.
fluoxetine was administered chronically for 14 days, the treatment caused increased swimming as the predominant active behavior in the modified FST. Similarly, chronic administration of desipramine for 14 days produced an increase in climbing behavior. These behavioral results are consistent with the idea that pharmacologically selective antidepressant drugs substantially maintain their selectivity even after chronic administration.

Antidepressants with dual actions on 5-HT and norepinephrine (SNRIs) have also been tested for their ability to produce different components of the modified rat FST. Venlafaxine, a dual norepinephrine and 5-HT reuptake inhibitor, increased swimming behavior at lower doses (20–40 mg/kg) but simultaneously increased both swimming and climbing behavior when given at 80 mg/kg, SC. Similar dual effects were found in rats treated with the combination of fluoxetine and desipramine. Thus, the deliberate combination of dual pharmacologic effects produced a subtle behavioral change by causing both active components (swimming and climbing) to contribute to reductions in immobility in the FST.

The differentiation of behavioral components has not yet been extended to the mouse FST. However, strain surveys have shown that only certain mouse strains (e.g., Swiss, DBA/2, BALB/c) are sensitive to the effects of SSRIs. Perhaps not surprisingly, mouse strains that do not respond easily to SSRIs will respond to NRIs in the same test. It is possible that genetic differences in key substrates for behavioral response underlie such dramatic pharmacologic differences. If so, the mouse FST may provide an animal model for predicting genes associated with pharmacogenetic differences in antidepressant response among different patients.

It has been suggested that pharmacologically distinct antidepressants act on overlapping but different components of human behavior. For example, noradrenergic antidepressants improved psychomotor activation most effectively and serotonergic antidepressants best improved symptoms related to anxiety, it would be logical to conclude that classes of antidepressant drugs may produce overlapping therapeutic effects but for distinct reasons. Moreover, drugs with dual pharmacologic effects could show benefits in ways that are different from those of antidepressants with pharmacologic selectivity. These predictions have not been tested in clinical studies.

INVOLVEMENT OF SEROTONIN AND NOREPINEPHRINE IN THE BEHAVIORAL EFFECTS OF ANTIDEPRESSANT DRUGS

Although antidepressant drugs can enhance 5-HT and/or norepinephrine transmission and produce behavioral responses, in agreement with their acute pharmacologic properties, they also produce effects on other neurotransmitters. Thus, it may be difficult to prove how potential neuropharmacologic actions of antidepressant drugs relate to their actual clinical effects. The best method for identifying neurochemical substrates that underlie clinical antidepressant drug effects derives from studies in which monoamine neurotransmitters have been depleted in recovering depressed patients. Clinical studies using para-chlorophenylalanine (PCPA) to interfere with 5-HT synthesis during initial treatment prevented the therapeutic effects of imipramine or tranylcypromine in depressed patients. More recently, functional depletion of 5-HT produced by short-term tryptophan depletion led to relapse in patients who had demonstrated clinical improvement with SSRIs, but not in patients who had been treated with NRIs. Conversely, inhibition of catecholamine synthesis was shown to interfere with the clinical response to NRIs but not SSRIs. These studies support the idea that pharmacologically diverse antidepressant drugs may produce a common clinical outcome through specific and distinct neurotransmitter mechanisms. The acute pharmacologic effects of antidepressants could be important in triggering separate sets of neural components that eventually contribute to their clinical efficacy following chronic treatment.

There is a substantial literature of animal studies that provide a type of laboratory parallel to the studies being conducted in depressed patients. These studies in which brain monoamines have been depleted selectively by synthesis inhibition or lesions can provide critical evidence for the contribution of individual neurotransmitters to the behavioral responses of different types of antidepressant drugs. Although it may seem obvious to hypothesize that an SSRI should be ineffective following 5-HT depletion, the counter-hypothesis—that the nonselective effects of SSRIs could sustain their behavioral effects—is also tested. Furthermore, the interaction between norepinephrine and 5-HT neurotransmitter systems and behaviors mediated by the complementary set of drugs can also be tested. Animal studies have exploited a broader array of depletion techniques with varying selectivity and anatomic specificity. The following section reviews animal behavior studies that have attempted to determine the roles of 5-HT and norepinephrine systems in the production of antidepressant effects. The review emphasizes studies of the rat and mouse FST because of the suitability of this behavioral tool to measure the acute pharmacologic effects of different types of antidepressants. This literature has not been reviewed elsewhere.

Depletion of Serotonin

The role of 5-HT in the behavioral effects of antidepressant drugs in animal studies is more definitive than that of norepinephrine. A number of techniques are available for producing a substantial depletion of central 5-HT. One of the most effective methods has been the use of PCPA, an irreversible inhibitor of the enzyme trypto-
phine hydroxylase. In addition, the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) has been used to destroy 5-HT neurons after intraventricular administration. Both treatments have been shown to produce substantial to near-complete depletions of 5-HT tissue content. Interestingly, baseline performances in antidepressant behavior tests, such as the FST, tail suspension test (TST), and learned helplessness, are not altered by the depletion of 5-HT in otherwise “normal” animals, which suggests that 5-HT may play a greater role in mediation of the behavioral actions of antidepressant drugs than in stress-evoked behavioral depression.

Studies of the effects of 5-HT depletion on the behavioral effects of antidepressants are summarized in Table 3. Page et al. reported that pretreatment with PCPA blocks the behavioral effects of the SSRI fluoxetine in the modified rat FST. The effects of fluoxetine in the FST were also attenuated in rats pretreated with methylenedioxyamphetamine (MDA), which caused a depletion of 5-HT. MDA reduced 5-HT tissue content to a lesser degree (27%–40%) than PCPA (86%–93%).

Depletion of Catecholamines

Despite the high affinity for the norepinephrine transporter by many antidepressants, evidence for the role of norepinephrine in the behavioral effects of antidepressants is more ambiguous and controversial than that for 5-HT. This uncertainty is due in part to the difficulty of producing a substantial and selective depletion of norepinephrine content using conventional pharmacologic tools without affecting other neurotransmitters, such as dopamine or 5-HT. Also, treatments that produce substantial depletion of norepinephrine content can cause motor impairment or toxicity that complicates interpretation of behavioral responses to antidepressants. For example, depletion of norepinephrine content by inhibiting tyrosine hydroxylase using α-methyl-para-tyrosine (AMPT) will affect dopamine and epinephrine content as well. The use of neurotoxins, such as 6-hydroxydopamine (6-OHDA) or N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), can produce more selective destruction of norepinephrine neurons when administered under conditions that protect other vulnerable neurons. However, the neurotoxins can block in Dbh knockout mice, which are unable to synthesize norepinephrine. Taken together, these results suggest that although 5-HT is ordinarily an important component of the behavioral effects of sertraline, the involvement of catecholamines can be shown under certain conditions.

The effects of PCPA pretreatment on behavioral effects produced by the dual-action SNRIs imipramine and venlafaxine have also been examined. PCPA pretreatment did not alter the effect of imipramine in the mouse TST. Recently we found that PCPA pretreatment in mice had no effect on the behavioral actions evoked by desipramine or reboxetine in the TST (O.F.O., I.L., unpublished observations). The destruction of 5-HT neurons with 5,7-DHT did not attenuate the behavioral effects of desipramine or imipramine in the rat FST. Similarly, 5,7-DHT pretreatment did not alter the ability of desipramine or clomipramine to reverse escape deficits in a learned helplessness model. Thus, evidence does not support a role for 5-HT in the behavioral effects of NRIs.
Table 3. Effects of 5-HT Depletion on the Behavioral Effects of Antidepressant Drugs

<table>
<thead>
<tr>
<th>Method of Depletion</th>
<th>Species</th>
<th>Test</th>
<th>Drugs Tested</th>
<th>Results</th>
<th>5-HT Depletion</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP</td>
<td>Rat</td>
<td>FST</td>
<td>Fluoxetine 10 mg/kg, SC</td>
<td>Blocked</td>
<td>93% decrease in frontal pole</td>
<td>Page et al., 1999&lt;sup&gt;63&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Desipramine 10 mg/kg, SC</td>
<td>Not blocked</td>
<td>86% decrease in prefrontal cortex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 15 mg/kg, SC</td>
<td>Not blocked</td>
<td>90% decrease in FC</td>
<td>Wieland and Lucki, 1990&lt;sup&gt;64&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>FST</td>
<td>Fluoxetine 32 mg/kg, IP</td>
<td>Blocked</td>
<td>NR</td>
<td>Eckeli et al., 2000&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine 15 mg/kg, IP</td>
<td>Partial blockade</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mouse</td>
<td>FST</td>
<td>Paroxetine 8 mg/kg, IP</td>
<td>Attenuated</td>
<td>61% decrease in whole brain</td>
<td>Redrobe et al, 1998&lt;sup&gt;66&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td></td>
<td>16 mg/kg, IP</td>
<td>Attenuated</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>32 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Fluoxetine 20 mg/kg, IP</td>
<td>Blocked</td>
<td>77% decrease in FC</td>
<td>O’Leary et al, 2001&lt;sup&gt;57&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Citalopram 20 mg/kg, IP</td>
<td>Blocked</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Fluoxetine 32 mg/kg, IP</td>
<td>Blocked</td>
<td>NR</td>
<td>Rodrigues et al, 2002&lt;sup&gt;58&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine 15 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Desipramine 20 mg/kg, IP</td>
<td>Not blocked</td>
<td>ND</td>
<td>O’Leary et al, unpublished data</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reboxetine 20 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>FST</td>
<td>Venlafaxine 8 mg/kg, IP</td>
<td>Attenuated</td>
<td>61% decrease in whole brain</td>
<td>Redrobe et al, 1998&lt;sup&gt;59&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 mg/kg, IP</td>
<td>Attenuated</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>32 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
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<tr>
<td>5,7-DHT</td>
<td>Rat</td>
<td>FST</td>
<td>Sertraline 80 mg/kg</td>
<td>Not blocked</td>
<td>92% decrease in hippocampus</td>
<td>Lucki et al, 1994&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Desipramine 10 mg/kg</td>
<td>Not blocked</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Rat</td>
<td>FST</td>
<td>Sertraline 64 µmol</td>
<td>Not blocked</td>
<td>73% decrease in whole brain</td>
<td>Cervo et al, 1991&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>64 µmol/d for 7 d</td>
<td>Not blocked</td>
<td>83% decrease in whole brain</td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 30 mg/kg, IP</td>
<td>Not blocked</td>
<td>79% decrease in amygdala</td>
<td>Araki et al, 1985&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine 30 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raphe</td>
<td>Rat</td>
<td>LH</td>
<td>Desipramine 24 mg/kg, IP</td>
<td>Not blocked</td>
<td>NR</td>
<td>Soubrie et al, 1986&lt;sup&gt;73&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td>Clomipramine 32 mg/kg, IP</td>
<td>Not blocked</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>32 mg/kg, IP for 5 d</td>
<td>Not blocked</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine 32 mg/kg, IP for 5 d</td>
<td>Not blocked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>Rat</td>
<td>FST</td>
<td>Fluoxetine 5 mg/kg, IP</td>
<td>Attenuated swimming</td>
<td>27% decrease in FC</td>
<td>Harkin et al, 2003&lt;sup&gt;74&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Attenuated</td>
<td>40% decrease in amygdala</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 mg/kg, IP</td>
<td></td>
<td>28% decrease in hippocampus</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 5-HT = serotonin, DHT = dihydroxytryptamine, FC = frontal cortex, FST = forced swimming test, ICV = intracerebroventricular, IP = intraperitoneal, LH = learned helplessness, MDA = methylenedioxymphetamine, ND = not determined, NR = not reported, PCPA = para-chlorophenylalanine, SC = subcutaneous, TST = tail suspension test.
also cause abnormal axonal growth or compensatory nor- 
epinephrine release at remaining intact terminals.77,78

Studies of the effects of catecholamine depletion on the 
behavioral effects of antidepressants are summarized in 
Table 5. Destruction of the locus ceruleus and its dorsal 
norepinephrine bundle by electrolytic lesions, systemic 
administration of the selective neurotoxin DSP-4, or local 
injections of 6-OHDA blocked the effects of desipramine 
after a single or subchronic administration in the rat 
FST,74,80 suggesting involvement of the dorsal norepineph- 
rine bundle. These studies are contradicted by others using 
local administration of 6-OHDA into the locus ceruleus 
or systemic DSP-4 administration, which failed to block 
the effects of subchronic reboxetine81 or desipramine.79 
Finally, norepinephrine depletion either attenuated by about 
50% or failed to block the effects of chronic desipramine 
treatment given for 14 days.80,82 Eliminating the behav- 
ioral effects of NRIs after global lesioning of norepineph- 
rine pathways would prove to be difficult if only a discrete 
norepinephrine pathway was involved. Cryan et al.81 re- 
ported that local treatment with 6-OHDA to destroy the 
ventral noradrenergic bundle blocked the effects of the 
selective NRI reboxetine in the modified rat FST. Some of 
the prior studies using lesions aimed at the dorsal norepi- 
 nephrine bundle did not evaluate whether the ventral nor- 
epinephrine bundle was also affected by their treatments. 
The specific circuitry underlying the effects of NRIs 
can be identified by destruction of relevant norepineph- 
rine terminals, as shown when local administration of 
6-OHDA into the amygdala blocked the effects of desipa-
mine.72 Additional studies could confirm the involvement 
of this or other brain regions.

Employing pharmacologic inhibitors of norepinephrine 
synthesis may require nearly complete depletion to pre- 
vent the effects of desipramine in the mouse TST. Substan- 
tial doses of AMPT failed to block the behavioral effects 
of 20 mg/kg of desipramine (O.F.O., I.L., unpublished ob- 
servations). Only after reserpine was given in combination 
with AMPT, to destroy catecholamine vesicles as well as 
as to deplete norepinephrine, were the behavioral effects 
of desipramine significantly blocked, although baseline 
immorbility values were also increased by the treatment 
(O.F.O., I.L., unpublished observations).

A supporting role for norepinephrine has been sug- 
gested in the mechanism of the dual-action SNRIs imipa- 
mine and venlafaxine. De Montis et al.83 reported that 
pretreatment with AMPT blocked the ability of imipra- 
mime to prevent the development of learned-helplessness 
behavior in rats. Pretreatment with 6-OHDA in the amyg- 
dala blocked the effects of imipramine in the rat FST.72 In 
the mouse FST,69 DSP-4 attenuated the effects of a low 
dose of venlafaxine (16 mg/kg) but did not alter the effects 
of a higher dose of venlafaxine (32 mg/kg) because both 
5-HT and norepinephrine may be active at this dose.

There is a study investigating the role of norepineph- ine in the mechanism of action of the SSRI fluoxetine in 
rats. Pretreatment of rats with 6-OHDA (administered into 
the locus ceruleus and surrounding pathways) did not alter 
fluoxetine-induced decreases of immobility in the modi- 
fied rat FST.54 

In view of the difficulties of using pharmacologic and 
neurotoxic agents to interfere with norepinephrine trans- 
mision, the generation of mice with a targeted disruption 
of the gene for the enzyme dopamine-β-hydroxylase (Dbh) 
provides an excellent model89 for examining a po- 
tential role of norepinephrine in the behavioral effects 
of antidepressant drugs. Dbh is the enzyme specifically 
responsible for the conversion of dopamine to norepineph- ine in the vesicles of noradrenergic neurons. The deletion 
of Dbh causes a complete and specific inhibition of nor- 
eeipinephrine (and epinephrine) while circumventing the 
problems associated with the use of nonselective and toxic 
depleting agents. In addition, norepinephrine levels in 
these mutants may be transiently restored, during devel- 
opment or in adulthood, by administration of L-threo-3, 
4-dihydroxyphenylserine (DOPS), a synthetic precursor 
of norepinephrine that is metabolized by L-aromatic amino 
decarboxylase into norepinephrine, thus bypassing the re- 
quirement for Dbh.85

In studies involving NRI antidepressants, Dbh mutant 
mice demonstrated no significant deficits in baseline per- 
formance in antidepressant tests when tested without 
drugs or on tests of locomotor activity. The behavioral 
effects of desipramine and reboxetine were completely 
blocked in both the FST84 and TST76 in the Dbh mutant
### Table 5. Effects of Catecholamine Depletion on the Behavioral Effects of Antidepressant Drugs

<table>
<thead>
<tr>
<th>Method of Depletion</th>
<th>Species</th>
<th>Test</th>
<th>Drugs Tested</th>
<th>Results</th>
<th>Monoamine Depletion</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OHDA</td>
<td>Amygdala</td>
<td>FST</td>
<td>Desipramine 30 mg/kg, IP</td>
<td>Blocked</td>
<td>61% decrease in NE in amygdala</td>
<td>Araki et al, 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine 30 mg/kg, IP</td>
<td>Blocked</td>
<td>32% decrease in DA</td>
<td></td>
</tr>
<tr>
<td>Locus ceruleus and NE pathway</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 10 mg/kg, IP</td>
<td>Blocked</td>
<td>59%–94% decrease in NE depending on brain region</td>
<td>Reneric et al, 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluoxetine 10 mg/kg, IP</td>
<td>Not blocked</td>
<td>6%–37% decrease in DA depending on brain region</td>
<td></td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 10 mg/kg, IP for 7 d</td>
<td>Not blocked</td>
<td>70% decrease in NE in hippocampus</td>
<td>Esposito et al, 1987</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 20 mg/kg, IP</td>
<td>Blocked</td>
<td>65% decrease in NE in forebrain</td>
<td>Danysz et al, 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Desipramine 10 mg/kg, IP for 14 d</td>
<td>Attenuated</td>
<td>40% increase in DA</td>
<td></td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>Rat</td>
<td>FST</td>
<td>Sertraline 64 µmol</td>
<td>Not blocked</td>
<td>73% decrease in NE in hippocampus</td>
<td>Cervo et al, 1991</td>
</tr>
<tr>
<td>Ventral bundle</td>
<td>Rat</td>
<td>FST</td>
<td>Reboxetine 10 mg/kg, SC</td>
<td>Blocked</td>
<td>42% decrease in NE in cortex</td>
<td>Cryan et al, 2002</td>
</tr>
<tr>
<td>Ventral bundle</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 10 mg/kg, IP for 7 d</td>
<td>Not blocked</td>
<td>70% decrease in NE in hypothalamus</td>
<td>Esposito et al, 1987</td>
</tr>
<tr>
<td>electrolytic lesion of locus ceruleus</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 20 mg/kg, IP</td>
<td>Blocked</td>
<td>71% decrease in NE in whole brain</td>
<td>Danysz et al, 1986</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Desipramine 10 mg/kg, IP for 14 d</td>
<td>Not blocked</td>
<td>35% decrease in DA</td>
<td></td>
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<tr>
<td>DSP-4</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 10 mg/kg, IP for 7 d</td>
<td>Not blocked</td>
<td>85% decrease in NE in hippocampus</td>
<td>Esposito et al, 1987</td>
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<td>Desipramine 10 mg/kg, IP for 14 d</td>
<td>Not blocked</td>
<td>35% decrease in hypothalamus</td>
<td>Danysz et al, 1986</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>FST</td>
<td>Reboxetine 10 mg/kg, SC</td>
<td>Not blocked</td>
<td>69% decrease in hypothalamus</td>
<td>Cryan et al, 2002</td>
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<tr>
<td>AMPT</td>
<td>Rat</td>
<td>FST</td>
<td>Desipramine 10 mg/kg, IP</td>
<td>Not blocked</td>
<td>26% decrease in NE in hypothalamus</td>
<td>Redrobe et al, 1998</td>
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<td>Imipramine 10 mg/kg, IP bid for 21 d</td>
<td>Not blocked</td>
<td>28% decrease in NE in whole brain tissue</td>
<td></td>
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<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Desipramine 20 mg/kg, IP</td>
<td>Not blocked</td>
<td>ND</td>
<td>O’Leary and Lucki, unpublished data</td>
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<td>400 mg/kg, IP</td>
<td>Not blocked</td>
<td>ND</td>
<td>O’Leary and Lucki, unpublished data</td>
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<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Desipramine 20 mg/kg, IP</td>
<td>Blocked</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>TST</td>
<td>Desipramine 5 and 20 mg/kg, IP</td>
<td>Blocked</td>
<td>Below limits of detection (Thomas et al, 1998)</td>
<td>Cryan et al, 2001</td>
</tr>
<tr>
<td></td>
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<td>Reboxetine 5 and 20 mg/kg, IP</td>
<td>Blocked</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Fluoxetine 20 and 40 mg/kg, IP</td>
<td>Blocked</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Sertraline 20 and 40 mg/kg, IP</td>
<td>Blocked</td>
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<td>Paroxetine 5 mg/kg, IP</td>
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<td>20 mg/kg, IP</td>
<td>Not blocked</td>
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<td></td>
<td></td>
<td></td>
<td>Citalopram 5 and 20 mg/kg, IP</td>
<td>Not blocked</td>
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</table>

Abbreviations: AMPT = α-methyl-para-tyrosine, DA = dopamine, DSP-4 = N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine, FST = forced swimming test, IP = intraperitoneal, LH = learned helplessness, ND = not determined, NE = norepinephrine, NR = not reported, OHDA = hydroxydopamine, SC = subcutaneous, TST = tail suspension test.
mice. Restoration of norepinephrine with DOPS restored the ability of desipramine to reduce immobility in both tests of antidepressant effects.

The effects of some SSRIs were examined using the TST in Dbh mutants. Unexpectedly, the effects of fluoxetine and sertraline were completely blocked in these norepinephrine-deficient mice. Behavioral responses to paroxetine were partially attenuated, and those to citalopram were unaffected in the Dbh mutant mice. Subsequent restoration of norepinephrine with DOPS completely reinstated the effects of paroxetine in the TST.76

Because of these behavioral deficits, microdialysis studies were conducted in Dbh mutants to measure extracellular levels of 5-HT in the hippocampus and the response to SSRIs. There were no differences in basal 5-HT levels between the norepinephrine-deficient mice and controls. Although fluoxetine (20 mg/kg, IP) produced a 4-fold increase in extracellular levels of 5-HT in control animals, hippocampal 5-HT levels were not increased in Dbh mutants by systemic administration of fluoxetine. In contrast, systemic injection of citalopram (20 mg/kg, IP) increased extracellular 5-HT 3-fold in controls, and this was reduced by only 25% in the Dbh mutants.87 Thus, fluoxetine may have been behaviorally inactive and citalopram may have been active in behavioral tests in Dbh mutants because of the different effects of the drugs on extracellular 5-HT levels. Importantly, these results suggest that an excitatory impact on norepinephrine transmission may participate in, or even be required for, full manifestation of the behavioral effects of some SSRIs. Serotonergic cells in the dorsal raphe are activated by α1 receptors from afferent noradrenergic input that provide important excitatory drive.26,88 Because citalopram is unique among the SSRIs in having the greatest selectivity for 5-HT transporters over norepinephrine transporters in vitro89 and does not increase extracellular norepinephrine levels,13 citalopram may be a truly selective SSRI antidepressant that is equally effective in manifesting its behavioral effects with and without norepinephrine.

Conclusions and Limitations of Depletion Studies

This section reviewed animal behavior studies that examined the role of 5-HT and norepinephrine as neural substrates underlying the behavioral effects of antidepressant drugs. Most of the studies involved the use of the FST and TST in rats or mice. The similar behavioral responses seen with antidepressants provide a common endpoint for identifying individual neurochemical substrates for different types of antidepressants.

The pattern of results suggests that 5-HT plays a critical role in the behavioral effects of SSRIs but not NRIs. The depletion of 5-HT prevented behavioral effects produced by SSRIs in the majority of studies. There was also evidence that 5-HT depletion attenuated the effects of dual-action SNRI antidepressants. In contrast, 5-HT depletion failed to alter the behavioral effects produced by antidepressants that selectively enhance norepinephrine transmission, such as desipramine and reboxetine.

Identifying the role of norepinephrine in the behavioral effects of different types of antidepressants has been complicated by the lack of drugs or treatments for producing selective depletion of norepinephrine without a compromising impact on behavioral performance. The role of norepinephrine depletion also appears to depend on the specific neurotransmitter pathway and region being affected. However, a number of studies demonstrated prevention of the effects of NRIs and dual-action SNRIs after the depletion of norepinephrine by neurotoxin treatment or genetic deletion. Recent results also challenge the assumption that the norepinephrine and 5-HT systems function independently of each other. The depletion of norepinephrine in Dbh knockout mice impacted changes in extracellular levels of 5-HT and behavioral effects produced by SSRIs, particularly those agents that are less selective between norepinephrine and 5-HT transporters, including fluoxetine, sertraline, and paroxetine.

Most of the animal behavior tests used so far, such as the FST and TST, are important because they are highly predictive of clinical antidepressant activity and as such are used in antidepressant drug discovery. The tests are not animal models of depression per se, but they may allow us to examine homologous trait behaviors shown by depressed patients, such as evolutionary components of escape persistence and entrapment.90 Moreover, the pattern of results for depletion studies in animals is consistent with clinical observations of the effects of neurochemical depletion on mood in depressed patients60–62; that is, the therapeutic effects of SSRIs and NRIs appear to require 5-HT and norepinephrine, respectively.

A major criticism of the literature developed so far is that the animal behavior paradigms mostly have been studied only after acute or short-term treatment with antidepressant drugs. However, these short-term paradigms are the most practical to use given the substantial difficulties in maintaining large neurochemical depletions for the extended period of time required to conduct chronic studies in rodents. Genetic methods for interfering with monoamine synthesis, such as the Dbh–/– mouse, may be the most efficient new development for producing long-term selective monoamine depletions, given the limitations of alternative procedures. Another paradigm that would be informative but has not yet been used is examination of the effects of monoamine depletion in animals after the chronic administration of antidepressant drugs for weeks. Constructed according to this experimental design, such studies would have the merit of being similar to the kinds of depletion studies that have been used with depressed patients receiving clinical treatment.

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CONCLUSIONS

Antidepressant drugs can be divided into broad drug classes according to whether they facilitate effects of the neurotransmitter serotonin (SSRIs), norepinephrine (NRIs), or both neurotransmitters (SNRIs). Examination of their acute effects in vivo suggests that pharmacologically selective antidepressants increase extracellular levels of the individual neurotransmitters that they target when administered systemically. Although there is evidence that pharmacologically selective antidepressants may affect transmission of the complementary neurotransmitter, or even a third neurotransmitter such as dopamine, antidepressants still seem to produce their greatest effects on the targeted neurotransmitter. Evidence for cross-talk between classes is greater for some SSRIs affecting norepinephrine transmission than for NRIs affecting 5-HT transmission. In the case of less selective drugs, non-selectivity may be derived from actions at a lower affinity target at behaviorally active doses. However, physiologic interactions between neurotransmitters make interactions between norepinephrine and 5-HT systems more likely in particular brain regions and corresponding behavioral functions. Although cellular targets have been suggested as a final common pathway for diverse antidepressant drugs in gross brain regions, additional work is necessary to determine how common these effects are at a more refined physiologic and anatomic level of resolution.

Animal models of stress-related behaviors, such as the modified FST, continue to be useful for examining the neural substrates involved in the behavioral actions of antidepressant drugs. Genetic paradigms may provide appropriate models to achieve more selective long-term neurotransmitter depletions and to identify pharmacogenomic variables that influence treatment response. The impact of 5-HT depletion appears to be most profound on SSRIs, but complete norepinephrine depletion appears capable of interfering with the effects of both NRIs and SSRIs. Additional models and paradigms that can evaluate antidepressant effects following chronic treatment will provide information that is complementary to homologous studies with acute neurotransmitter depletion conducted on depressed patients.

It remains to be established whether the overall efficacy or behavioral actions of dual-action antidepressants will differ from those of antidepressants that are selective for a single mechanism. Even if overall efficacy is not different, studies should consider whether the quality of behavioral change differs between treatments. This approach has been modeled in some animal studies, such as the modified rat FST. The impact of different classes of antidepressants on clinical treatment could be more clear under provocative circumstances in which the impact of norepinephrine and 5-HT systems may differ. For example, variations at the pharmacogenomic level resulting in disparities in 5-HT or norepinephrine neurotransmission may be treated more successfully with antidepressants that are targeted for an individual patient. Physical conditions or diseases that are comorbid with depression, such as diabetes, cardiovascular disease, cancer, and head injury, may also differentially involve biogenic amine systems and produce selective outcomes between different types of antidepressant drugs. The rational tailoring of pharmacologically selective treatments to appropriate patients could improve overall clinical care and is a worthwhile target for future research.

**Drug names:** amitriptyline (Elavil and others), citalopram (Celexa), clomipramine (Anafranil and others), desipramine (Norpramin and others), escitalopram (Lexapro), fluoxetine (Prozac and others), imipramine (Tofranil and others), maprotiline (Ludiomil and others), nor- triptyline (Aventyl, Pamelor, and others), paroxetine (Paxil and others), phenelzine (Nardil), protriptyline (Vivactil), reserpine (Diapres, Renese-R. and others), sertraline (Zoloft), tranylcypromine (Parnate), venlafaxine (Effexor).

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