

# How Antidepressants Help Depression: Mechanisms of Action and Clinical Response

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The monoamine hypothesis of depression suggests that depressive symptoms can be moderated by enhancing monoamine neurotransmission. Targeted neurotransmitters include serotonin and norepinephrine, and a number of medications are available that can selectively enhance the actions of one or both of these substances. Although laboratory tests have validated the pharmacologic effects of these compounds, much less is known about how these effects translate into clinical response. Therapeutic research and experience show clearly that the medications help patients, although the individual and potential cooperative or complementary effects of stimulating each neurotransmitter system remain unclear. Depletion studies have reinforced the validity of targeting these systems and, at the same time, underscored that monoamines most likely are not the only factor driving the clinical presentation of depression.

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A large body of data conclusively demonstrates that all medications currently approved by the U.S. Food and Drug Administration for use in the treatment of major depression have potent effects on monoamine neurotransmission. Furthermore, more than 30 years of clinical research strongly suggests that effects on monoamine neurotransmission are the essential pharmacologic properties for antidepressant response and that effects on acetylcholine and/or histamine neurotransmission are not necessary.<sup>1</sup>

Most antidepressants have potent pharmacologic effects that increase synaptic levels of the monoamine neurotransmitters norepinephrine and/or serotonin (5-HT) and, in some cases, dopamine. Monoamine concentrations can be increased by blocking reuptake as well as by inhibiting metabolism that occurs via the enzyme monoamine oxidase. Associated data reviewed by Owens<sup>2</sup> and Lucki and O'Leary<sup>3</sup> in this supplement show that, depending on the paradigm used, certain antidepressants appear to have relatively more selective effects on 5-HT or norepinephrine neurotransmission and others exhibit dual effects by increasing both 5-HT and norepinephrine. Much of this

evidence is based on radioligand binding studies measuring the in vitro affinity of drugs for monoamine transporters. There are, however, relatively few direct human data with which to assess the pharmacologic effects most important for a particular drug.

Furthermore, many important questions regarding the interpretation of radioligand binding data remain unanswered. How accurately do measurements of binding affinity estimate the actual pharmacologic effects to be expected in a living person being treated for major depression? Even assuming that the binding data are an accurate reflection of relative pharmacologic selectivity, how do we know which, if any, of these pharmacologic effects is responsible for antidepressant efficacy? We have learned that antidepressant selectivity is relative and dose-dependent, so which drugs truly are 5-HT-selective or norepinephrine-selective at usual clinical doses? Which drugs are dual-acting at the usual clinical doses? Getting at these answers may still leave a central issue unresolved. What is the clinical significance, if any, of being more or less selective for norepinephrine or 5-HT or of having dual action? This article will review selected clinical data relevant to these questions.

## EVIDENCE SUPPORTING THE VALIDITY OF NOREPINEPHRINE AND SEROTONIN ANTIDEPRESSANT MECHANISMS OF ACTION

### Drugs Selective for Norepinephrine or Serotonin Are Effective Antidepressants

All available antidepressants increase monoamine neurotransmission by blocking reuptake or inhibiting metabolism of the neurotransmitter(s), or else blocking receptors that secondarily increase signal transduction through other

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**Table 1. Some Relatively Neurotransmitter-Selective Antidepressants**

Norepinephrine reuptake inhibitors	
Desipramine	
Nortriptyline	
Protriptyline	
Maprotiline	
Atomoxetine	
Reboxetine	
5-HT/Norepinephrine reuptake inhibitors	
Imipramine	
Doxepin	
Amtriptyline	
Trimipramine	
Venlafaxine	
Duloxetine	
Milnacipran	
5-HT reuptake inhibitors	
Fluoxetine	
Sertraline	
Paroxetine	
Fluvoxamine	
Citalopram	
Escitalopram	

Abbreviation: 5-HT = serotonin.

receptors, increase neuronal firing rate, or stimulate release of neurotransmitters.<sup>1</sup> These initial effects on monoamines are well characterized, and there are several converging lines of evidence supporting the view that they are essential for antidepressant efficacy.

First and foremost is the fact that drugs synthesized to selectively block 5-HT (i.e., selective serotonin reuptake inhibitors [SSRIs]) or norepinephrine reuptake (i.e., norepinephrine reuptake inhibitors [NRIs]) are effective in the treatment of major depression. Furthermore, the effects on histamine, acetylcholine,  $\gamma$ -aminobutyric acid (GABA), or ion channel blockade seen with older antidepressants have been eliminated in selective agents with no apparent reduction in efficacy. Thus, it is reasonable to conclude that although such effects might contribute directly or indirectly to antidepressant efficacy in some patients, they are not essential.<sup>1</sup>

We now have a large number of selective 5-HT and norepinephrine reuptake inhibitors, some of which are listed in Table 1. The evolution of SSRIs has been in the direction of increasing selectivity and potency. The recently developed escitalopram is the most potent and selective SSRI available and has demonstrated equal or greater efficacy compared with its slightly less selective and potent parent compound, citalopram.<sup>4</sup>

Although the ability to engineer these medications has sharpened, our understanding of the clinical differences between SSRIs and NRIs with regard to the symptom profiles that each improves is less clear. Nelson<sup>5</sup> published a comprehensive review of 16 studies comparing an NRI and an SSRI in patients with major depression. Most of the agents listed in Table 1 and 1563 patients were involved in the studies. Response rates were similar for the SSRIs and

NRIs (65% and 60%, respectively), and there were no consistent findings across studies when baseline symptoms that predict response were examined. Some research, however, has shown that baseline anxiety predicts preferential response to SSRIs versus NRIs.<sup>6,7</sup>

The topic of whether NRIs are as effective for treating anxiety symptoms and disorders as SSRIs continues to be an important focus of debate. This debate is fueled in part by the lack of efficacy of NRIs for obsessive-compulsive disorder<sup>8-10</sup> and posttraumatic stress disorder,<sup>11-13</sup> as well as a generally held perception that SSRIs are more effective than NRIs in depressed patients with moderate-to-severe anxiety symptoms.<sup>14</sup> Arguing against a significant class difference are the studies showing NRIs to be effective in panic disorder<sup>15-19</sup> and a recent study showing that baseline Hamilton Rating Scale for Anxiety score did not predict response to either sertraline or bupropion during treatment of depression.<sup>20</sup>

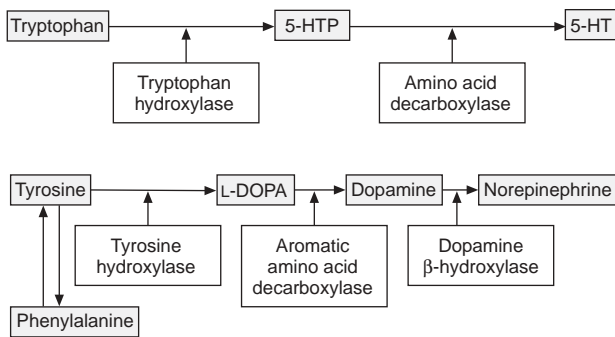
Whether or not clinical differences in the efficacy profiles of SSRIs and NRIs beyond obsessive-compulsive disorder are understood clearly, the bulk of evidence indicates that these drug classes are comparably effective in the treatment of major depression. Also supporting the notion that the primary mechanisms of these compounds are linked to antidepressant response is the fact that most of the newest compounds listed in Table 1 do not have clinically relevant effects on acetylcholine, histamine, or GABA but still are effective. The most parsimonious explanation from these data is that norepinephrine or 5-HT reuptake inhibition is the most important pharmacologic property underlying the antidepressant actions of these drugs.

### Depletion of Monoamines Selectively Reverses Antidepressant Action

A second line of evidence supporting the primary role of increased levels of norepinephrine or 5-HT in antidepressant action comes from neurotransmitter depletion studies.<sup>21-23</sup> Discovery of the synthetic pathways for monoamines in the early 1960s made it possible to deplete monoamines pharmacologically by either blocking a particular step in synthesis or limiting the availability of an essential amino acid precursor. Figure 1 shows a representation of the synthetic pathways for monoamines. The methods derived from this knowledge for neurotransmitter depletion in humans are relatively straightforward and safe.

Several reversible monoamine synthesis inhibitors have been developed and studied in humans. This work evolved from research exploring the various substrates for the key enzymes involved in monoamine synthesis. Experimentation revealed that chemical modification of some precursors caused them to act as inhibitors of key rate-limiting enzymes. The development of  $\alpha$ -methyl-*para*-tyrosine (AMPT), a reversible inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in norepinephrine and dopamine

Figure 1. Metabolic Pathways of Serotonin (5-HT), Dopamine, and Norepinephrine



Abbreviations: L-DOPA = L-dihydroxyphenylalanine,  
5-HTP = 5-hydroxytryptophan.

synthesis, marked the advent of such an approach.<sup>24</sup> AMPT treatment in man (600–4000 mg/day) decreases urinary excretion of catecholamine metabolites by up to 75%.<sup>24</sup> A 3-g/day dose of AMPT reliably reduces urinary 3-methoxy-4-hydroxyphenylethylene glycol (a major norepinephrine metabolite) by 70% and reduces cerebrospinal fluid (CSF) levels of the primary dopamine metabolite, homovanillic acid, by 61% with no change in the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA).<sup>25</sup> Maximum reduction of catecholamine metabolites during AMPT treatment occurs within 2 to 3 days of initiation of treatment, with levels returning to normal within 3 to 4 days after withdrawal of the drug.<sup>24,25</sup> Since its development, AMPT has been used extensively in laboratory animals and humans to rapidly deplete norepinephrine and dopamine.<sup>22,24–27</sup>

The synthesis of 5-HT can be inhibited similarly by ingestion of *para*-chlorophenylalanine (PCPA). PCPA inhibits the enzyme tryptophan hydroxylase, the rate-limiting step in 5-HT synthesis.<sup>28</sup> In a study of nonpsychiatric patients with 5-HT-producing carcinoid tumors, PCPA led to a variety of acute behavioral changes ranging from lethargy, irritability, anxiety, and depression to psychosis, although many subjects demonstrated little behavioral change.<sup>29</sup>

Two mid-1970s clinical studies reported that PCPA appeared to rapidly reverse the antidepressant effects of both imipramine<sup>30</sup> and tranylcypromine<sup>31</sup> in patients with major depression. Antidepressant-remitted subjects experienced a depressive relapse within 24 hours of the initiation of PCPA treatment and a return to remission within 24 hours of discontinuation. The studies were highly criticized for a lack of placebo control and the small number of patients tested, and no attempts at replication have been reported. PCPA use was discontinued in the late 1970s because of concerns over tolerability and safety, and the substance no longer is available.

Another strategy for 5-HT depletion arose from research on dietary requirements. The essential amino acid L-tryptophan is the direct precursor of 5-HT synthesis. By definition, an essential amino acid is one that cannot be synthesized by humans, making dietary intake the sole source of its availability. One implication is that 5-HT can be depleted by rapid dietary depletion of tryptophan.<sup>32</sup> L-Tryptophan depletion has become the most commonly used method for 5-HT depletion experiments.

L-Tryptophan-free or low-L-tryptophan diets administered to healthy humans cause reductions of plasma L-tryptophan levels.<sup>33–39</sup> Maintenance for up to 1 month on such diets has been reported without serious medical or psychological consequences.<sup>33</sup> Diets that reduce the plasma ratio of L-tryptophan to large neutral amino acids (LNAA) increase the competition of LNAA with L-tryptophan for passage across the blood-brain barrier, resulting in decreased levels of CSF 5-HIAA.<sup>40</sup> A 200-mg/day, low-L-tryptophan diet maintained for 8 days enhanced the prolactin response to intravenous L-tryptophan in healthy humans, suggesting the development of post-synaptic 5-HT receptor supersensitivity.<sup>38</sup>

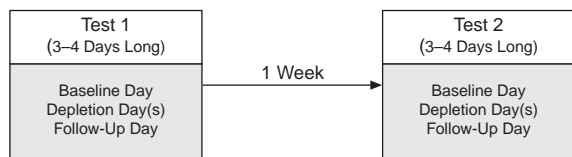
Reduction in plasma L-tryptophan of up to 80% also can be accomplished within 3 to 5 hours reliably and consistently by administering an oral L-tryptophan-free amino acid solution.<sup>21,34,35,39,41–43</sup> The drink induces hepatic protein synthesis and thereby depletes available plasma L-tryptophan, which is used in the production of new proteins.<sup>28,39</sup> Additionally, the large increase in LNAA that occurs after ingestion of an L-tryptophan-free amino acid solution competitively blocks any remaining L-tryptophan from being transported into the brain.

Data from human studies using this paradigm have been highly consistent and show that the plasma changes are associated with changes in brain 5-HT synthesis. An L-tryptophan-free amino acid drink leads to a 25% decrease in CSF 5-HIAA in healthy volunteers during continuous CSF sampling,<sup>44</sup> and similar data have been observed with single CSF sampling.<sup>45</sup> A study using positron emission tomography in healthy subjects reported an 87% reduction in 5-HT synthesis in men and a 97% reduction in women within 5 hours of ingesting the drink.<sup>46</sup>

Clinical research with neurotransmitter depletion relevant to antidepressant action has focused on 5 distinct groups: healthy people without personal or family history of depression,<sup>35–38,40,45–48</sup> medication-free people with a history of depression who were not clinically depressed,<sup>48–50</sup> symptomatic depressed patients,<sup>41,42,51</sup> SSRI-treated depressed patients,<sup>21–23,27</sup> and NRI-treated depressed patients.<sup>21–23,27</sup>

The protocol is similar across studies and is composed of a double-blind, placebo-controlled sequence of 2 test periods 1 week apart. Figure 2 shows an overview of these procedures. Each test period is 3 to 4 days in duration and involves a baseline day, depletion day(s), and a follow-up

Figure 2. Protocol for Neurotransmitter Depletion Studies



day. During one of the two test periods, a real depletion occurs and during the other, no depletion occurs. For L-tryptophan depletion, subjects are administered an amino acid drink that either has or does not have L-tryptophan. For depletion of norepinephrine and dopamine, subjects are administered either AMPT or the “active” placebo diphenhydramine, 25 mg t.i.d.<sup>22</sup>

In studies of the relative importance of norepinephrine or 5-HT to maintaining the effects of antidepressants,<sup>21–23,27</sup> patients received up to 12 weeks of antidepressant therapy and entered the depletion phase if they achieved and maintained a 50% reduction in Hamilton Rating Scale for Depression score from baseline for 2 consecutive weeks. For the duration of the studies, these subjects also continued taking antidepressant medication at dose levels that yielded response. Table 2 summarizes the study results.

One of the most striking findings from these studies is how swiftly subjects react to the depletions. Antidepressant response takes several weeks to occur, and yet in these patients depressive symptoms returned acutely during depletion and then disappeared quickly as the depletion wore off. Making the results all the more remarkable is the fact that these reactions occurred in spite of antidepressant medication maintenance. Mood changes that occurred typically lasted from 4 to 36 hours. In addition, the symptoms reported by patients during depletion often were conspicuously similar to those experienced prior to treatment.<sup>21</sup>

These results confirm that monoamines are necessary for maintaining antidepressant therapeutic response. Furthermore, in spite of the fact that interactions between norepinephrine and 5-HT in the brain are well known,<sup>52</sup> these systems appear to be independent of one another in the context of maintaining antidepressant action. The ability to selectively deplete and reverse the antidepressant effects in people taking single-acting agents suggests that, at least in those patients, only one particular neurotransmitter had a role in the mechanism of action of that medication.

### CONCLUSIONS

Basic science studies have shown that all current antidepressants have potent effects on monoamine systems,

Table 2. Neurotransmitter Depletion and Antidepressant Effects in Responders to SSRI or NRI Treatment<sup>a</sup>

Intervention	Statistically Significant Increase in Depressive Symptoms Compared With Placebo?	
	SSRI Responders	NRI Responders
Norepinephrine/dopamine depletion <sup>b</sup>	No	Yes
5-HT depletion <sup>c</sup>	Yes	No
Sham <sup>d</sup>	No	No

<sup>a</sup>Data from Delgado et al.<sup>21–23</sup> and Miller et al.<sup>27</sup> Patients had achieved and for 2 consecutive weeks maintained a 50% reduction in Hamilton Rating Scale for Depression score from baseline.

<sup>b</sup>Via ingestion of  $\alpha$ -methyl-*para*-tyrosine, 1 g t.i.d.

<sup>c</sup>Via ingestion of a 100-g, tryptophan-free, 15-amino acid drink.

<sup>d</sup>For 5-HT depletion, a tryptophan-containing amino acid drink was administered; for norepinephrine/dopamine depletion, diphenhydramine was administered.

Abbreviations: 5-HT = serotonin, NRI = norepinephrine reuptake inhibitor, SSRI = selective serotonin reuptake inhibitor.

although the paths between these effects and clinical outcomes remain poorly understood. Two converging lines of evidence provide a convincing body of data supporting the primary role for norepinephrine and 5-HT in antidepressant actions: Drugs that selectively increase either norepinephrine or 5-HT are effective in the treatment of depression, and the effects of these agents can be reversed selectively by depleting norepinephrine or 5-HT.

The evidence proves that increasing monoamines is the initial, necessary step for antidepressant action. Identification of which biological change is most proximal to the actual therapeutic response, however, remains elusive. The answer may be related to the fact that, while not achieving full potential, monoamine levels increase by 100% to 300% within hours of the initial dose of medication,<sup>53</sup> and yet therapeutic response lags by at least 2 to 4 weeks. Current theories aimed at explaining this discrepancy focus on the adaptive changes that occur in non-monoamine neurons as a result of increased monoamine levels.<sup>54–56</sup> More recent data suggest that vulnerability to major depression may relate to cellular dysfunction in key limbic areas of the brain.<sup>57</sup> It is hypothesized that the effects of increased monoamines on dysfunctional neurons of the limbic system may partially restore their ability to function.<sup>23</sup> The rapid return of depression in antidepressant-treated patients during neurotransmitter depletion may be a reflection of the fragility of these neurons.

Also unresolved is whether the separate effects of increasing norepinephrine and 5-HT could be additive in causing antidepressant action, or if perhaps they are 2 separate and distinct pathways to the same endpoint. A somewhat ancillary question is whether selective norepinephrine or 5-HT drugs have different profiles of symptom response. In spite of failed prior efforts<sup>5,20</sup> to find differences in symptom profile between patients responding to SSRIs or NRIs, I would argue that such effects are likely to be present. To address these questions directly,

prospective studies that specifically focus on key symptoms thought to differ between patients responding to SSRIs and NRIs are required. Vital to such studies are rating scales that are more sensitive to changes in symptoms. Obtaining the answers to these and other questions will help us to refine antidepressant treatments to yield better outcomes.

*Drug names:* amitriptyline (Elavil and others), atomoxetine (Strattera), bupropion (Wellbutrin and others), citalopram (Celexa), desipramine (Norpramin and others), doxepin (Sinequan and others), escitalopram (Lexapro), fluoxetine (Prozac and others), imipramine (Tofranil and others), maprotiline (Ludiomil and others), nortriptyline (Pamelor, Aventyl, and others), paroxetine (Paxil and others), sertraline (Zoloft), tranylcypromine (Parnate), trimipramine (Surmontil), venlafaxine (Effexor).

## REFERENCES

1. Bolden-Watson C, Richelson E. Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. *Life Sci* 1993;52:1023–1029
2. Owens MJ. Selectivity of antidepressants: from the monoamine hypothesis of depression to the SSRI revolution and beyond. *J Clin Psychiatry* 2004;65(suppl 4):5–10
3. Lucki I, O'Leary OF. Distinguishing roles for norepinephrine and serotonin in the behavioral effects of antidepressant drugs. *J Clin Psychiatry* 2004;65(suppl 4):11–24
4. Gorman JM, Korotzer A, Su G. Efficacy comparison of escitalopram and citalopram in the treatment of major depressive disorder: pooled analysis of placebo-controlled trials. *CNS Spectr* 2002;7[4 suppl 1]:40–44
5. Nelson JC. A review of the efficacy of serotonergic and noradrenergic reuptake inhibitors for treatment of major depression. *Biol Psychiatry* 1999;46:1301–1308
6. Tyrer PJ, Lee I, Edwards JG, et al. Prognostic factors determining response to antidepressant drugs in psychiatric out-patients and general practice. *J Affect Disord* 1980;2:149–156
7. Aberg-Wistedt A. A double-blind study of zimelidine, a serotonin uptake inhibitor, and desipramine, a noradrenaline uptake inhibitor, in endogenous depression, I: clinical findings. *Acta Psychiatr Scand* 1982;66:50–65
8. Goodman WK, Price LH, Delgado PL, et al. Specificity of serotonin reuptake inhibitors in the treatment of obsessive-compulsive disorder: comparison of fluvoxamine and desipramine. *Arch Gen Psychiatry* 1990;47:577–585
9. Leonard BE. The comparative pharmacology of new antidepressants. *J Clin Psychiatry* 1993;54(suppl 8):3–15
10. Lelliott PT, Monteiro WO. Drug treatment of obsessive-compulsive disorder. *Drugs* 1986;31:75–80
11. Reist C, Kauffmann CD, Haier RJ, et al. A controlled trial of desipramine in 18 men with posttraumatic stress disorder. *Am J Psychiatry* 1989;146:513–516
12. Dow B, Kline N. Antidepressant treatment of posttraumatic stress disorder and major depression in veterans. *Ann Clin Psychiatry* 1997;9:1–5
13. Canive JM, Clark RD, Calais LA, et al. Bupropion treatment in veterans with posttraumatic stress disorder: an open study. *J Clin Psychopharmacol* 1998;18:379–383
14. Blackwell B. Side effects of antidepressant drugs. In: Hales RE, Frances AJ, eds. *The American Psychiatric Association Annual Review*. Arlington, Va: American Psychiatric Press; 1987:724–725
15. Kalus O, Asnis GM, Rubinson E, et al. Desipramine treatment in panic disorder. *J Affect Disord* 1991;21:239–244
16. Lydiard RB, Morton WA, Emmanuel NP, et al. Preliminary report: placebo-controlled, double-blind study of the clinical and metabolic effects of desipramine in panic disorder. *Psychopharmacol Bull* 1993;29:183–188
17. Villarreal G. Desipramine plasma levels and treatment response in panic disorder [letter]. *Can J Psychiatry* 1995;40:110–111
18. Versiani M, Cassano G, Perugi G, et al. Reboxetine, a selective norepinephrine reuptake inhibitor, is an effective and well-tolerated treatment for panic disorder. *J Clin Psychiatry* 2002;63:31–37
19. Sasson Y, Iancu I, Fux M, et al. A double-blind crossover comparison of clomipramine and desipramine in the treatment of panic disorder. *Eur Neuropsychopharmacol* 1999;9:191–196
20. Rush AJ, Batey SR, Donahue RM, et al. Does pretreatment anxiety predict response to either bupropion SR or sertraline? *J Affect Disord* 2001;64:81–87
21. Delgado PL, Charney DS, Price LH, et al. Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry* 1990;47:411–418
22. Delgado PL, Miller HL, Salomon RM, et al. Monoamines and the mechanism of antidepressant action: effects of catecholamine depletion on mood of patients treated with antidepressants. *Psychopharmacol Bull* 1993;29:389–396
23. Delgado PL, Miller HL, Salomon RM. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry* 1999;46:212–220
24. Engelman K, Horwitz D, Jequier E, et al. Biochemical and pharmacologic effects of alpha-methyltyrosine in man. *J Clin Invest* 1968;47:577–594
25. Bunney WE, Brodie HK, Murphy DL, et al. Studies of alpha-methyl-para-tyrosine, L-dopa, and L-tryptophan in depression and mania. *Am J Psychiatry* 1971;127:872–881
26. Bunney WE, Davis JM. Norepinephrine in depressive reactions: a review. *Arch Gen Psychiatry* 1965;13:483–494
27. Miller HL, Delgado PL, Salomon RM, et al. Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. *Arch Gen Psychiatry* 1996;53:117–128
28. Harper AE, Benevenga NJ, Wohlhueter RM. Effects of ingestion of disproportionate amounts of amino acids. *Physiol Rev* 1970;50:428–558
29. Serotonin now: clinical implications of inhibiting its synthesis with para-chlorophenylalanine. *Ann Intern Med* 1970;73:607–630
30. Shopsin B, Gershon S, Goldstein M, et al. Use of synthesis inhibitors in defining a role for biogenic amines during imipramine treatment in depressed patients. *Psychopharmacol Commun* 1975;1:239–249
31. Shopsin B, Friedman E, Gershon S. Parachlorophenylalanine reversal of tranylcypromine effects in depressed patients. *Arch Gen Psychiatry* 1976;33:811–819
32. Curzon G. Relationships between plasma, CSF and brain tryptophan. *J Neural Transm Suppl* 1979;15:81–92
33. Rose WC, Haines WJ, Warner DT. The amino acid requirements of man. *J Biol Chem* 1954;206:421–430
34. Young SN, Ervin FR, Pihl RO, et al. Biochemical aspects of tryptophan depletion in primates. *Psychopharmacology (Berl)* 1989;98:508–511
35. Young SN, Smith SE, Pihl RO, et al. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)* 1985;87:173–177
36. Young VR, Hussein MA, Murray E. Tryptophan intake, spacing of meals, and diurnal fluctuations of plasma tryptophan in men. *Am J Clin Nutr* 1969;22:1563–1567
37. Young VR, Hussein MA, Murray E. Plasma tryptophan response curve and its relation to tryptophan requirements in young adult men. *J Nutr* 1971;101:45–59
38. Delgado PL, Charney DS, Price LH, et al. Neuroendocrine and behavioral effects of dietary tryptophan restriction in healthy subjects. *Life Sci* 1989;45:2323–2332
39. Moja EA, Cipolla P, Castoldi D, et al. Dose-response decrease in plasma tryptophan and in brain tryptophan and serotonin after tryptophan-free amino acid mixtures in rats. *Life Sci* 1989;44:971–976
40. Perez-Cruet J, Chase TN, Murphy DL. Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans. *Nature* 1974;248:693–695
41. Delgado PL, Price LH, Miller HL, et al. Serotonin and the neurobiology of depression: effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatry* 1994;51:865–874
42. Delgado PL, Price LH, Miller HL, et al. Rapid serotonin depletion as a provocative challenge test for patients with major depression: relevance to antidepressant action and the neurobiology of depression. *Psychopharmacol Bull* 1991;27:321–330
43. Moreno FA, Gelenberg AJ, Heninger GR. Tryptophan depletion and depressive vulnerability. *Biol Psychiatry* 1999;46:498–505

44. Carpenter LL, Anderson GM, Pelton GH. Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology* 1998;19:26–35
45. Moreno FA, McGavin C, Malon TP. Tryptophan depletion selectively reduces CSF 5-HT metabolites in healthy young men: results from single lumbar puncture sampling technique. *Int J Neuropsychopharmacol* 2000;3:277–283
46. Nishizawa S, Benkelfat C, Young SN, et al. Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* 1997;94:5308–5313
47. Benkelfat C, Ellenbogen MA, Dean P. Mood-lowering effect of tryptophan depletion: enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* 1994;51:687–697
48. Smith KA, Fairburn CG, Cowen PG. Relapse of depression after rapid depletion of tryptophan. *Lancet* 1997;349:915–919
49. Leyton M, Young SN, Blier P. The effect of tryptophan depletion on mood in medication-free, former patients with major affective disorder. *Neuropsychopharmacology* 1997;16:294–297
50. Moreno FA, Heninger GR, McGahuey CA. Tryptophan depletion and risk of depression relapse: a prospective study of tryptophan depletion as potential predictor of depressive episodes. *Biol Psychiatry* 2000;48:327–329
51. Miller HL, Delgado PL, Salomon RM, et al. Effects of (alpha)-methyl-para-tyrosine (AMPT) in drug-free depressed patients. *Neuropsychopharmacology* 1996;14:151–157
52. Sulser F, Sanders-Bush E. The serotonin-norepinephrine link hypothesis of affective disorders: receptor-receptor interactions in the brain. In: Ehrlich YH, ed. *Molecular Basics of Neuronal Responsiveness*. New York, NY: Plenum Press; 1997:439–502
53. Bymaster FP, Zhang W, Carter PA, et al. Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl)* 2002;160:353–361
54. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry* 1997;54:597–606
55. Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* 1999;46:1181–1191
56. Hyman SE, Nestler EJ. Initiation and adaptation: a paradigm for understanding psychotropic drug action. *Am J Psychiatry* 1996;153:151–162
57. Rajkowska G. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol Psychiatry* 2000;48:766–777