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Comparative Serotonin Neurotoxicity of the Stereoisomers of Fenfluramine and Norfenfluramine

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JOHNSON, M. P. AND D. E. NICHOLS. *Comparative serotonin neurotoxicity of the stereoisomers of fenfluramine and norfenfluramine*. PHARMACOL BIOCHEM BEHAV **36**(1) 105–109, 1990.—The optical isomers of fenfluramine and norfenfluramine were administered to rats to examine their relative potency for destruction of serotonin neurons. Rats were sacrificed one week following a single 10 mg/kg SC injection of one of the four compounds and monoamine and metabolite levels in the frontal cortex and hippocampus brain regions were examined by HPLC-EC techniques. In addition, [^{3}H]-paroxetine binding to homogenates of these brain regions was determined. With the exception of hippocampal 5-HT levels following *d*-fenfluramine treatment, there was a decrease in all the serotonergic markers assayed, following treatment with the *d*-enantiomers of fenfluramine and norfenfluramine. No decrease in any serotonergic marker was seen at this dose following treatment with the *l*-enantiomers of fenfluramine or norfenfluramine. Also, none of the drug treatments resulted in a significant decrease in catecholamines or their metabolites. With all the serotonergic markers examined, *d*-norfenfluramine was found to cause a significantly greater decrease than *d*-fenfluramine. The significance of these results is discussed in terms of the hypothesis that the long-term serotonergic deficits observed with *d*-fenfluramine may result from its metabolite, *d*-norfenfluramine.

Fenfluramine Norfenfluramine Serotonin HPLC-EC [^{3}H]-Paroxetine Neurotoxicity

RACEMIC fenfluramine is a substituted amphetamine which shows anorectic activity but lacks the psychostimulant effects of amphetamine (19). There is increasing evidence that the anorectic actions of fenfluramine are a result of enhancement of central serotonergic function (15,36). One of the important properties of this drug is that each stereoisomer appears to have a different primary action. While it is the *d*-isomer of fenfluramine that is a more potent anorectic, *l*-fenfluramine is reported to be an anti-dopaminergic agent similar to haloperidol in its actions (5). However, mechanistic studies are complicated by the fact that fenfluramine is metabolized to its N-dealkylated analog, norfenfluramine, which is an active metabolite (3, 15, 29). *d*-Norfenfluramine has been found to be both an effective anorectic agent and a serotonergic releaser (4,22).

Several recent articles have dealt with the significance of a potentially harmful side effect of fenfluramine, its action as a serotonin neurotoxin (1,9). Earlier work by Clineschmidt and co-workers (8) and Steranka and Sanders-Bush (33), that has been more recently confirmed by Wagner and Peroutka (35), and by Contrera and co-workers (7), indicates that long-term depletion of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) occurs after administration of racemic or *d*-fenfluramine. It has been suggested that this is due to a selective degeneration of the ascending fine-axon serotonergic projections from the dorsal raphe nucleus (23).

An important consideration regarding neurotoxicity is the possibility that the primary metabolite, *d*-norfenfluramine, is responsible for the long-term serotonergic depletion seen with *d*-fenfluramine. Several observations seem to support the validity of this hypothesis. For instance, while fenfluramine has been found to be more potent as a serotonin uptake inhibitor than as a releaser, norfenfluramine is a much more potent releaser of 5-HT than it is an uptake inhibitor (4,22). Serotonin neurotoxicity has been associated with other serotonergic releasers such as *p*-chloroamphetamine (PCA) (18), 3,4-methylenedioxyamphetamine (MDA) (27) and 3,4-methylenedioxymethamphatamine (MDMA) (31,34), but not with serotonin uptake inhibitors. Indeed, administration of a 5-HT uptake inhibitor can actually block the neurotoxicity induced by PCA, MDMA or fenfluramine (11,30). Thus, the idea that norfenfluramine may be involved in the neurotoxic effects of fenfluramine seems reasonable.

However, experiments designed to test this hypothesis directly have been inconclusive (12,32). Considering the differences in the pharmacology of the optical isomers of fenfluramine and norfen-

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fluramine, it would be useful to compare the neurotoxic activity of these four compounds. It was anticipated that if norfenfluramine is critically involved in the neurotoxic effects of fenfluramine, then a similar stereoselectivity would be apparent for both compounds. Since fenfluramine is only partially metabolized to norfenfluramine (24), *d*-norfenfluramine would also have to be more potent than *d*-fenfluramine as a neurotoxin. Therefore, in the present report, the neurotoxic potential of the stereoisomers of fenfluramine and norfenfluramine was examined. One week following a single injection, the neurotoxic potency of the test compounds was assessed using two procedures: the measurement of the levels of 5-HT and 5-HIAA using HPLC with electrochemical detection, and changes in the parameters of homogenate binding of [^{3}H]-paroxetine, a serotonin uptake inhibitor. In combination, these procedures correlate well with the degree of neurotoxicity seen with other substituted amphetamines (2,26).

METHOD

HPLC With Electrochemical Detection

Individually housed male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175–200 g were given free access to food and water. Drugs were administered as a single subcutaneous injection, with a solution of the HCl salt equivalent to 10 mg/kg of the free bases, in a volume of 1.0 ml/kg of sterile saline. The animals were sacrificed by decapitation one week following drug treatment and the frontal cortex and hippocampus brain regions were dissected (16) over ice and immediately frozen in liquid nitrogen for storage at −70°C until assay. One hemisphere from each brain region was used in [^{3}H]-paroxetine experiments described below, and monoamine and metabolite levels were assayed in the other hemisphere.

The brain regions were weighed and placed in 0.5 ml of 0.4 N $HClO_4$ containing 0.05% Na_2EDTA, 0.1% $Na_2S_2O_5$, and 50 ng/ml of N-acetyl-5-hydroxytryptamine as an internal standard. The samples were then homogenized with a motor-driven teflon pestle specially designed to fit a 1.5 ml eppendorf centrifuge tube and were then centrifuged at 15,000 × g for 4 min with a tabletop centrifuge. The supernatant was assayed for catecholamines, 5-HT, and their metabolites using the following conditions. A mobile phase containing 0.05 M NaH_2PO_4, 0.03 M citric acid, 0.1 mM Na_2EDTA, 0.25% sodium octyl sulfate and 25% methanol (pH 2.75) was eluted through a reverse phase C_{18} cartridge column (Brownlee Laboratories, Ann Arbor, MI) at a flow rate of 0.7 ml/min. A Princeton 400 electrochemical detector with a Ag/AgCl electrochemical cell (Lawrenceville, NJ) was set at a voltage of −850 mV with a pre-electric voltage of −60 mV. The following were identified by their retention times and quantitated using a Hitachi D-2000 integrator: NE, 4.2 min; DOPAC, 4.7 min; 5-HIAA, 5.7 min; N-Ac-5-HT, 6.2 min; DA, 7.2 min; HVA, 9.2 min; and 5-HT, 13.2 min, respectively.

[^{3}H]-Paroxetine Binding Studies

A modified procedure of Habert and co-workers (17) was employed. The hippocampus regions from 2 rats were combined and weighed to provide a sufficient amount of tissue. The frontal cortical region from each rat was assayed separately. The tissue sample was homogenized in 15 ml of 50 mM tris, containing 120 mM NaCl and 5 mM KCl (pH 7.4) using a Brinkman polytron (setting 6, 2 × 20 sec). The homogenates were centrifuged twice at 30,000 × g for 10 min, with an intermittent wash.

The resulting tissue homogenate was added to increasing concentrations of [^{3}H]-paroxetine (0.1 to 2.5 nM) to give a final volume of 2 ml. Fluoxetine (10 μM) was utilized to define specific binding. The tubes were allowed to equilibrate for 1 hr at 24°C before filtering through GF/C filters presoaked in 0.05% polyethyleneimine using a Brandel cell harvester. The tubes were washed twice with 5 ml of cold buffer and the filters were air dried before being placed into scintillation vials with 10 ml of Aqueous Counting Scintillant (ACS, Amersham). Samples were allowed to sit overnight before counting at an efficiency of 46% with a Packard 4000 Scintillation counter. Data analysis employed the least squares curve fitting routines embodied in the programs EBDA and LIGAND as described by McPherson (20). The protein concentrations were determined by the method of Bradford (6).

Drugs

Each group of rats received a single subcutaneous injection of saline vehicle or the hydrochloride of either *d*-fenfluramine, *l*-fenfluramine, *d*-norfenfluramine or *l*-norfenfluramine. The dosages were corrected for the salt forms so that drug treatments gave 10 mg/kg of the free base. Eight animals were used in each of the five treatment groups. The enantiomers of fenfluramine·HCl and norfenfluramine·HCl were synthesized in this laboratory by modifications of a previously described method (25). The HPLC standards were purchased from Sigma (St. Louis, MO). [^{3}H]-Paroxetine (29.4 Ci/mmol) was purchased from New England Nuclear (Boston, MA).

Statistical Analysis

Analysis of variance was utilized to examine all the data. Individual treatment comparisons versus control were made using Dunnett's procedure. Comparison between the *d*-fenfluramine and *d*-norfenfluramine treatment groups was made using Student's *t*-test with the Bonferroni adjustment (28).

RESULTS

As seen in Table 1, there is a decrease in the levels of 5-HT and 5-HIAA one week following a single injection of *d*-fenfluramine or *d*-norfenfluramine compared with saline. With the exception of hippocampal 5-HT and 5-HIAA levels and cortical 5-HIAA levels following *d*-fenfluramine administration, these decreases are statistically significant in both the frontal cortex and hippocampus. This is a marked stereoselective effect since there was no significant decrease in any markers following treatment with the *l*-enantiomers. Importantly, there is significantly more depletion of 5-HT and 5-HIAA in both brain regions following treatment with *d*-norfenfluramine as compared to *d*-fenfluramine. These decreases are apparently selective for serotonin neurons in that no changes in the levels of NE, DA or their metabolites were observed with any of the drug treatments.

The results of the [^{3}H]-paroxetine binding shown in Fig. 1 parallel those described above. Compared with controls, there was a significant decrease in the B_{max} values following treatment with the *d*-isomers of fenfluramine or norfenfluramine but not with the *l*-isomers. K_D values associated with [^{3}H]-paroxetine binding were not altered except following treatment with *d*-norfenfluramine. The K_D (± SE) values in rat frontal cortex and hippocampus were: saline, 123 (12) and 167 (15) pM; *d*-fenfluramine, 136 (15) and 136 (13) pM; *l*-fenfluramine, 122 (13) and 157 (7) pM; *d*-norfenfluramine, 232 (28) and 416 (80) pM; *l*-norfenfluramine, 141 (14) and 190 (10) pM, respectively.

DISCUSSION

Several recent articles have discussed the significance of potential neurotoxicity associated with fenfluramine (1,9). Although the mechanism of this toxicity has not been fully elucidated, fenfluramine's acute anorectic activity has been extensively

TABLE 1

THE EFFECTS OF THE ENANTIOMERS OF FENFLURAMINE AND NORFENFLURAMINE AT ONE WEEK POSTTREATMENT*

Treatment	Monoamine Levels (pg/mg wet wt.) NE	5-HT	5-HIAA	DA	DOPAC	HVA
Cortex						
Saline	447 ± 17	204 ± 17	293 ± 15	82 ± 16	91 ± 12	103 ± 10
d-fenfluramine	427 ± 16	122 ± 6†	242 ± 13	83 ± 26	75 ± 10	111 ± 15
l-fenfluramine	477 ± 20	226 ± 23	292 ± 17	77 ± 9	66 ± 8	95 ± 16
d-norfenfluramine	413 ± 18	49 ± 7‡§	99 ± 16‡§	79 ± 18	88 ± 10	100 ± 12
l-norfenfluramine	464 ± 16	176 ± 26	254 ± 24	64 ± 9	67 ± 6	96 ± 14
Hippocampus						
Saline	418 ± 15	119 ± 12	455 ± 23	—	—	—
d-fenfluramine	415 ± 36	95 ± 13	381 ± 22	—	—	—
l-fenfluramine	465 ± 22	116 ± 20	530 ± 32	—	—	—
d-norfenfluramine	382 ± 22	51 ± 5†§	170 ± 20‡§	—	—	—
l-norfenfluramine	473 ± 20	136 ± 17	457 ± 42	—	—	—

*Saline, or 10 mg/kg of the test drugs was injected SC and the animals sacrificed one week later. The monoamines and their metabolites in the frontal cortex and hippocampus of each rat were determined by HPLC-EC. The DA, DOPAC and HVA levels in the rat hippocampus were below the sensitivity level of the detector. The values represent the mean ± SE for n = 8.

†Significantly decreased from control ($p<0.05$, Dunnett's comparison).

‡Significantly decreased from control ($p<0.001$, Dunnett's comparison).

§Significantly decreased from *d*-fenfluramine ($p<0.05$, Student's *t*-test with Bonferroni adjustment).

investigated [for review see (15) and (19)]. Fenfluramine is reported to be a serotonin uptake inhibitor and vesicular releaser, while norfenfluramine releases serotonin from a nonvesicular pool (4,22). One theory has been developed that the anorectic activity of fenfluramine may actually be due to the 5-HT releasing effect of its metabolite, norfenfluramine (15,19). However, previous reports [e.g., (14)] and a more recent study (13) suggest that it is a combination of the actions of fenfluramine and norfenfluramine that accounts for the short-term anorectic activity.

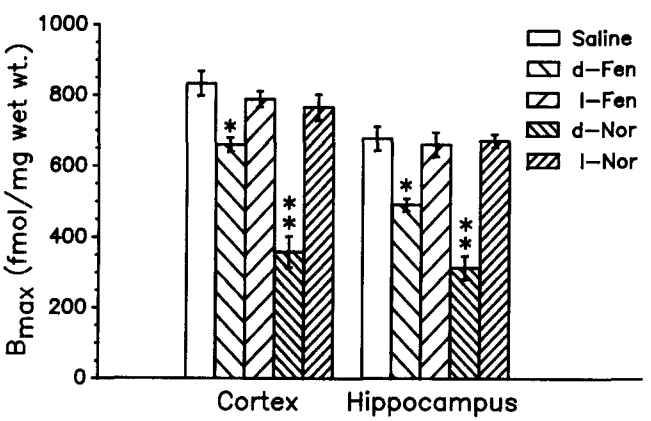

FIG. 1. The B_{max} value for [^{3}H]-paroxetine binding one week following drug treatment. The concentration of [^{3}H]-paroxetine ranged from 0.1 to 2.5 nM. The tissue from two rats was combined in hippocampus experiments to give an n = 4. In frontal cortex experiments n = 8 was used. The values represent the mean ± S.E. *Indicates a significant decrease from control ($p<0.001$, Dunnett's comparison). **Indicates a significant decrease from control ($p<0.001$, Dunnett's comparison), and a significant decrease from *d*-fenfluramine treatment [$p<0.001$, Student's *t*-test with Bonferroni adjustment (28)].

In contrast, the importance of metabolism in regard to the long-term effects of fenfluramine has not been well studied. Given the apparent correlation between neurotoxic and serotonin releasing activity among substituted amphetamines such as *p*-chloroamphetamine (18) and 3,4-methylenedioxyamphetamine (27), one might anticipate that the more potent serotonin releaser, norfenfluramine, would be the more potent neurotoxic agent. Schuster and co-workers (32) have attempted to examine this issue by observing the long-term effects of racemic fenfluramine in guinea pigs, since N-dealkylation was thought not to occur in that species. Those investigators found that fenfluramine retained its long-term serotonin neurotoxic effects, concluding that fenfluramine metabolism to norfenfluramine is not required. Schuster *et al.* (32) also cited Garattini *et al.* (14) as providing evidence that blockade of fenfluramine metabolism by pretreatment with the P-450 inhibitor SKF-525A failed to attenuate the long-term toxicity. However, Garattini and co-workers (14) examined only the acute 5-HT reduction by fenfluramine and neither Schuster *et al.* nor Garattini *et al.* provided conclusive evidence that fenfluramine was not metabolized to norfenfluramine in their experiments. Furthermore, a recent report by Fuller and co-workers (12) found that brain levels of norfenfluramine greatly exceeded those of fenfluramine at 1 to 4 hr posttreatment with fenfluramine in the guinea pig. Therefore, to date there has been no conclusive evidence to suggest that metabolism to norfenfluramine is not required for the long-term toxicity associated with fenfluramine.

We attempted a more direct investigation of this hypothesis by pretreating rats with SKF-525A (data not shown). A 45-min pretreatment with 50 mg/kg SKF-525A or saline (IP) preceded a subcutaneous injection of either 10 mg/kg *d*-fenfluramine, *d*-norfenfluramine or saline, with sacrifice one week later. In that experiment SKF-525A had no effect on the long-term serotonin deficits produced either by *d*-norfenfluramine or by *d*-fenfluramine. However, the ability of SKF-525A to actually block the in

vivo metabolism of fenfluramine was not established. Furthermore, an earlier investigation by Fuller and Baker (10) found that microsomal oxidase inhibitors were ineffective in blocking the N-dealkylation of several N-alkyl-*p*-chloroamphetamines, suggesting that SKF-525A may not be an effective inhibitor of N-dealkylation for substituted amphetamines. Without studies establishing brain levels of fenfluramine and norfenfluramine following SKF-525A, no reliable conclusions can be drawn.

Despite this problem, several lines of evidence suggest that metabolism to norfenfluramine is required for neurotoxicity. As previously mentioned, fenfluramine is predominately an uptake inhibitor at 5-HT neurons, and no "pure" 5-HT uptake inhibitors are serotonin neurotoxins. In contrast, several nonvesicular serotonin releasers, such as PCA (18) and norfenfluramine (present study), result in long-term depletion of 5-HT and 5-HIAA. As apparent from the results obtained here, the *d*-enantiomers of both fenfluramine and norfenfluramine are more potent serotonin depletors than their *l*-antipodes. This also parallels the stereoselectivity seen for anorectic activity and for serotonin release/uptake inhibition. Finally, *d*-norfenfluramine is a more potent neurotoxin than *d*-fenfluramine. Therefore, the long-term effects of *d*-fenfluramine might be explained by the actions of *d*-norfenfluramine.

It should be noted that the K_D values were found to be significantly higher ($p<0.001$, Dunnett's comparison) one week following treatment with *d*-norfenfluramine. This phenomenon has been previously observed in our laboratory following treatment with potent serotonergic neurotoxins such as *p*-chloroamphetamine (unpublished results). It seems possible that the levels of 5-HT uptake sites normally measured may be so low that [^{3}H]-paroxetine is now labeling some other type of site. Alternatively, the lower affinity of [^{3}H]-paroxetine in *d*-norfenfluramine-treated animals may reflect binding to a 5-HT uptake site associated with neurotoxin-resistant thick axon serotonergic projections from the median raphe nucleus (21,23). Although there is no present evidence to support this, it seems possible that the binding and kinetics of the 5-HT uptake site on the thick axon terminals may differ from those on the neurotoxin-sensitive class of 5-HT neurons. In either case, the B_{max} observed (Fig. 1) following *d*-norfenfluramine may not be an accurate representation of the number of 5-HT uptake sites remaining on the fine-axon serotonergic neurons.

One possible explanation for the decreased levels of serotonin following either *d*-fenfluramine or *d*-norfenfluramine treatment is the decreased dietary intake caused by their potent anorectic effects. However, other reports indicate that *d*-norfenfluramine is not a significantly more potent anorectic than *d*-fenfluramine (22). By contrast, in the present study (Table 1), norfenfluramine significantly decreased 5-HT to a greater extent than fenfluramine. In addition, one would not expect dietary decreases to profoundly alter the levels of the serotonin uptake carrier as was seen in the present study (Fig. 1).

In conclusion, the evidence to date is suggestive that metabolism of fenfluramine to norfenfluramine plays a partial and possibly critical role in the long-term toxic effects observed. However, a definitive proof for the role of metabolism must await the development of a means to block reliably the metabolic formation of norfenfluramine.

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REFERENCES

1. Barnes, D. M. Neurotoxicity creates regulatory dilemma. Science 243:29–30; 1989.
2. Battaglia, G.; Yeh, S. Y.; O'Hearn, E.; Molliver, M. E.; Kuhar, M. J.; De Souza, E. B. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroys serotonin terminals in rat brain: Quantification of neuro degeneration by measurement of [^{3}H]-paroxetine-labeled serotonin uptake sites. J. Pharmacol. Exp. Ther. 242: 911–916; 1987.
3. Beregi, L. G.; Hugon, P.; Le Douarec, J. C.; Laubie, M.; Duhault, D. Structure-activity relationships in CF_3 substituted phenethylamines. In: Costa, E.; Garattini, S., eds. Amphetamines and related compounds. New York: Raven Press; 1970:21–61.
4. Borroni, E.; Ceci, A.; Garattini, S.; Mennini, T. Differences between *d*-fenfluramine and *d*-norfenfluramine in serotonin presynaptic mechanisms. J. Neurochem. 40:891–893; 1983.
5. Bettini, E.; Ceci, A.; Spinelli, R.; Samanin, R. Neuroleptic-like effects of the *l*-isomer of fenfluramine on striatal dopamine release in freely moving rats. Biochem. Pharmacol. 36:2387–2391; 1987.
6. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
7. Contrera, J. F.; Battaglia, G.; Zaczek, R.; De Souza, E. B. Fenfluramine neurotoxicity: Selective degeneration and recovery of brain serotonin neurons. Soc. Neurosci. Abstr. 14:222.1; 1988.
8. Clinschmidt, B. V.; Totaro, J. A.; McGuffin, J. C.; Pflueger, A. B. Fenfluramine: Long-term reduction in brain serotonin (5-hydroxytryptamine). Eur. J. Pharmacol. 35:211–214; 1976.
9. Derôme-Tremblay, M.; Nathan, C. Fenfluramine studies. Science 243:901; 1989.
10. Fuller, R. W.; Baker, J. C. The role of metabolic N-dealkylation in the action of *p*-chloroamphetamine and related drugs on brain 5-hydroxytryptamine. J. Pharm. Pharmacol. 29:561–562; 1977.
11. Fuller, R. W.; Perry, K. W.; Molloy, B. B. Reversible and irreversible phases of serotonin depletion by 4-chloroamphetamine. Eur. J. Pharmacol. 33:119–124; 1975.
12. Fuller, R. W.; Snoddy, H. D.; Perry, K. W. Metabolism of fenfluramine to norfenfluramine in guinea-pigs. J. Pharm. Pharmacol. 40:439–441; 1988.
13. Fuller, R. W.; Snoddy, H. D.; Robertson, D. W. Mechanisms of effects of *d*-fenfluramine on brain serotonin metabolism in rats: Uptake inhibition versus release. Pharmacol. Biochem. Behav. 30: 715–721; 1988.
14. Garattini, S.; Jori, A.; Buczka, W.; Samanin, R. The mechanism of action of fenfluramine. Postgrad. Med. J. 51(Suppl. 1):27–35; 1975.
15. Garattini, S.; Caccia, S.; Mennini, T.; Samanin, R.; Consolo, S.; Ladinsky, H. Biochemical pharmacology of the anorectic drug fenfluramine. Curr. Med. Res. Opin. 1(Suppl. 6):15–27; 1979.
16. Glowinski, J.; Iverson, L. L. Regional studies of catecholamines in rat brain. I. The disposition of [^{3}H]-norepinephrine, [^{3}H]-dopamine, and [^{3}H]-DOPA in various regions of the brain. J. Neurochem. 13: 665–669; 1966.
17. Habert, E.; Graham, D.; Tahraoui, L.; Claustre, Y.; Langer, S. Z. Characterization of [^{3}H]-paroxetine binding to rat cortical membranes. Eur. J. Pharmacol. 118:107–114; 1985.
18. Harvey, J. A.; McMaster, S.; Yunger, L. *p*-Chloroamphetamine: Selective neurotoxic action in brain. Science 187:841–843; 1975.
19. Le Douarec, J. C.; Neveu, C. Pharmacology and biochemistry of fenfluramine. In: Costa, E.; Garattini, S., eds. Amphetamines and related compounds. New York: Raven Press; 1979:75–105.
20. McPherson, G. A. Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. J. Pharmacol. Methods 14:213–228; 1985.
21. Mamounas, L. A.; Molliver, M. E. Evidence for dual serotonergic projections to neocortex: Axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin *p*-chloroamphetamine (PCA). Exp. Neurol. 102:23–36; 1988.
22. Mennini, T.; Garattini, S.; Caccia, S. Anorectic effect of fenfluramine isomers and metabolites: Relationship between brain levels and in vitro potencies on serotonergic mechanisms. Psychopharmacology (Berlin) 85:111–114; 1985.

23. Molliver, D. C.; Molliver, M. E. Selective neurotoxic effects of (±)-fenfluramine upon 5-HT axons in rat brain: Immunocytochemical evidence. Soc. Neurosci. Abstr. 14:88.8; 1988.
24. Morgan, C. D.; Cattabeni, F.; Costa, E. Methamphetamine, fenfluramine and their N-dealkylated metabolites: Effect on monoamine concentrations in rat tissues. J. Pharmacol. Exp. Ther. 180:127–135; 1972.
25. Nichols, D. E.; Barfknecht, C. F.; Rusterholz, D. B.; Benington, F.; Morin, R. D. Asymmetric synthesis of psychotomimetic phenyisopropylamines. J. Med. Chem. 16:480–483; 1973.
26. O'Hearn, E.; Battaglia, G.; De Souza, E. B.; Kuhar, M. J.; Molliver, M. E. Systemic MDA and MDMA, psychotropic substituted amphetamines, produce serotonin neurotoxicity. Soc. Neurosci. Abstr. 12: 336.2; 1986.
27. Ricaurte, G.; Bryan, G.; Strauss, L.; Seiden, L.; Schuster, C. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science 229:986–988; 1985.
28. Samuels, M. L. Statistics for the life sciences. San Francisco: Dellen Publishing Company; 1989:504–509.
29. Schechter, M. D. Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol. Biochem. Behav. 31:817–824; 1988.
30. Schmidt, C. J. Neurotoxicity of the psychedelic amphetamine, Methylenedioxymethamphetamine. J. Pharmacol. Exp. Ther. 240: 1–7; 1987.
31. Schmidt, C. J.; Wu, L.; Lovenberg, W. Methylenedioxyamphetamine: A potentially neurotoxic amphetamine analogue. Eur. J. Pharmacol. 124:175–178; 1986.
32. Schuster, C. R.; Lewis, M.; Seiden, L. S. Fenfluramine: Neurotoxicity. Psychopharmacol. Bull. 22:148–151; 1986.
33. Steranka, L. R.; Sanders-Bush, E. Long-term effects of fenfluramine on central serotonergic mechanisms. Neuropharmacology 18:895–903; 1979.
34. Stone, D. M.; Stahl, D. C.; Hansen, G. R.; Gibb, J. W. The effects 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur. J. Pharmacol. 128:41–48; 1986.
35. Wagner, J. A.; Peroutka, S. J. Comparative neurotoxicity of fenfluramine and 3,4-methylenedioxymethamphetamine (MDMA). Soc. Neurosci. Abstr. 14:135.12; 1988.
36. Wurtman, J. J.; Wurtman, R. J. Fenfluramine and other serotoninergic drugs depress food intake and carbohydrate consumption while sparing protein consumption. Curr. Med. Res. Opin. 6(Suppl. 1): 28-33; 1979.