Long-Term Amphetamine Treatment Decreases Brain Serotonin Metabolism: Implications for Theories of Schizophrenia

Abstract. *Long-term amphetamine administration to cats (a mean of 8.75 milligrams per kilogram twice daily for 10 days) produced large decreases (40 to 67 percent in serotonin and its major metabolite, 5-hydroxyindoleacetic acid, in all brain regions examined. This treatment also produced several behaviors that are dependent on depressed central serotonergic neurotransmission, and which normally are elicited exclusively by hallucinogenic drugs. Short-term amphetamine administration (15 mg/kg) did not produce these behaviors and resulted in small decreases in brain serotonin and no change in 5-hydroxyindoleacetic acid. These data are discussed in the context of monoamine theories of schizophrenia.*

Amphetamine is a widely used psychomotor stimulant whose effects are thought to be mediated primarily through an action on brain catecholamines (*1*). In fact, the behavioral effects of long-term amphetamine administration to humans and animals provides an important underpinning for the catecholamine theory of schizophrenia (*2, 3*). A recent report by Ellinwood (*4*), however, led us to examine the effects of long-term amphetamine administration on brain serotonin metabolism in the cat. Ellinwood reported that administration of high doses of amphetamine to cats for 11 days led to a number of unusual behaviors. Since we had previously found that two of these behaviors—limb flick and abortive groom—were elicited exclusively by manipulations that depressed central serotonergic neurotransmission (*5*), we hypothesized that long-term administration of amphetamine to cats might decrease the brain concentrations of serotonin (5-hydroxytryptamine) and of its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA).

Adult, female cats were individually housed in stainless steel cages and exposed to a photoperiodic cycle of 12 hours of light and 12 hours of darkness (lights were turned on at 8:00 a.m.). The cats were injected intraperitoneally twice daily (at 9:00 a.m. and at 9:00 p.m.) with *d*-amphetamine sulfate ($N = 6$) or saline ($N = 6$) for ten consecutive days. To avoid any sudden toxic effects, the initial dose (in salt form) was 5 mg/kg and then increased every other day by 2.5 mg/kg up to 15 mg/kg on day 9 (*4*). The animal was observed 30 minutes and 3 1/2 hours after each amphetamine injection and was scored for 30 minutes for such activities as limb flicking, abortive grooming, and head shaking (*6*), as well as for dopamine-related stereotyped behaviors (*7*). On day 10, the cats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) 4 to 5 hours after the final amphetamine injection. Their brains were rapidly removed and dissected into left and right cortex, hippocampus, striatum, diencephalon, brainstem, and spinal cord (first cervical through sixth thoracic vertebrae). The right hemispheric regions were assayed for serotonin and 5-HIAA by using the method of Curzon and Green (*8*) and for dopamine and norepinephrine by using the method of Jacobowitz and Richardson (*9*). The left hemispheric regions were assayed for tryptophan by using the method of Bloxam and Warren (*10*), and for homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) by using the method of Westerink and Korf (*11*). Each cat in a second group was given a single injection of *d*-amphetamine sulfate (15 mg/kg, intraperitoneally) or saline ($N = 4$ for each kind of injection). Behavior was observed 30 minutes and 3 1/2 hours after the injection, and 4 to 5 hours after the injection the cats were killed for the neurochemical assays described above.

Long-term amphetamine treatment produced large decreases (40 to 67 percent) in serotonin and 5-HIAA concentrations in all brain regions examined (Table 1). Short-term amphetamine administration, however, produced small decreases in serotonin (approximately 20 percent) and no change in 5-HIAA (*12*). Both long- and short-term amphetamine administration resulted in large changes in concentrations of norepinephrine, dopamine, and dopamine metabolites. Long-term amphetamine administration produced large decreases (85 to 94 percent) in norepinephrine concentration in all brain regions examined and a 95 percent decrease in striatal dopamine (*13*).

Initially, amphetamine (5 or 15 mg/kg) produced strong sympathomimetic signs (mydriasis, panting, and salivation) and stereotyped, rhythmic bobbing and weaving of the head and upper body. With repeated administration of amphetamine, both the sympathomimetic effects and the stereotyped behaviors became less pronounced, even though the dosage was progressively increased (*14*). Behaviors (limb flicking and abortive grooming) characteristic of depressed central serotonergic neurotransmission also began to be produced. From a mean baseline rate of 0.3 ± 0.2 flicks per hour after injection of saline, the rate of limb flicking gradually increased and achieved statistical significance on day 5 (2.3 ± 0.5 flicks per hour, $P < .05$, two-tailed Student's *t*-test, compared to saline baseline). Asymptotic rates of 8 to 10 flicks per hour ($P < .01$, compared to saline baseline) were reached on days 8 to 10. Abortive grooming increased similarly, from a saline baseline of 0 to an asymptotic rate of 2 to 4 occurrences per hour ($P < .05$) after 7 to 10 days of amphetamine treatment. The rate of occurrence of these behaviors 30 minutes after amphetamine injection did not differ significantly from the rate 3 1/2 hours after injection. Limb flicking and abortive grooming were never seen after the first injections of amphetamine (5 or 15 mg/kg) or after any saline injections. During long-term amphetamine treatment (especially from days 3 through 7), the cats simultaneously displayed prominent stereotyped behaviors (indicating overactivity in the dopamine system) and high rates of limb flicking and abortive grooming (indicating inactivation of serotonergic neurotransmission).

These data indicate that in addition to the well-known effects of *d*-amphetamine on norepinephrine and dopamine in the brain (*15, 16*), there are dramatic changes in the concentration of serotonin. The effects of amphetamine on brain serotonin are dependent on repeated administration, since a single injection produced only a small decrease in brain serotonin concentration and no change in 5-HIAA (*17*). By contrast, large changes in the amounts of brain norepinephrine, dopamine, and dopamine metabolites were produced by a single amphetamine injection. Previous studies (*15, 18*) may not have recorded large effects of amphetamine on brain serotonin because of lower doses, fewer injections, and the use of different species (mostly rodents).

The changes in brain serotonin concentration produced by long-term amphetamine administration are especially interesting because of their behavioral significance. In a series of studies (*5, 19*), we showed that the emergence of several behavioral effects, such as limb flicking and abortive grooming, is elicited exclusively by hallucinogenic drugs and is specifically dependent on the functional inactivation of the brain's serotonin system. The gradual onset of these behaviors over several days is consistent with the notion that the decrease in brain

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serotonin concentration is progressive (*20*).

Although the cats in this study received a large amount of amphetamine, the doses were comparable to those ingested by human amphetamine abusers. Therefore, it is reasonable to assume that amphetamine, consumed in large quantities over a long time, may also produce alterations in human brain chemistry. Amphetamine abusers frequently take as much as 300 mg/day, and doses of 900 to 1200 mg/day have been reported (*21*). Assuming a body weight of 50 to 80 kg, this represents a dose range of 5 to 25 mg/kg-day for abusers, compared with a mean of 17.5 mg/kg-day during the 10-day treatment period for the cats. Also, the daily dose administered to a cat can be lowered and still produce functionally significant reductions in brain serotonin, although the threshold for this has not been determined. We recently found that administration of 7.5 mg/kg every 12 hours for 10 days produced an average reduction of 35 to 40 percent in serotonin and 5-HIAA concentrations, and elicited high rates of limb flicking and abortive grooming. Further studies are needed to determine the permanency of these changes in brain serotonin (*22*) and the means by which they are mediated. The fact that 5-HIAA concentration is decreased only after long-term amphetamine treatment indicates that the chronic decrease in brain serotonin is not simply due to the serotonin-releasing effects of amphetamine.

These findings have important scientific and clinical implications. Since amphetamine is used as an investigative tool in so many experiments that are aimed at elucidating the role of catecholamines in behavior, studies in which there are repeated injections of this drug will have to be considered in light of possible changes in brain serotonin (*23*). Clinically, our data are applicable to hypotheses that concern the neurochemical bases of schizophrenia. The similarity of the behavioral effects of long-term amphetamine administration in animals and humans to paranoid schizophrenia is a fundamental piece of evidence favoring the catecholamine theory of schizophrenia (*2, 3*). Our results indicate that consideration of the role played by decreased brain serotonin concentration in the etiology of amphetamine psychosis is also warranted. Whether these data have relevance to idiopathic schizophrenia is less clear, since the results of clinical studies on the role of serotonin in schizophrenia are equivocal (*24*). However, single doses of hallucinogenic drugs such as lysergic acid diethylamide (LSD) produce behavioral effects in humans that resemble the acute, florid phase of schizophrenia (*3, 25*). It is thought that LSD works primarily by inactivating serotonin-containing neurons in the brain (*26*), but LSD is also known to mimic the effects of dopamine (*27*). Thus, acute psychotic episodes and the effects of long-term amphetamine administration

Table 1. Neurochemical effects of long- and short-term amphetamine treatment. Values are expressed as micrograms per gram of tissue (mean ± standard error). The statistical significance of differences between saline- and amphetamine-treated groups was determined with a two-tailed *t*-test.

Brain region	Treatment	Serotonin	5-HIAA	Norepinephrine	Tryptophan	Dopamine	HVA	DOPAC
				Long-term treatment				
Cortex	Saline	0.16 ± 0.01	0.13 ± 0.01	3.11 ± 0.41	0.18 ± 0.01			
	Amphetamine	0.10 ± 0.01	0.06 ± 0.01	3.16 ± 0.59	0.02 ± 0.01			
	(percent change)	(−42)†	(−52)†	(+1)	(−89)‡			
Hippocampus	Saline	0.50 ± 0.04	0.42 ± 0.02	3.69 ± 0.34	0.19 ± 0.01			
	Amphetamine	0.25 ± 0.01	0.19 ± 0.02	4.21 ± 0.45	0.02 ± 0.01			
	(percent change)	(−50)†	(−55)‡	(+14)	(−90)‡			
Striatum	Saline	0.58 ± 0.05	0.53 ± 0.02	4.58 ± 0.29	0.28 ± 0.03	5.65 ± 0.40	3.50 ± 0.35	2.34 ± 0.19
	Amphetamine	0.31 ± 0.03	0.32 ± 0.03	5.23 ± 0.64	0.02 ± 0.01	0.26 ± 0.08	1.37 ± 0.31	0.87 ± 0.06
	(percent change)	(−47)†	(−40)†	(+14)	(−94)‡	(−95)‡	(−61)†	(−63)†
Diencephalon	Saline	0.69 ± 0.05	0.90 ± 0.06	3.39 ± 0.30	0.39 ± 0.01			
	Amphetamine	0.23 ± 0.02	0.30 ± 0.03	4.14 ± 0.73	0.05 ± 0.01			
	(percent change)	(−67)‡	(−66)‡	(+22)	(−87)‡			
Brainstem	Saline	0.67 ± 0.03	0.92 ± 0.07	5.12 ± 0.77	0.29 ± 0.02	0.15 ± 0.02	0.14 ± 0.01	0.29 ± 0.03
	Amphetamine	0.30 ± 0.02	0.35 ± 0.01	5.98 ± 0.39	0.04 ± 0.01	0.06 ± 0.03	0.08 ± 0.01	0.17 ± 0.02
	(percent change)	(−55)‡	(−62)‡	(+17)	(−85)‡	(−61)*	(−43)*	(−40)*
Spinal cord	Saline	0.34 ± 0.01	0.29 ± 0.01	2.98 ± 0.27	0.12 ± 0.01			
	Amphetamine	0.18 ± 0.01	0.14 ± 0.02	3.28 ± 0.29	0.02 ± 0.01			
	(percent change)	(−48)‡	(−51)†	(+10)	(−86)†			
				Short-term treatment				
Cortex	Saline	0.23 ± 0.01	0.12 ± 0.01	2.98 ± 0.05	0.12 ± 0.01			
	Amphetamine	0.18 ± 0.02	0.13 ± 0.01	7.12 ± 0.99	0.04 ± 0.01			
	(percent change)	(−23)	(+9)	(+139)*	(−67)‡			
Hippocampus	Saline	0.55 ± 0.02	0.44 ± 0.04	4.51 ± 0.11	0.15 ± 0.02			
	Amphetamine	0.38 ± 0.02	0.38 ± 0.02	9.66 ± 2.17	0.05 ± 0.01			
	(percent change)	(−32)†	(−12)	(+114)*	(−69)*			
Striatum	Saline	0.63 ± 0.02	0.59 ± 0.01	3.82 ± 0.23	0.26 ± 0.01	5.65 ± 0.22	4.06 ± 0.53	2.00 ± 0.18
	Amphetamine	0.48 ± 0.03	0.68 ± 0.03	8.13 ± 1.00	0.10 ± 0.02	1.25 ± 0.18	7.53 ± 1.25	1.04 ± 0.12
	(percent change)	(−24)†	(+15)	(+112)*	(−60)‡	(−78)‡	(+86)*	(−48)†
Diencephalon	Saline	0.63 ± 0.02	0.84 ± 0.03	4.10 ± 0.13	0.36 ± 0.03			
	Amphetamine	0.44 ± 0.03	0.67 ± 0.05	9.58 ± 1.73	0.14 ± 0.03			
	(percent change)	(−30)†	(−21)	(+129)*	(−61)†			
Brainstem	Saline	0.67 ± 0.01	0.89 ± 0.01	3.48 ± 0.24	0.29 ± 0.01	0.12 ± 0.02	0.16 ± 0.01	0.17 ± 0.02
	Amphetamine	0.55 ± 0.03	0.77 ± 0.03	6.94 ± 0.71	0.15 ± 0.03	0.09 ± 0.02	0.34 ± 0.01	0.11 ± 0.02
	(percent change)	(−17)*	(−13)*	(+99)†	(−47)*	(−29)	(+120)‡	(−36)
Spinal cord	Saline	0.30 ± 0.01	0.27 ± 0.02	3.82 ± 0.10	0.11 ± 0.01			
	Amphetamine	0.30 ± 0.04	0.28 ± 0.01	8.25 ± 1.36	0.05 ± 0.01			
	(percent change)	(0)	(+5)	(+116)*	(−59)*			

*$P < .05$. †$P < .01$. ‡$P < .001$.

and single doses of hallucinogenic drugs may all be based on neurochemical processes involving dopamine and serotonin.

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Auditory Brainstem Frequency Following Responses to Waveform Envelope Periodicity

Abstract. *An auditory frequency following response (FFR) was recorded to four complex stimuli. The FFR corresponded to the waveform envelope periodicity but not to the "missing fundamental" pitch of the stimuli. The FFR may be significant for timbre perception and sound lateralization.*

The importance of periodicity for the pitch perception of complex sounds has long been of interest to auditory investigators. In the 19th century, Seebeck (*1*) showed that the pitch derived from a series of overtones was identical to the pitch of the "missing fundamental." Helmholtz (*2*) later incorporated this finding into his pitch-place theory by supposing that the fundamental was introduced to the ear through distortion. An alternative to the spectrally based theory of Helmholtz was offered when Wundt (*3*) proposed that the cue for pitch was periodicity of nerve impulses that was synchronous with stimulus periodicity. Much later, Schouten (*4*) and Licklider (*5*) disproved the distortion hypothesis of Helmholtz, showing that the fundamental did not have to be present in the ear when its pitch was heard during complex stimulation. This finding generated new interest in periodicity as a pitch cue, and theories of pitch perception that emphasized the periodicity of the waveform envelope (*6*) or the fine structure of the stimulus (*7*) were developed.

Complex waveform periodicity has also been proposed as a cue for the perception of timbre and the lateralization of high-frequency (> 2000 Hz) complex stimuli. In timbre perception, the perceived roughness of a complex sound changes as the prominence of the stimulus envelope is altered (*8*). In sound lateralization, low-frequency sine waves can be lateralized by periodicity cues, but high-frequency sine waves cannot. When the high-frequency signal is complex and has a low-frequency envelope, however, the signal can be lateralized on the basis of its envelope periodicity (*9*). Thus the inherent periodicity of complex stimuli may have significance for the perception of pitch and timbre and for the lateralization of high-frequency sounds.

Recently Smith *et al.* (*10*) recorded a frequency following response (FFR) to the missing fundamental. The FFR is an auditory brainstem response that is generally accepted to be a representation of neural periodicity. The finding of Smith *et al.* is important because it shows that the periodicity of the missing fundamental is available at the brainstem and that it could be used as a temporal cue for

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