Behavioral Consequences of Partial Monoamine Depletion in the CNS After Methamphetamine-Like Drugs: The Conflict Between Pharmacology and Toxicology

Lewis S. Seiden, William L. Woolverton, Stanley A. Lorens, Joseph E.G. Williams, Rebecca L. Corwin, Norio Hata, and Mary Olimski

INTRODUCTION

There is considerable evidence that dopamine (DA) and serotonin (5-hydroxytryptamine [5-HT]), by virtue of their role as tonic neurotransmitters in the central nervous system (CNS), play an important role in locomotion and motor control, food intake, mood, sexual behavior, aggression, sleep, and pain perception (Seiden et al. 1979; Carlsson 1988; Iversen et al. 1990; Seiden and Dykstra 1977). Part of that evidence is derived from pharmacological studies: Drugs that either directly or indirectly stimulate DA receptors have a characteristic profile of behavioral effects (Creese and Iversen 1973, 1975; Goodman and Gilman 1990). For example, amphetamine (AMPH) is an indirect DA agonist. At low-to-moderate doses, AMPH increases locomotion, apparently involving DA in the limbic forebrain; decreases food and water intake; has rate-dependent effects on schedule-controlled behavior; and functions as a positive reinforcer. At higher doses, AMPH induces species-specific stereotypic behaviors involving striatal DA (Cooper et al. 1991; Ellinwood 1974; Kelleher and Morse 1964; Scheel-Kruger and Randrup 1967). In humans, AMPH increases positive mood, has a high abuse liability, and when used in high doses, can induce stereotypic and maladaptive behaviors. When AMPH is used repeatedly, paranoid psychosis develops that may also be related to a hyperdopaminergic state. AMPH can also prevent sleep and is prescribed in the treatment of narcolepsy (Goodman and Gilman 1990). Although some of the effects of AMPH may involve 5-HT and/or the norepinephrine (NE) mechanisms, DA appears to play a particularly prominent role (Ando et al. 1985; Azzaro et al. 1974; Chiueh and Moore 1975; Heikkila et al. 1975; Raiteri et al. 1979). It also should be noted in this context that DA antagonists are used as antipsychotic agents and DA agonists are used to treat Parkinson's disease.

Similarly, pharmacological studies suggest that 5-HT plays an important role in the behavioral effects of antidepressant, antipsychotic, and antianxiety drugs (Asberg and Wagner 1986; Garattini and Samanin 1988; Marek et al. 1988; Peroutka and Snyder 1980; Pilc 1987; Seiden and O'Donnell 1985; Sulser 1983*a*; Vetulani et al. 1976). Several clinically efficacious antidepressant drugs block the 5-HT transporter (e.g., chlorimpramine, imipramine, fluoxetine) (Cowen 1990; Richelson 1990; Sulser 1983*b*; Willner 1985; Crews et al. 1983; Marek and Seiden 1988*a*, 1988*b*). These compounds alter locomotor activity, food and water intake, and pain perception. In addition, they increase time spent in the forced swim test, increase the number of reinforcers obtained on a differential reinforcement of low rate 72-second (DRL 72-s) schedule, and have been reported to affect sleep. The above effects are consistent with the idea that 5-HT plays a role in the maintenance of physiological and behavioral function (Curzon 1990; Humphrey et al. 1990; Jimerson et al. 1990; Morin et al. 1990; Richardson 1990; Wauquier and Dugovic 1990).

Data from experiments with neurotoxins also suggest important behavioral functions for DA and 5-HT. Lesions to DA systems induced by 6-hydroxydopamine (6-OHDA) have profound behavioral effects shortly after the toxin is administered. These include severe decreases in food and water intake and locomotion. In addition, DA lesions alter the behavioral effects of drugs that act via DA mechanisms (Kostrzewa and Jacobowitz 1974). Depletion of 5-HT by neurotoxins or electrolytic lesions have been shown to have effects on sleep, reaction to painful stimuli, sexual behavior, and aggression, although there has been some difficulty in interpreting some of these findings (Seiden and Dykstra 1977). It is reasonable to suspect, therefore, that chronic depletion of DA or 5-HT would lead to long-term changes in behavior.

Methamphetamine (MA) and certain related phenethylamines are drugs of abuse that are toxic to DA- and/or 5-HT-containing neurons in the brain (Hotchkiss et al. 1979; Koda and Gibb 1973; Seiden et al. 1976; Seiden and Kleven 1989). When administered repeatedly to animals, these compounds induce long-lasting monoamine depletions on the order of 50 percent. It seems likely that these drugs are neurotoxic in humans. Therefore, it is important to determine whether monoamine depletions of the magnitude produced by these drugs have significant behavioral consequences. Although these compounds have been found to have little or no obvious long-lasting effect on behavior, behavioral testing has not been extensive. Therefore, the present series of experiments was designed to examine the effects of small-to-moderate depletions of DA and 5-HT induced by neurotoxins or neurotoxic amphetamines on a wide range of behaviors that were predicted to be sensitive to small changes in monoamine concentrations. Those behaviors included locomotion, feeding, drinking, avoidance, escape, and open-field testing as well as several different operant tasks that are known to be sensitive to antidepressant, anxiolytic, and antipsychotic drugs.

METHODS

Male Sprague-Dawley rats (3 to 4 months old) were housed individually in a temperature- and humidity-controlled facility on a 12-hour light-dark cycle (lights on at 7 a.m.). Rat chow and water were available ad lib, except in experiments with schedule-controlled behavior (fixed conservative number [FCN], variable interval [VI], and DRL) and eight-arm radial maze when the rats were maintained at 80 to 85 percent of their predeprivation body weights.

Neurotoxins

Rats were pretreated with pargyline (20 or 30 mg/kg, intraperitonally [IP] 60 to 75 minutes prior to the neurotoxin injections) and anesthetized with sodium pentobarbital (30 mg/kg, IP, 40 to 55 minutes prior) supplemented with methoxyflurane. Desipramine (20 mg/kg, IP) was administered 30 to 45 minutes prior to neurotoxins. 5,7-Dihydroxtryptamine creatinine sulfate (5,7-DHT) (25, 50, 75, or 100 μg/ventricle, as the base) (Regis Chemical Co., Morton Grove, IL) or 6-OHDA (25, 37.5 and 50 μg/ventricle, as the base) (Sigma Chemical Co., St. Louis, MO) was injected bilaterally intracerebroventricularly (ICV). Ampicillin (50 mg/kg, intramuscularly 25 to 40 minutes prior) was given to prevent infection. Postoperatively, the 6-OHDA lesion rats were given access to preferred foods (vanilla custard with whole milk and vanilla wafers) and gradually returned to standard chow.

Neurotoxic Drugs

(+)-MA HCl (12.5, 25, and 50 mg/kg/injection) or (±)-3,4-methylenedioxymethamphetamine (MDMA) HCl (MDMA) (10, 20, and 40 mg/kg/injection) was injected subcutaneously (sc), b.i.d. (morning and evening, 12 hours apart), for 4 consecutive days. Drugs were dissolved in 0.9 percent saline, and doses are expressed as the salts. Parachloroamphetamine (PCA) HCl was purchased from Sigma.

Behavioral Testing

A variety of behavioral tests were conducted. Methods and results from each test are described below. In most cases, testing began 2 weeks after treatment and continued for at least 2 weeks. The major exception was food and water intake for which one group was tested approximately 18 months after treatment.

Analysis of Monoamines

After behavioral testing, rats were sacrificed by decapitation and their brains were rapidly dissected over ice; the striatum, hippocampus, and frontal cortex were assayed for 5-HT and DA (Heffner et al. 1980). Concentrations of DA and

5-HT were analyzed using high-performance liquid chromatography with electronic detection, by the method of Kotake (Kotake et al. 1985).

RESULTS

Neurochemical effects of neurotoxins and neurotoxic drugs were dose related. Generally, only the maximum depletion that was observed is reported. Behavioral changes are summarized in table 1.

Locomotor Activity

Locomotor activity (Erinoff et al. 1979) was measured in a stabilimeter apparatus for a period of 24 hours (12 hours light, 12 hours dark) once per week in rats treated with 6-OHDA or 5,7-DHT and in appropriate control rats.

TABLE 1. *Summary of behavioral effects of neurotoxins and neurotoxic drugs*

	Treatment			
Behavioral Paradigm*	5,7-DHT	6-OHDA	MDMA	MA
Locomotor activity	0	0	—	—
Food intake				
Total (g)	0	↓	0	0
Number of meals	↓	0	0	0
Meal duration	0	↓	0	0
Meal size	ϕ	0	0	0
Intermeal interval	0	ϕ	0	0
Water intake				
Total (mL)	0	0	0	0
Number of bouts	0	0	0	0
Bout duration	ϕ	0	0	0
Bout size	0	↓	0	0
Interbout interval	0	0	0	0
Schedule-controlled behavior				
FCN	0	0	—	—
VI 90-s	0	ϕ	0	0
DRL 72-s	ϕ	ϕ	0	0
Open-field behavior	0(ϕ)†	0(ϕ)‡	0	0
One-way avoidance				
Acquisition	0	0(ϕ)‡	0	0
Retention	0	—	—	—

TABLE 1. *(continued)*

Behavioral Paradigm*	Treatment			
	5,7-DHT	6-OHDA	MDMA	MA
Two-way avoidance acquisition	0[§]	0(ϕ)[‡]	0	0
Swim test	0(ϕ)[‖]	0	0	0
8-Arm radial maze				
Acquisition	0	—	0	—
Extinction	—	—	0	—
Morphine analgesia	¡[**]	—	0[††]	0
Home cage intrusion	¡	0	—	—

KEY: ¡ = increased; 0 = no effect; ϕ = decreased/impaired; — = not tested.

NOTES:

* See Methods section above for abbreviations, doses, routes, and schedules of drug administration.

† A decrease in center squares entered was seen only in one experiment (Lorens et al. 1990) and only after the 50 mg, ICV total dose. This finding was not replicated nor observed at higher doses (100 to 200 mg, ICV).

‡ Alterations produced by 6-OHDA were not consistent across groups (see text).

§ PCA (5 mg/kg × 2d) also was ineffective.

‖ Impaired swimming ability was reported by Lorens and colleagues (1990) but was not replicated.

** Morphine (5.0 mg/kg, SC) analgesia was potentiated by 200 mg (ICV total dose) but not 50 mg 5,7-DHT. This effect was observed 8 but not 2 weeks postinjection.

†† This finding is in contrast to the report by Nencini and colleagues 1988.

No changes were observed in the face of an approximately 50-percent depletion of DA or 50 percent of 5-HT. Rats showed a low level of activity during the light and a slightly higher level during the dark.

Food and Water Intake

Food (45 mg BioServ dustless pellets) and water intake were measured using photo-beam detection units (Coulbourn Instruments, Lehigh, PA) and monitored by a computer in an adjacent room. Food intake was analyzed in terms of total grams, number of meals, meal duration, meal size, and intermeal interval. Water intake was analyzed in terms of total mL, number of bouts, bout duration, bout size, and interbout interval. The room was on a 12-hour light-dark cycle

and the last 5 days of stable intake were used for data analysis. After treatment, one group of rats (short term) was returned to the experimental chambers after a 2-week recovery period, for at least 2 weeks. A second group (long term) was singly housed and maintained at stable body weight (approximately 350 g) for 18 months, after which it was returned to the experimental chambers for at least 2 weeks. For both groups, the last 5 days of stable intake were compared based on drug dose received.

Short-Term Studies. In rats given 6-OHDA, DA was depleted to 44 percent of control in the caudate, 60 percent of control in the frontal cortex, 73 percent of control in the hypothalamus, and 63 percent of control in the hippocampus; DA was unaffected in the spinal cord. Total grams, meal duration, and bout size increased. Intermeal interval decreased. In rats given 5,7-DHT, 5-HT was reduced to 30 percent of control in the caudate, 14 percent of control in the hypothalamus, 7 percent of control in the hippocampus, and 16 percent of control in the spinal cord. 5-HT was not affected in the frontal cortex. Number of meals increased, whereas meal size and bout duration decreased.

The MDMA regimen depleted 5-HT to 61 percent of control in the caudate, 37 percent of control in the frontal cortex, and 49 percent of control in the hippocampus. 5-HT in other regions was unaffected. There were no effects on food intake. In rats given MA, DA was depleted to 70 percent of control in the caudate; DA in other regions was unaffected. 5-HT was depleted to 43 percent of control in the caudate, and 29 percent of control in the frontal cortex; it was unaffected in other regions. There were no changes in food intake.

Long-Term Studies. In rats given MDMA and tested approximately 18 months after the last injection, DA in the spinal cord was depleted to 55 percent of control, whereas DA in other regions was unaffected. 5-HT was not affected in any region. There were no changes in food intake. In rats given MA and tested approximately 18 months after the last injection, 5-HT was depleted to 16 percent of control in the frontal cortex and 26 percent of control in the hippocampus, increased to 131 percent in the hypothalamus, and was unaffected in other regions. DA was increased to 221 percent of control in the spinal cord and unaffected in other regions. There were no changes in food intake.

Schedule-Controlled Behavior

Three schedules of reinforcement were used to study the effects of neurotoxins on schedule-controlled behavior: an FCN, a VI 90-second (VI 90-s), and a DRL 72-s. After stable behavior was established under each schedule, neurotoxins were administered. Retesting began approximately 2 weeks after administration of neurotoxins.

Fixed Consecutive Number

The FCN was conducted in standard two-lever operant chambers (Gerbrands Corp., Arlington, MA). Five or more consecutive responses on the left lever followed by one response on the right lever were required for delivery of a food pellet (45 mg, Noyes). A response on the right lever before five consecutive responses on the left lever reset the response requirement on the left lever. This schedule contained a signalled (SD) and an unsignalled (SO) component; each component consisted of 10 trials. The components (SD-SO) alternated until a total of 10 components (5 each) were completed. During the SD, the left lever light was illuminated until five consecutive responses were made; then the left lever light was extinguished, and the right lever light was illuminated. During the SO component, only the houselight was illuminated. The probability of reinforcement during the SD component was adjusted so that the number of reinforcers for both components was approximately equal. Responding was analyzed as percent correct for each component.

In rats that received 6-OHDA, DA was depleted to 14 percent of control in caudate, increased to 172 percent of control in the spinal cord, and was unaffected in other regions. There was no effect on FCN performance. In rats that received 5,7-DHT, 5-HT was depleted to 20 percent of control in caudate, 18 percent of control in frontal cortex, 34 percent of control in hypothalamus, 12 percent of control in hippocampus, and 15 percent of control in the spinal cord. DA was reduced to 76 percent of control in caudate, 51 percent of control in hypothalamus, and 14 percent of control in spinal cord; it was unaffected in other regions. NE was depleted to 4 percent of control in the hippocampus but was unaffected in other regions. FCN performance was unaffected.

VI 90-s responding (Levine et al. 1980*a*, 1980*b*) was unchanged following approximately 50-percent depletions of either DA or 5-HT. The rates of both responding and reinforcement were the same between the treated and the sham-treated rats.

Responding under the DRL 72-s was also unchanged (response rate and reinforcement rate did not differ) as a result of approximately 50-percent depletions of either DA or 5-HT.

Behavioral Test Battery

An additional battery of behavioral tests, designed to assess various aspects of behavioral function, was used to examine the effects of neurotoxins and neurotoxic drugs. The test battery included open-field behavior, one-way avoidance, discriminated two-way avoidance, and the forced swim test (Lorens et al. 1990). The effects of both neurotoxins and neurotoxic drugs were examined using this test battery. The effects of 5,7-DHT and MDMA on

eight-arm radial maze performance and morphine-induced analgesia also were determined. In addition, the effects of 6-OHDA on a home-cage-intrusion test were examined. Groups of rats were treated with neurotoxins and neurotoxic drugs and evaluated in multiple tests.

In rats that received 5,7-DHT, 5-HT was depleted to about 20 to 40 percent of control. MDMA also produced dose-dependent and selective reductions in regional CNS 5-HT concentrations that were virtually equivalent (18 to 34 percent reductions) in all CNS regions assayed (catecholamine concentrations were not altered). The lowest dose (50 μg) of 5,7-DHT significantly decreased open-field behavior and impaired swimming ability. MDMA did not alter open-field behavior or swimming ability. There was no effect of 5,7-DHT or MDMA on one-way (spatial, unsignalled) or visually discriminated two-way (nonspatial, signaled) avoidance conditioning. Additionally, neither treatment affected acquisition of one- or two-way avoidance or performance in the eight-arm radial maze.

Although dose-dependent effects of 6-OHDA were obtained, the changes in regional DA concentrations were highly variable. Thus, the 6-OHDA-treated rats were grouped according to their caudate-putamen DA levels: 6-OHDA-1, 83 percent of control; 6-OHDA-2, 66 percent of control; and 6-OHDA-3, 34 percent of control. In the frontal cortex, DA reductions reached 56 percent, but the variability precluded statistical significance. Decreases in hypothalamic DA were more modest but statistically significant. No systematic alterations were detected in hippocampal or spinal cord monoamines and their metabolites. Some significant behavioral changes were observed that varied as a function of lesion group and test. In the open field, the 6-OHDA-2 group exhibited a reduction in center squares entered. The 6-OHDA-3 group made more errors during one-way conditioned avoidance response (CAR) training, whereas the 6-OHDA-1 group returned to the shock compartment (errors of commission) more often during two-way CAR acquisition. There was no effect in the swim test, and there were no changes in aggressive or social behavior in the home-cage-intrusion test.

In rats treated with MA, DA levels were reduced only in the neostriatum (by 34 percent). Additionally, MA reduced 5-HT in the frontal cortex (by 61 percent), neostriatum (by 57 percent), hippocampus (by 81 percent), and spinal cord (by 29 percent) but not in the hypothalamus. NE concentrations were not altered in any CNS region. There was no effect on open-field exploration, the acquisition of a one-way or visually discriminated two-way CAR, swimming ability, or morphine analesia (5 mg/kg, sc).

DISCUSSION

An overview of the behavioral results obtained in the present series of studies shows that although neurotoxins had some long-lasting behavioral effects (e.g., food intake, VI and DRL performance), a wide variety of behaviors were unaffected by partial denervation of the DA or 5-HT systems by MDMA or MA. In addition, some of the changes that were observed with neurotoxins were generally unsystematic with regard to dose and, therefore, may have been the result of random variation. One might draw the conclusion from these results that DA and/or 5-HT do not play an important role in mediating these behaviors. However, these results should be interpreted with caution because the effects of drugs on 5-HT and DA systems strongly suggest that these systems play a role in behavior.

An account of the results presented here must, at this point, remain largely speculative. It may be that the functional reserve capacity of the CNS readily compensated for neurotransmitter changes. Within a particular neurotransmitter system, the amount of release that is compromised by drug-induced toxicity may be too small to be reflected in behavior. There is some evidence to support this view in that larger depletions of DA or 5-HT cause more persistent changes in behavior. VI 90-s responding has been found to increase with time as a result of 6-OHDA lesions that depleted both DA and NE by greater than 70 percent. Similarly, large depletions of DA cause response rate increases and reinforcement rate decreases in DRL 72-s behavior, and 5,7-DHT lesions that produce a large depletion of 5-HT increase the response rate and decrease the rate of reinforcement on the DRL 72-s schedule.

Alternatively, it may be that the behaviors studied, rather than relying on the function of any particular neurotransmitter system, rely on several synergistic transmitter systems. A partial reduction in the presynaptic terminal field of one neurotransmitter may be readily compensated for by one of the synergistic systems.

Other possibilities involve functional compensation in CNS neurotransmission. It may be that transmission in DA and 5-HT neurons was normal or at least adapted to maintain normal behavioral function. Although the concentrations of monoamines in the brain were decreased, this measure may not reflect the amount that is released. If synthesis and release were increased as a compensatory mechanism, then the same number of receptors would be stimulated and function would not change. Although it is difficult to understand how postsynaptic tone would be maintained if one assumes that the DA and 5-HT systems comprise tight chemical junctions, the assumption of volume transmission makes the results more reasonable. In volume transmission, the

released transmitter can diffuse many millimeters from the release site and thereby stimulate receptors not located within microns of the presynaptic terminal. Thus, one could readily see why function would not be compromised even in the face of significant decreases in the levels of DA and/or 5-HT. It is also possible that the number of receptors changed in such a way as to give a normal response in the face of only partial agonism. These events do occur in the face of denervation, but there have not been adequate studies to determine how these changes translate into effects on brain function and behavior.

Regardless of the precise mechanisms involved, one is left with the somewhat surprising conclusion that drug-induced CNS monoamine neurotoxicity had few, if any, long-lasting behavioral consequences in these studies. However, this should not be construed as meaning that CNS neurotoxicity is inconsequential. It is possible that the behavioral tasks used were not sensitive to functional changes. Moreover, behavioral consequences of neurotoxicity may be more apparent under different conditions. For example, depletions of DA in neonatal rats have been shown to cause hyperactivity that persists into adulthood (Erinoff et al. 1979). It may also be the case that the appearance of age-related deficits is hastened by drug-induced neurotoxicity. It remains for future research to examine these possibilities.

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DISCUSSION

Q: I was wondering if you saw any long-term changes in body weight?

A: Not when they are compared with their own rate of growth. In other words, you would get some initial losses, particularly with methamphetamine [meth], in the body weight, but then if you compared them with controls in a relatively free-feeding situation, they will gain weight at about the same rate as the controls. So it does lag a little bit.

Q: Second question: On one of your slides you said something about the recovery of the serotonin, and I was wondering, are those changes that you were talking about relatively permanent or were you looking at them in some point in the recovery phase?

A: Now, that is a good question. It depends upon which drug you are talking about. For the meth, we've looked for long times after we have treated these animals, and they are down in serotonin, and they are down in dopamine. But for fenfluramine, we do see a certain amount of recovery in certain regions in the brain as we do for MDMA; furthermore, it depends on what brain region you are talking about and at what aspect of the system you are talking about. Mainly, we look at levels, and the levels will tend to come back toward normal in certain brain regions and stay flat in other brain regions. Dr. Molliver will talk about this in more detail; there seems to be some recovery in levels. Nate Appel has seen some recovery in the uptake sites. It is a matter of controversy as to how to interpret some of these data, and maybe we can get some of that worked out here.

Q (Gibb): Lew, having spent my life looking at changes in neurochemistry, I find your results discouraging. On the other hand, the fact is that we do know that fluoxetine is effective in the treatment of depression. Also, as I understand it, it is very effective in the treatment of obesity. If my information is correct, the primary focus of that drug's effect is on the serotonergic system. So I would conclude that 5-HT is important, even though at this point none of the data that we have available from animal studies would suggest that. That would be the skeptic's view. It seems to me that the question that really needs to be addressed now (and I think you have said it) is whether the tests that we are using are sophisticated enough to pick up those kinds of changes. That is the important issue here. You would think that the feeding behavior would address the issue. Obviously, I am not a behaviorist, and I wondered if you care to comment on that?

A: The project took a period of 3 years. We honestly thought that the tests that we were using would be sensitive enough to pick up changes as they occurred.

Q: Have you looked at sex at all? Because as I understand it, I don't know . . .

A: Lorens looked at sexual behavior, but he had a very limited trial. And there were no changes.

Q (Heimer): Lew, I was wondering about the definition of the term "neurotoxicity" in relation to the techniques you have covered here. I suppose that you take for granted, as does everybody who uses the so-called "suppressive" silver methods, that when you see heavily argyrophilic particles or a strongly argyrophilic neuron, this neuron is degenerating and will eventually

die. When you do see black "measles" representing degenerating terminals, you take for granted that they will die and that the related neuronal cell bodies will also die. Although many people have been obsessed by silver methods, there are only a few who have been experimenting with more and more refined techniques. Maybe you saw the material at the Anatomy Meeting that Dr. de Olmos and his colleagues from Argentina produced. They kept modifying their silver technique to the point where, in quinolinic acid experiments, they used the same dose that people have used to make "restricted" lesions in the striatum. De Olmos and his colleagues detected degenerating neurons not only in striatum but in cerebral cortex, thalamus, and various other places even 15 minutes after the injection. I was just wondering if the silver methods have become so sensitive that they might even pick up changes that are reversible. I am sure that there are many who know this area better, so they might even have thought about these things, but as I remember from the time I used silver techniques, there are few people studying the ultrastructure of these argyrophilic particles and neurons. I wonder what you think about this problem?

A: George Ricaurte spent a lot of time working at the level of the electron microscope and did most of the silver staining procedures along with Ray Guillery. I thought that the changes using the electron microscope were not reversible. I must say that, since that time, Phil Groves has obtained evidence showing very nicely that you can see a silver-positive neuron in a cell that has been stained immunologically for tyrosine hydroxylase, indicating that it is the same cell that takes up silver. Now, if you combine that with the idea that the uptake site is down and that the levels are down, I think the conclusion becomes inescapable. But I would agree with you that any single benchmark in and of itself may not be a hallmark for neurotoxicity. We have converging evidence from chemistry, from function (if you take uptake as a function of the cell), and the argyrophilic nature of the fiber that degeneration can occur. But taken singly, I think that any one of these things could lead us astray.

Q (Molliver): About 10 years ago, S. Ögren and colleagues published a series of reports on animals treated with PCA [parachloroamphetamine] that have a selective serotonin deficiency. They found profound behavioral changes: a tenfold decrease in conditioned avoidance learning. Would you comment on these data?

A: We have never looked at it using PCA. We have looked at conditioned avoidance acquisition using other 5-HT toxins, but we have not been able to replicate these data; but we have never tried it with PCA.

Q (Molliver): That was a profound effect. Have others been able to replicate these data?

A: I don't think so. I think they have tried.

Q (Gibb): Lew, one other thing that I think needs to be taken into consideration here is (I am sure George will tell us about this) that the rat is relatively less sensitive to these compounds than primates. And I wonder, therefore, if there is any possibility that there is impairment of behavior in primates subsequent, down the line. What I think your data show is that there is a tremendous amount of plasticity in the system. And we know that if you use 6-hydroxydopamine, you have to deplete more than 80 percent or so of the dopamine to show any parkinsonian symptoms. I wonder if the same thing applies here, and if so, is it worthwhile conducting tests. I guess you couldn't do it in rats, maybe, but to see whether with age there is a deficit that develops. I think the concern that all of us have with regard to the abuse of these compounds is what is going to happen to these individuals in their sixties or earlier, possibly, as they abuse these amphetamine compounds; will they be early parkinsonian patients? Is there anything that we can do as far as tests are concerned?

COMMENT (Schuster): We did look extensively at the behavior of the primate after serotonin and dopamine depletions induced by high levels of methamphetamine. And I must say that we were as completely disappointed in those investigations as Lew had been with these more recent rat studies. Our studies used very sophisticated behavioral methods such as force-lever techniques in which you are able to measure hand tremor, and despite the fact that the monkeys had 60 to 70 percent depletion of dopamine, we did not see any changes in their performance.

COMMENT (Ricaurte): It is a nice idea. However, I actually favor the other possibility you mentioned, and that is that there are functional deficits but that you need the right task and a sufficient degree of damage within a given neurotransmitter system to detect it. So, for example, in the case of the force-lever paradigm, I would maintain that the animals didn't have as much dopamine depleted from the basal ganglia that one would have liked; I also would maintain that perhaps the task, although it is a motor task, may not be the appropriate task to get at extrapyramidal motor dysfunction. Before going on to other systems, I would favor the notion of stressing a given system and selecting the appropriate task to see whether it is possible to detect subclinical deficits.

A (Seiden): These monkeys were impaired when they were challenged with physostigmine, as I recall.

Q (Molliver): I have a question about Dr. Gibb's comment regarding fluoxetine. You must continue to give fluoxetine to see its effects on the depression, so this is a pharmacological action as opposed to a permanent alteration induced by the given compound. If you give MDMA every day, you will see behavioral

changes. However, if you give a course of it, wait for 3 weeks, and then look at the system, you don't see any permanent changes in behavior.

A (Seiden): That takes you back to the dilemma again. If you are modifying the presynaptic level pharmacologically and you get a change, and then you deprive the system of the presynaptic transmitter and you don't get a change, I have trouble interpreting those data.

Q (Landfield): I think this issue you raised on effects in animals is really very important, and it is going to be a recurring issue because we see the same thing in our THC [tetrahydrocannabinol] studies—the same issues. There are two factors that make me think that one shouldn't be too discouraged about the discrepancies. We have to remember that human behavior has a lot of redundancy built in, a lot of subtlety. Even in the case of Alzheimer's disease, there has to be massive neuronal degeneration before a patient is institutionalized. Human behavior is much more complex; at least, we like to think so. We can see subtle differences in human behavior that we might not see in the rat behavior. A few cocktails can change people's speech; we can notice changes. Whereas to observe an alcohol effect on behavior in a rat, you might need to induce a level of alcohol toxicity that would have a human completely knocked out. We have to remember that with human behavior, we can detect very subtle changes. The ratio between toxicity and observable behavioral effect may be very different in different species. Now, on the other side of the coin, some conditions allow us to detect a larger effect in a rat. In rats, one can damage the nervous system and see much more degeneration from certain toxic drugs because of experimental design; for example, dosage can be controlled to obtain a much higher rate of delivery. As you said earlier, if you don't see that effect in humans, it can be disappointing or seem less interesting. However, that may not be so, because toxicity in animals can at least provide clues to which brain systems are being changed by the drug or what is happening to membranes. These toxic effects in animals can provide clues to mechanisms, even though they are not seen in humans. You might have to treat for 20 years in humans to see the same effect. We also have to remember that the lifespan of the rat is 2 years, so everything is telescoped. What takes 2 months in a rat may take 10 years in human beings. I don't think we should be disappointed about discrepancies—just recognize the large differences in the experiments with different species. The discrepancies are providing important clues, and we shouldn't expect to see the same things in rats and humans exactly. I think the points that were made here are very important. You need different types of tests, more subtle tests, to see subtle changes in rat behavior.

A (Seiden): I couldn't agree more. I think that is exactly what I wanted to do here. I think that I've stimulated our discussion. Tom?

Q (Sobotka): Could I suggest looking at more local areas of the brain for depletion? I was working in the area of norepinephrine and noticing that Dr. Molliver and his colleagues have shown us that there is quite a bit of differentiation in where norepinephrine was located in the brain. I tried to locally deplete norepinephrine in rabbits. At least with conditioned avoidance tests, many people have looked for behavioral effects of depletion of norepinephrine, and there really isn't a lot of consensus in that area. I thought that since you tried a global depletion, there may be some kind of global compensation. Norepinephrine and serotonin are interacting with many other things locally in the brain, so what if we depleted norepinephrine locally and found significant behavioral changes in our task?

AUTHORS

Lewis S. Seiden, Ph.D.
Professor

William L. Woolverton, Ph.D.
Associate Professor

Department of Pharmacological and Physiological Sciences
University of Chicago
947 East 58th Street
Chicago, IL 60637

Stanley A. Lorens, Ph.D.
Professor
Department of Pharmacology
Loyola University Medical Center
2160 First Avenue
Maywood, IL 60153

Joseph E.G. Williams, Ph.D.
Assistant Professor
Queen Mary and Westfield College
Mile End Road
London E1 4NS
ENGLAND

Rebecca L. Corwin, Ph.D.
Research Associate
Behavioral Neuropharmacology Unit
Experimental Therapeutics Branch
National Institute of Mental Health
National Institutes of Health
Building 10, Room 4N 214
Bethesda, MD 20892

Norio Hata, Ph.D.
Instructor
Department of Psychiatry
Shinshu University School of Medicine
3-1-1 Asahi
Matsumato 390
JAPAN

Mary Olimski
Research Technician
4629 North Winchester
Chicago, IL 60640