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Methamphetamine, Physostigmine, Atropine and Mecamylamine: Effects on Force Lever Performance

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PRESTON, K. L., C. R. SCHUSTER AND L. S. SEIDEN. *Methamphetamine, physostigmine, atropine and mecamylamine: Effects on force lever performance.* PHARMACOL BIOCHEM BEHAV **23**(5) 781–788, 1985. —Dose response functions for *d*-methamphetamine (MA), physostigmine, atropine, and mecamylamine on force lever performance (a measure of motor control) were determined in three rhesus monkeys. The rhesus monkeys were then treated with a repeated high dose regimen of MA, and the effects of the four drugs were redetermined. Following the completion of the behavioral studies, the monkeys were killed and brain monoamine concentrations were measured. It was found that each of the four drugs produced differential effects on force lever performance indices. Following the MA regimen, the MA-treated monkeys were less sensitive to the effects of MA on force lever performance but showed no change in sensitivity to any of the cholingergic agents. The monkeys were subsequently shown to have decreased brain dopamine and serotonin levels.

Methamphetamine Physostigmine Atropine Mecamylamine Force lever Motor control

THE repeated high dose administration of *d*-methamphetamine (MA) or amphetamine has been shown to cause long-lasting alterations in dopaminergic and serotonergic metabolism in various species. These alterations include decreased dopamine (DA) and/or serotonin (5-hydroxytryptamine; 5HT) levels in various regions of the brain, decreased tyrosine hydroxylase and trytophan hydroxylase activity, decreased DA and 5HT high-affinity uptake sites, an increase in transmitter turnover, and terminal degeneration [16, 24, 29, 30, 38, 39, 43, 46, 49, 50, 51, 52, 53].

Studies examining the functional consequences of these neuronal changes have demonstrated that tolerance to the acute effects of MA on force lever and DRL performance and locomotor activity develops during and persists for at least several months after a high dose regimen of MA [14, 15, 26, 31]. Several studies have also shown that decreased sensitivity to apomorphine, a dopaminergic agonist, and increased sensitivity to haloperidol, a dopaminergic antagonist, accompany this tolerance to the effects of MA [14, 25, 31].

The present study was conducted to determine whether changes in sensitivity to the effects of drugs acting on a different neurotransmitter might also be altered following the repeated high dose administration of MA. There is a great deal of evidence, both biochemically and behaviorally, to indicate that there is an interaction between the cholinergic and dopamine nervous systems in the brain. DA antagonists generally increase acetylcholine (ACh) release and turnover and decrease ACh levels; agonists generally decrease ACh release and turnover and increase ACh levels [7, 21, 28, 32, 44, 45, 48]. Conversely, cholinergic agonists increase DA release and turnover and enhance haloperidol-induced increases in DA turnover [2, 5, 18, 22, 34] while cholinergic antagonists block haloperidol- and ACh-induced increases in DA turnover and, alone, slightly decrease DA turnover [1,22]. In addition, anticholinergic agents have been shown to enhance the behavioral effects of DA agonists [42] and reverse the effects of DA antagonists [36] while cholinergic agonists inhibit the effects of DA agonists [9] and are inhibited by DA antagonists [47]. In addition, a cholinergic-dopaminergic imbalance has been hypothesized to be important in motor control, and disruptions of this balance are thought to lead to a number of motor diseases, such as Parkinson's disease, Huntington's chorea and, perhaps, tardive dyskinesia [3, 4, 8, 10, 17].

To determine whether changes in sensitivity to drugs acting on cholinergic neurons occur following a high dose MA regimen, the effects of physostigmine (an acetylcholinesterase inhibitor), atropine (a muscarinic antagonist), mecamylamine (a nicotinic antagonist), and MA were tested in rhesus monkeys performing in a force lever apparatus for water reinforcement before and after a repeated high dose regimen of MA. Following completion of the behavioral studies, the monkeys were killed, and brain levels of monoamines were measured to confirm that neurochemical alteration had been induced by the MA regimen.

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TABLE 1
PERFORMANCE INDICES

Efficiency*	=	Number of Reinforcers Delivered × Minimum Time Requirement (3 sec) / In Band Responding Time (sec)
Tonic Accuracy*	=	In Band Responding Time (sec) / Total Responding Time (sec)
Work Rate	=	Total Responding Time (sec) / Session Time (sec)
Mean Band Entrances*	=	Total Band Entrances / Number of Reinforcers Delivered
Mean in Band Time*	=	In Band Responding Time (sec) / Total Band Entrances
Reinforcers	=	Number of Reinforcers Delivered Within 30 minutes (Maximum of 50)

*If responding is completely eliminated by any manipulation, this index cannot be calculated.

METHOD

Subjects

The subjects were 1 male and 2 female adult rhesus monkeys weighing between 4 and 8 kg at the beginning of the study. One monkey was experimentally naive; the other 2 monkeys had previous lever pressing experience with responding maintained by intravenous drugs and shock punishment experience but had not received any drug nor were in any experiment for one year prior to this experiment. Each monkey was housed individually in a standard metal cage. The colony room temperature was 21°C.

Each monkey received a total of 175 to 225 ml of water daily (75 ml maximum during the session, the rest given in the home cage after the daily session and titrated to the animal's needs to avoid dehydration). The monkeys were given free access to Purina Monkey Chow and two sugar cubes saturated with liquid vitamins (Vitol, Vet-A-Mix, Inc., Shanandoah, IA) daily in the home cage.

Apparatus

The force lever system is the same as that described by Johanson *et al.* [25] and is a modified version of an apparatus described by Falk and Haas [12] designed to monitor discriminative motor control in rats. The apparatus consisted of a standard metal cage which had one side removed so that the monkey could place its arm through an adjustable Plexiglas tube and press on a conical shaped manipulandum (maximum displacement 0.1 mm) located at the end of the tube and attached to a force transducer. This apparatus was situated in two sound attenuating wooden boxes, one housing the animal chamber and one housing the Plexiglas tube, manipulandum and force transducer. A Plexiglas wall separated the two chambers. A water cup was located on the Plexiglas wall to the left of the Plexiglas tube and was attached by plastic tubing to a peristaltic infusion pump (7540X Cole-Parmer Co., Chicago, IL) located outside the wooden boxes. Four amber indicator lights were located to the left of the water cup on the Plexiglas wall in a vertical arrangement.

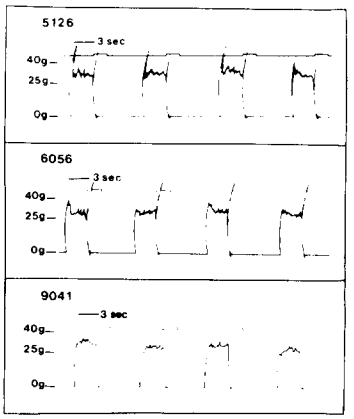

FIG. 1. Analog recordings of responding of subjects 5126, 6056, and 9041 after the IM administration of saline. The tracing moves horizontally with time and vertically with changes in force exerted on the lever. The notches in the line at the top of each recording indicate delivery of water.

The amount of force applied to the manipulandum was monitored by a force transducer (Statham Instruments, Oxnard, CA, Model UC3), a Beckman Dynograph (Beckman Instruments, Inc., Lincolnwood, IL, Type R411), and BRS/LVE solid-state programming and recording equipment (BRS/LVE, Beltsville, MD). Calibration of the apparatus was checked regularly by suspending weights from the manipulandum.

Teminal Schedule

The monkey was required to extend its arm through the Plexiglas tube and press on the lever with a force greater than 25 grams and less than 40 grams for 3 continuous seconds. If the force exerted was outside these limits (less than 25 grams or greater than 40 grams) for more than 30 msec, the time requirement was reset. When a trial was suc-

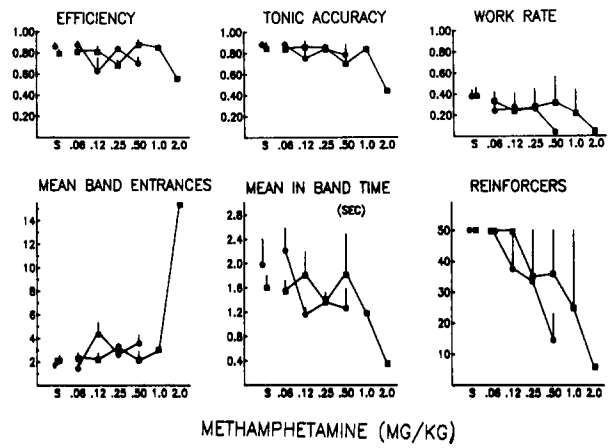

FIG. 2. Effects of methamphetamine on force lever performance before and after repeated high dose administration of methamphetamine. Combined data from 3 monkeys for 6 performances indices (see Table 1) after the IM administration of methamphetamine before (circles) and after (squares) a methamphetamine regimen. S=saline control. Vertical lines represent the standard deviation of the means.

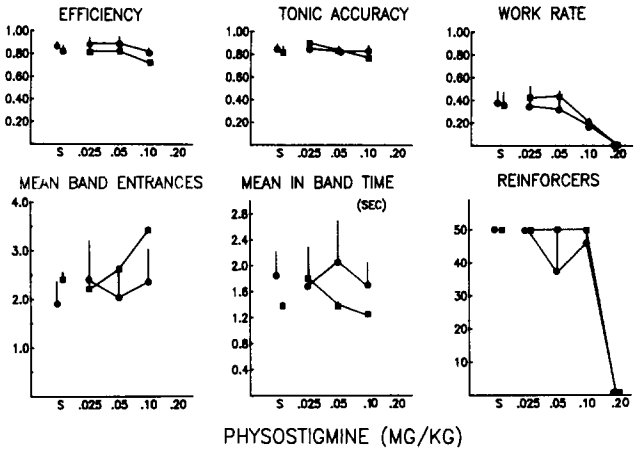

FIG. 3. Effects of physostigmine on force lever performance before and after repeated high dose administration of methamphetamine. Combined data from 3 monkeys for 6 performance indices (see Table 1) after the IM administration of physostigmine before (circles) and after (squares) a methamphetamine regimen. S=saline control. Vertical lines represent the standard deviation of the means.

cessfully completed (i.e., the 3 second requirement was met), the pump delivered 1.5 ml of water. During water delivery, all lights were extinguished in the cubicle, and responding had no programmed consequence. A session ended after 50 completed trials, or after 30 minutes had elapsed, whichever came first.

Indicator lights provided feedback for responding. One of 3 lights was on when the pressure on the lever was between 10 and 25 grams, 25 and 40 grams, or above 40 grams, respectively. A light located above the indicator lights in the experimental chamber indicated when the session was on.

Total session time and time spent responding between 10 and 25 grams (below band), between 25 and 40 grams (in band), and above 40 grams (above band) were recorded on four timers. Counters recorded the number of times the response force entered the required band width (from either above or below band) and the number of water deliveries obtained within the 30 minutes time limit. Total time responding was calculated by summing the times recorded for below, in, and above band responding.

Data Analysis

Using the above measures, the indices in Table 1 were calculated for each experimental session. These indices have previously been shown by Falk [11] and Johanson *et al.* [25] to be sensitive to the effects of various psychotropic drugs.

Efficiency, tonic accuracy, and work rate (Table 1) are relatively independent and vary between 0 and 1.0 with higher values corresponding to better performance. Efficiency is a measure of how well the monkey performed relative to a perfect score but independent of the number of reinforcers it earned; that is, it is the time spent pressing the lever with between 25 and 40 grams of force relative to the minimum in band time required to earn a given number of reinforcers. Tonic accuracy is a measure of how much time the monkey spent responding within the 25 to 40 gram range relative to the total amount of time it spent pressing the lever with 10 or more grams of pressure. Work rate measures the amount of time the monkey spent pressing the lever with 10 or more grams of pressure relative to the total session time. While work rate is not a measure of motor control, it is a good measure of general performance. Mean band entrances is the average number of band entrances made per reinforcer earned. A drug which caused a tremor would be expected to increase mean band entrances. Mean in band time gives the average length of time spent responding in the required band width per band entrance. Although these last two indices are related, it should be possible to differentially affect the two scores. For example, a drug which caused a gradual drift in the force exerted on the lever could result in an increase in the in band time without increasing to as large an extent the total number of band entrances.

Drugs

For the acute injections, *d*-methamphetamine HCl (National Institute on Drug Abuse), physostigmine SO_4 (Sigma), mecamylamine HCl (MSD), and atropine SO_4 (Aldrich) were given intramuscularly in 0.1 ml/kg of physiological saline. Pretreatment times were 20 minutes for MA and 30 minutes for all other drugs and were determined in pilot studies. Drug was given no more frequently than every fourth session (day) and only after responding returned to baseline. Saline injections were given intramuscularly (0.1 ml/kg) one or two sessions prior to each drug session. For the repeated administration regimen MA was given subcutaneously in a concentration of 50 mg/ml in physiological saline, as described below. Doses of each drug are expressed in terms of the salt.

Experiment

Experimental sessions were conducted once daily, seven days per week at the same time of day. Following training and when responding had become stable, single injections of MA were given to each monkey in a dose range of 0.062 to 0.5 mg/kg. Following the determination of the MA dose response curves, the effects of a range of doses of atropine, mecamylamine, and physostigmine were determined in each subject. The order in which the drugs were given was varied between subjects. The dose range of each drug varied be-

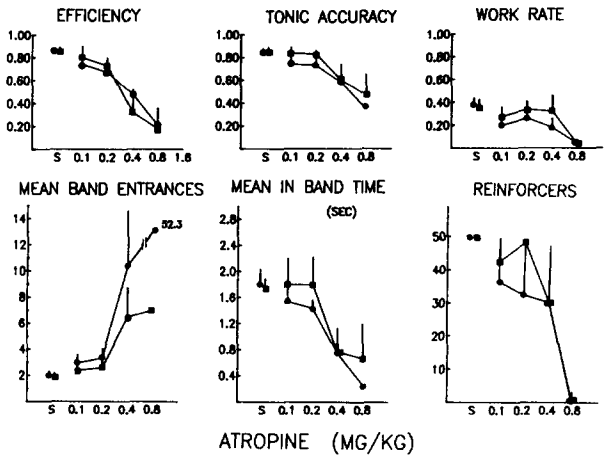

FIG. 4. Effects of atropine on force lever performance before and after repeated high dose administration of methamphetamine. Combined data from 3 monkeys for 6 performance indices (see Table 1) after the IM administration of atropine before (circles) and after (squares) a methamphetamine regimen. S=saline control. Vertical lines represent the standard deviation of the means.

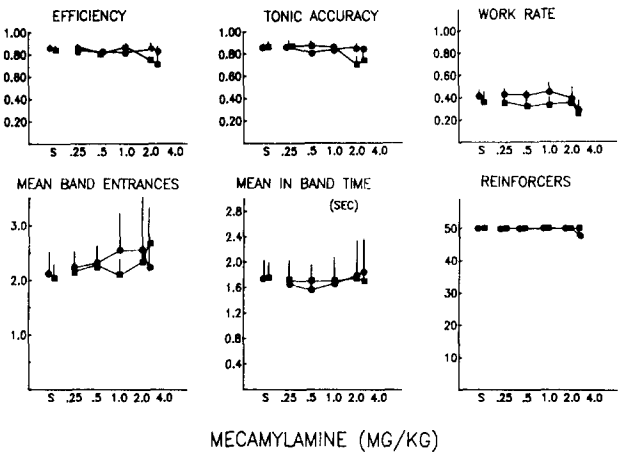

FIG. 5. Effects of mecamylamine on force lever performance before and after repeated high dose administration of methamphetamine. Combined data from 3 monkeys for 6 performance indices (Table 1) after the IM administration of mecamylamine before (circles) and after (squares) a methamphetamine regimen. S=saline control. Vertical lines represent the standard deviation of the means.

tween monkeys but was determined so that a dose which had little or no effect on responding and a dose which completely disrupted responding were given. Mecamylamine at a dose of 2.5 mg/kg produced such profound ataxia and sedation that higher doses were not tested, despite the fact that this dose had only minimal effects on force lever responding.

After all dose-response curves were determined, subjects were given free access to food and water for at least one week prior to the start of the repeated high dose regimen of MA. MA was given in four divided doses at 6 hour intervals starting at 8.0 mg/kg/day. Over a 14 day period, doses were gradually increased to a final dose of 40 mg/kg/day. These dose increases were based on food intake, body weight, and general condition of each monkey and varied between monkeys depending on individual sensitivity to the drug. The lab chow diet was supplimented with fruit when food intake was decreased. One subject developed severe lesions on her feet, tail, and hands during the regimen. Due to these potentially life threatening lesions, the regimen was modified and shortened to 13 days, with a final daily dose of 32 mg/kg/day. The monkey's lesions were cleaned and bandaged under light ketamine anesthesia every other day for one week after the MA was discontinued.

At the end of the two week MA regimen, monkeys were allowed one month to recover. Water intake was then again restricted, and monkeys were begun in daily force lever sessions. When responding again became stable, dose-response curves for MA, atropine, mecamylamine, and physostigmine were redetermined. Drugs were given to each monkey in the same order as they had been given before the MA regimen.

Assay

At least 2 months after the last drug administration, each monkey was anesthetized with an intraperitoneal injection of pentobarbital NA (Abbott), approximately 25 mg/kg. The monkey was then killed by exsanguination; a circumferential incision made in the skull, and the brain removed. The brain was dissected immediately in the following manner: the brain was first divided in the midsagittal plane. One side was chosen randomly and placed with its medial side facing up. A sample of frontal cortex was taken by making a perpendicular cut through the arcuate gyrus. A horizontal incision was then made anteriorly from the genu of the corpus callosum to the plane of the first incision. The head of the caudate nucleus was then isolated by visualization inside the lateral ventrical and dissected free. The white internal capsule was trimmed from the caudate tissue. The cerebellum was removed by severing the cerebellar peduncles close to the brain stem. The pons-medulla was isolated by making a cut from the caudal border of the corpora quadrigemina to the intra-peduncular fossa. The midbrain was dissected free by an anterior cut from the caudal border of the corpora quadrigemina to the caudal border of the infundibulum. A sample of hippocampus was obtained by making a coronal incision through the temporal lobe approximately 1 cm from the caudal end of the lobe. The hippocampus was then isolated by visualization, and a sample was dissected free. Finally, a small section of the cortex (1×1×1 cm) dorsal to the thalamus was dissected free. The second side was then immediately dissected in a like manner. Brain parts were stored in liquid nitrogen until assayed.

The DA and 5HT content of each brain region were determined using reverse phase high performance liquid chromatography coupled with electrochemical detection as modified by Kotake and Heffner (personal communication) from Felice *et al.* [13]. The significance of differences between group means was assessed with a one-tailed Mann-Whitney U test. Significance was accepted at the $p<0.05$ level.

RESULTS

The task took several days for each monkey to learn and several months for responding to become stable. After responding became stable, all 50 reinforcers were usually earned during the session. Figure 1 shows representative Beckman analog recordings of performance of the 3 monkeys after saline injections. The topography of responding differed somewhat between animals and accounted for individual differences in baseline scores. For example unlike

TABLE 2

THE CONCENTRATION OF DA AND 5-HT (μg/g TISSUE) IN SEVERAL BRAIN AREAS OF MONKEYS REPEATEDLY INJECTED WITH MA

	Dopamine		5-HT	
	C	E	C	E
Caudate	12.62 ±0.71	8.93* ±0.34	0.424 ±0.016	0.365 ±0.020
Cortex	0.102 ±0.059	0.120 ±0.020	0.077 ±0.011	0.049* ±0.007
Frontal cortex	0.043 ±0.021	0.033 ±0.006	0.074 ±0.009	0.051* ±0.005
Hippocampus	†	†	0.162 0.023	0.079* 0.001
Midbrain	0.360 ±0.044	0.429 ±0.111	0.408 ±0.104	0.500 ±0.219
Pons-medulla	0.034 ±0.014	0.041 ±0.013	0.196 ±0.040	0.225 ±0.059

*$p<0.05$ Mann-Whitney U-Test; C=Control; E=Methamphetamine-treated.

Data are Mean ± S.E. (N=5), †Not measured.

monkeys 6056 and 9041, monkey 5126 exceeded the 40 gram upper limit and entered the band from above on each trial. This difference in topography was reflected by generally higher mean band entrance and lower mean in band time baseline scores.

The data for the 3 monkeys were combined and presented here as a group. Figure 2 shows the dose-response curves of MA before and after the repeated MA regimen. Acute doses of MA produced a dose-dependent disruption of performance as the dose of MA was increased. Increased band entrances and lower mean in band times at higher doses of MA indicate the presence of tremor. Comparison of the pre- and post-MA treatment dose response curves for MA shows that the number of reinforcers earned was decreased to less than 15 pre-chronically by a dose 0.5 mg/kg and post-chronically by a dose of 2.0 mg/kg. Changes in work rate also demonstrate this decreased sensitivity. There was a four fold shift to the right in the post-chronic dose-response curves for work rate and number of reinforcers earned.

Figure 3 shows the dose-response curves for physostigmine in the 3 monkeys before and after the MA regimen. Physostigmine caused decreases in work rate and the number of reinforcers earned but only minor effects on the other performance indices. Comparison of the pre- and post-MA regimen dose-response curves shows no changes in sensitivity to physostigmine following the MA regimen in any of the six performance indices. The differences between the pre- and post-MA regimen dose response curves for mean band entrances and mean in band time appear to reflect a difference in baseline rather than a change in sensitivity.

Figure 4 shows the results of atropine administration before and after the MA regimen. The effects of atropine were dose dependent, such that efficiency, tonic accuracy, work rate, mean in band time, and number of reinforcers earned all decreased with increasing doses while mean band entrances per reinforcer increased with increasing doses of drugs. Atropine 0.8 mg/kg increased the number of mean band entrances to approximately 25 times the baseline number, however, this number is based on a very limited sample of responding as indicated by a low work rate. No change in the shape of the dose-response curve nor the dose of atropine at which responding was almost eliminated was seen following the MA regimen.

Figure 5 shows the dose response curves for mecamylamine in the 3 monkeys before and after the MA regimen. At nearly all doses, mecamylamine had only minimal effects on force lever responding. After the administration of all doses of this drug, the monkeys were ataxic and sedated. In spite of these profound gross changes in behavior, force lever performance was only minimally affected. Due to the gross effects of the drug and concern about the possible lethal affects of the drug, the maximum dose was limited to 2.5 mg/kg even though this dose did not disrupt responding. a comparison of the pre- and post-MA regimen dose-response curves show them to be nearly identical for all performance indices.

The results of the assays for DA and 5HT for 6 brain regions are given in Table 2. DA levels in MA-treated monkeys were significantly decreased in the caudate nucleus to 71% of control. No significant changes in DA levels were found in the cortex, frontal cortex, midbrain, or pons-medulla. No measurable amounts of hippocampal DA were found. Hippocampal 5HT levels were significantly decreased in MA-treated monkeys to 49% of control. 5HT levels in the cortex and frontal cortex were significantly decreased in MA-treated monkeys to 64% and 69% of control, respectively. 5HT levels in the caudate, midbrain, and pons-medulla were not changed.

DISCUSSION

The acute effects of the different drugs tested are interesting in that the types of alteration in performance produced by each drug was different. MA produced increases in the number of band entrances and decreases in the mean in band times, indicating the presence of tremor in acutely treated monkeys. This finding is in agreement with the force lever study by Johanson *et al.* [25] in which MA produced increases in band entrances without affecting efficiency or tonic accuracy. Physostigmine did not produce any indications of tremor (for example, increases in mean band entrances) in the force lever performance at doses that did not nearly eliminate responding. Cholinergic agonists, such as physostigmine, oxotremorine, and tremorine, however, have been shown to produce tremors in rats and mice [35,54] in studies using visual rating scales. It is possible that the doses of physostigmine at which tremors are produced are greater than the doses at which force lever responding is eliminated. If such were the case, tremor would not be detected by this procedure. Mecamylamine produced neither marked effects on motor control nor decreased responding. This is striking in view of the grossly observable effects which the drug produced, such as ptosis, ataxia, and sedation. Atropine, on the other hand, had profound effects on force lever performance at doses which produced few grossly observable effects. All performance indices indicated a decline in motor control with increasing doses of atropine. The increases in mean band entrances indicate the presence of tremor in the atropine treated monkeys while decreases in tonic accuracy reflect a decreased ability to hold the lever with the correct amount of force for the required time. The effects of atropine

on motor control as measured by this procedure appear to be centrally mediated since the administration of atropine methylnitrate has little or no effect on force lever performance [37].

Following the MA regimen, during which the monkeys did not perform in the force lever paradigm, the baseline performance of the monkeys were, for the most part unchanged (Figs. 2, 3, 4, and 5). A change in force lever performance might have been predicted based on studies showing motor changes following lesion-induced brain DA depletions in rhesus monkeys and other species [6, 19, 20, 27] and motor disorders involving decreased brain DA levels, such as Parkinson's disease [55]. In fact, the neurochemical changes following the repeated administration of MA are remarkably similar to those exhibited by Parkinsonian patients. Parkinsonian patients manifest a depletion of striatal DA and 5HT, a decrease in tyrosine hydroxylase activity, an increase in DA turnover, a decrease in the number of DA transport sites, and neuronal degeneration in the striatum [23]. Cell body degeneration in the substantia nigra found in Parkinsonian patients, however, was not found in MA-treated rats [38]. The lack of a change in baseline performance may be due to the relatively smaller decreases in brain DA and/or 5HT levels found in the monkeys in the present study (Table 2) than those found in 6-hydroxydopamine treated animals or Parkinsonian patients. A further study in which more extensive transmitter decreases than those found in the MA-treated monkeys used in the present study would be interesting to determine whether changes in force lever performance will result from more substantial DA and/or 5HT level decreases.

Although no changes in baseline force lever performance were seen following the repeated high dose administration of MA, the functional consequences of MA-induced neuronal alterations were evidenced by decreased sensitivity to the acute effects of MA on performance when measured more than one month after the last injection of the MA regimen. This long-term MA-induced tolerance presumably occurs because less DA and/or 5HT is available for release by the acute doses of MA in animals which have previously received high dose administration of MA. A given dose of MA would cause a smaller increase in DA concentration at receptors and, therefore, a smaller effect in subjects with MA-induced DA depletions. A similar effect would occur in 5HT terminals. Such tolerance would be expected to last for as long as a decrease in transmitter level persisted. Both tolerance and neuronal alterations produced by the repeated administration of MA have been shown to be long-lasting ([14,31] present study).

Neuronal alterations in the dopaminergic nervous system might be predicted to alter the effects of cholinergic agents on motor control. A cholinergic-dopaminergic imbalance has been hypothesized to be involved in motor disorders such as Parkinson's disease, Huntington's chorea, and tardive dyskinesia [3, 4, 8, 10, 17]. Parkinsonian patients, as mentioned above, exhibit neurochemical alterations of the dopaminergic and serotonergic systems similar to MA-induced alterations [23] with no alterations in cholinergic enzymes [33]. Duvosin [10] has shown that Parkinsonian symptoms are exacerbated by the administration of physostigmine and attenuated by the administration of scopolamine. In addition, increased response to the motor effects of atropine and scopolamine has been demonstrated in 6-hydroxydopamine treated animals [36, 40, 41]. The monkeys in the present force lever study, however, displayed neither changes in baseline motor function nor changes in sensitivity to physostigmine, atropine, or mecamylamine following the MA regimen.

The administration of repeated high doses of MA produced decreases in sensitivity to the acute effects of MA on force lever performance when measured more than one month after the last injection of the MA regimen. Previous studies have shown shifts in sensitivity to the effects of dopaminergic agents in animals previously treated with repeated administration MA that were consistent between agents and between animals [14,26]. In these studies, the MA regimen produced tolerance to the effects of DA agonists and hypersensitivity to a DA antagonist, indicating a specific effect not explained by overtraining or behavioral tolerance. Furthermore, while individual animals showed differences in the degree of shift in sensitivity to DA agents, the direction of the shift to a particular agent was the same in all animals (tolerance to agonists; hypersensitivity to antagonists). In the present study, no changes in sensitivity to the effects of any of the cholinergic agents tested were detected. It is probable that while the neuronal alterations produced by the MA regimen were large enough to produce tolerance to an agent (MA) acting on dopaminergic and serotonergic neurons, the alterations were not sufficient to produce an imbalance between the dopaminergic and cholinergic systems, such as is seen in Parkinson's disease, and, therefore, produce a change in sensitivity to agents acting on the cholinergic system.

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REFERENCES

1. Anden, N. E. and P. Bedard. Influences of cholinergic mechanisms on the function and turnover of brain dopamine. *J Pharm Pharmacol* **23:** 460–462, 1971.
2. Anden, N. E. and H. Wachel. Increase in the turnover of brain dopamine by stimulation of muscarinic receptors outside the dopamine nerve terminals. *J Pharm Pharmacol* **29:** 435–437, 1977.
3. Aquilonius, S. M. and R. Sjostrom. Cholinergic and dopaminergic mechanisms in Huntington's chorea. *Life Sci* **10:** 405–414, 1971.
4. Baldessarini, R. J, and D. Tarsy. Mechanisms underlying tardive dyskinesia. In: *The Basal Ganglia*, edited by M. D. Yahr. New York: Raven Press, 1976, pp. 433–446.
5. Bartholini, G., H. H. Keller and A. Pletscher. Drug-induced changes of dopamine turnover in striatum and limbic system of the rat. *J Pharm Pharmacol* **27:** 439–441, 1975.
6. Beleslin, D. B., R. Samardzic, S. K. Krstic, D. Micic and B. Terzic. Comparison of behavioral changes in cats treated with intracerebroventricular 6-hydroxydopamine and reserpine. *Brain Res Bull* **6:** 285–287, 1981.

7. Buccafusco, J. J. Kinetics of 3H choline and 3H acetylcholine metabolism in several regions of rat brain following intracerebro ventricular injection of 3H choline. *Biochem Pharmacol* **31:** 1599–1605, 1982.
8. Davis, K. L. and P. A. Berger. Pharmacologic investigations of the cholinergic imbalance hypothesis of movement disorders and psychosis. *Biol Psychiatry* **13:** 23–49, 1978.
9. Davis, K. L., L. E. Holister and J. Tepper. Cholinergic inhibition of methylphenidate-induced stereotypy: Oxotremorine. *Psychopharmacology* **56:** 1–4, 1978.
10. Duvosin, R. C. Cholinergic-anticholinergic antagonism in parkinsonism. *Arch Neurol* **17:** 124–136, 1967.
11. Falk, J. L. Drug effects on discriminative motor control. *Physiol Behav* **4:** 421–427, 1969.
12. Falk, J. L. and W. O. Haas. The control and recording of response force. *J Exp Anal Behav* **8:** 195–197, 1965.
13. Felice, L., J. Felice and P. Kissinger. Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. *J Neurochem* **31:** 1461–1465, 1978.
14. Finnegan, K. T., G. Ricaurte, L. S. Seiden and C. R. Schuster. Altered sensitivity to *d*-methylamphetamine, apormorphine, and haloperidol in rhesus monkeys depleted of caudate dopamine by repeated administration of *d*-methylamphetamine. *Psychopharmacology (Berlin)* **77:** 43–52, 1982.
15. Fischman, M. W. and C. R. Schuster. Long-term behavioral changes in the rhesus monkey after multiple daily injections of *d*-methylamphetamine. *J Pharmacol Exp Ther* **3:** 593–605, 1977.
16. Fuller, R. W. and S. Hemrick-Luerke. Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole treated rats. *Science* **209:** 305–306, 1980.
17. Gerlach, J., N. Reisby and A. Randrup. Dopaminergic hypersensitivity and cholinergic hypofunction in the pathophysiology of tardive dyskinesia. *Psychopharmacologia* **34:** 21–35, 1974.
18. Giorguieff, M. F., M. L. LeFloch, J. Glowinski and M. J. Besson. Involvement of cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of spontaneous release of dopamine from striatal dopaminergic terminals in the rat. *J Pharmacol Exp Ther* **200:** 535–544, 1977.
19. Goldstein, M., A. F. Battista, S. Nakatani and B. Anagnoste. Drug-induced relief of the tremor in monkeys with mesencephalic lesions. *Nature* **224:** 382–384, 1969.
20. Goldstein, M., A. F. Battista, S. Nakatani and B. Anagnoste. The effects of centrally acting drugs on tremor in monkeys with mesencephalic lesions. *Proc Ntl Acad Sci USA* **63:** 1113–1116, 1969.
21. Guyenet, P. G., Y. Agid, F. Javoy, J. C. Beaujouan, J. Rossier and J. Glowinski. Effects of dopaminergic receptor agonists and antagonists on the activity of the neostriatal cholinergic system. *Brain Res* **84:** 227–244, 1975.
22. Hery, F., M. F. Giorguieff, M. Hamon, M. J. Besson and J. Glowinski. Role of cholinergic receptors in the release of newly synthesized amines from serotonergic and dopaminergic terminals. In: *Interactions Between Putative Neurotransmitters in the Brain*, edited by S. Garattini, J. F. Pujol and R. Saminin. New York: Raven Press, 1978, pp. 39–51.
23. Hornykiewicz, O. Dopamine (3-hydroxytyramine) and brain function. *Pharmacol Rev* **18:** 925–964, 1966.
24. Hotchkiss, A. J., M. E. Morgan and J. W. Gibb. The long-term effects of multiple doses of methamphetamine on neostriatal tryptophan hydroxylase, tyrosine hydroxylase, choline acetyltransferase and glutamate decarboxylase activities. *Life Sci* **25:** 1373–1378, 1979.
25. Johanson, C. E., T. G. Aigner, L. S. Seiden and C. R. Schuster. The effects of methamphetamine on fine motor control in rhesus monkeys. *Pharmacol Biochem Behav* **11:** 273–278, 1979.
26. Johanson, C. E., C. R. Schuster, T. G. Aigner and L. S. Seiden. Effects of methamphetamine and haloperidol on fine motor control before and after repeated injections of methamphetamine. *Fed Proc* **37:** 659, 1978.
27. Kraemer, G., G. Breese, A. Prange, E. Moran, J. Lewis, J. Kemnitz, P. Bushnell, J. Howard and W. McKinney. Use of 6-hydroxydopamine to deplete brain catecholamines in the rhesus monkey: Effects on urinary catecholamine metabolites and behavior. *Psychopharmacology (Berlin)* **73:** 1–11, 1981.
28. Ladinsky, H., S. Consolo, S. Bianchi, D. Ghezzi and R. Samanin. Link between dopaminergic and cholinergic neurons in the striatum as evidenced by pharmacological, biochemical, and lesion studies. In: *Interactions Between Putative Neurotransmitters in the Brain*, edited by S. Garattini, J. F. Pujol and R. Samanin. New York: Raven Press, 1978, pp. 3–21.
29. Levine, M. S., C. D. Hull, E. Garcia-Rill, L. Erinoff, N. A. Buchwald and A. Heller. Long term decreases in spontaneous firing of caudate neurons induced by amphetamine on cats. *Brain Res* **194:** 263–268, 1980.
30. Lorez, H. Fluorescence histochemistry indicates damage of striatal dopamine nerve terminals in rats after multiple doses of methamphetamine. *Life Sci* **28:** 911–916, 1981.
31. Lucot, J. B., G. C. Wagner, C. R. Schuster and L. S. Seiden. The effects of dopaminergic agents on the locomotor activity of rats after high doses of methylamphetamine. *Pharmacol Biochem Behav* **13:** 409–413, 1980.
32. McGeer, P. L., D. S. Grewaal and E. G. McGeer. Influence of non-cholinergic drugs on rat striatal acetylcholine levels. *Brain Res* **80:** 211–217, 1974.
33. McGeer, P. L. and E. G. McGeer. Enzymes associated with the metabolism of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. *J Neurochem* **26:** 65–76, 1976.
34. Millington, W. R. and R. J. Wurtman. Choline and physostigmine enhance haloperidol-induced HVA and DOPAC accumulation. *Eur J Pharmacol* **80:** 431–434, 1982.
35. Ohmori, K. and T. Kojima. Effect of L-5HTP on tremorine- or oxotremorine-induced tremor in mice and rats. *Jpn J Pharmacol* **23:** 901–903, 1973.
36. Ondrusek, M. G., C. D. Kilts, G. D. Frye, R. B. Mailman, R. A. Mueller and G. R. Breese. Behavioral and biochemical studies of the scopolamine-induced reversal of neuroleptic activity. *Psychopharmacology (Berlin)* **73:** 17–22, 1981.
37. Preston, K. and C. R. Schuster. A comparison of the central and peripheral effects of atropine on force lever performance. *Pharmacol Biochem Behav* **16:** 423–427, 1982.
38. Ricaurte, G. A., R. W. Guillery, L. S. Seiden, C. R. Schuster and R. Y. Moore. Dopamine nerve terminal degeneration produced by high doses of methylamphetamine in the rat brain. *Brain Res* **235:** 93–103, 1982.
39. Ricaurte, G. A., C. R. Schuster and L. S. Seiden. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: A regional study. *Brain Res* **193:** 153–163, 1980.
40. Schallert, T., I. G. Whishaw, V. D. Ramirez and P. Teitelbaum. Compulsive, abnormal walking caused by anticholinergics in akinetic, 6-hydroxydopamine treated rats. *Science* **199:** 1461–1462, 1978.
41. Schallert, T., I. Q. Whishaw, V. D. Ramirez and P. Teitelbaum. 6-Hydroxydopamine and anticholinergic drugs. *Science* **202:** 1215–1217, 1978.
42. Scheel-Kruger, J. Central effects of anticholinergic drugs measured by the apomorphine gnawing test in mice. *Acta Pharmacol Toxicol* **28:** 1–16, 1970.
43. Seiden, L. S., M. W. Fischman and C. R. Schuster. Long-term methamphetamine induced changes in brain catecholamine in tolerant rhesus monkeys. *Drug Alcohol Depend* **1:** 215–219, 1975.
44. Sethy, M. H. and V. H. VanWoert. Modification of striatal acetylcholine concentration by dopamine receptor agonists and antagonists. *Res Commun Chem Pathol Pharmacol* **8:** 13–28, 1974.
45. Stadler, H., K. G. Lloyd, M. Gadea-Ciria and G. Bartholini. Enhanced striatal acetylcoline release by chlorpromazine and its reversal by apomorphine. *Brain Res* **55:** 476–480, 1973.

46. Steranka, L. R. and E. Sanders-Bush. Long-term effects of continuous exposure to amphetamine on brain dopamine concentration and synaptosomal uptake in mice. *Eur J Pharmacol* **65:** 439–443, 1980.
47. Taha, E. B. and P. Redgrave. Neuroleptic suppression of feeding and oral stereotypy following microinjections of carbachol into the substantia nigra. *Neurosci Lett* **20:** 357–361, 1980.
48. Trabucchi, M., D. L. Cheney, G. Racagniand and E. Costa. *In vivo* inhibition of striatal acetylcholine turnover by l-dopa, apomorphine, and (+)-amphetamine. *Brain Res* **85:** 130–134, 1975.
49. Trulson, M. E. and B. L. Jacobs. Chronic amphetamine administration to cats: behavioral and neurochemical evidence for decreased central serotonergic function. *J Pharmacol Exp Ther* **211:** 375–384, 1979.
50. Trulson, M. E. and B. L. Jacobs. Chronic amphetamine administration decreases tryptophan hydroxylase activity in cats. *Life Sci* **26:** 329–335, 1980.
51. Wagner, G. C., G. A. Ricaurte, C. E. Johanson, C. R. Schuster and L. S. Seiden. Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. *Neurology* **30:** 547–550, 1980.
52. Wagner, G. C., G. A. Ricaurte, L. S. Seiden, C. R. Schuster, R. J. Miller and J. Westley. Long-lasting depletion of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. *Brain Res* **181:** 151–160, 1980.
53. Wagner, G. C., L. S. Seiden and C. R. Schuster. Methamphetamine-induced changes in brain catecholamines in rats and guinea pigs. *Drug Alcohol Depend* **4:** 435–438, 1979.
54. Weinstock, M., A. P. Zavadil, A. J. Rosin, C. C. Chuich and I. J. Kopin. The role of peripheral catecholamines in oxotremorine tremor in the rat and its antagonism by *beta* adrenoceptor blocking agents. *J Pharmacol Exp Ther* **206:** 91–96, 1978.
55. Yahr, M. D., R. C. Duvosin, M. M. Hoehn, M. J. Schear and R. E. Barrett. l-Dopa (1–3, 4-dihydroxyphenylalanine), its clinical effects in Parkinsonism. *Trans Am Neurol Assoc* **93:** 56–63, 1968.