Exhibit 3

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LONG-TERM METHAMPHETAMINE INDUCED CHANGES IN BRAIN CATECHOLAMINES IN TOLERANT RHESUS MONKEYS

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d-Amphetamine, methamphetamine and other phenethylamines cause decreases in food intake, stereotypic behavior, disruption of behavior that is under stimulus control, as well as general sympathomimetic effects such as hyperthermia, increased blood pressure and piloerection. The doses required to produce different effects vary. In addition, most psychomotor stimulants are subject to abuse in man and will be self-administered by animals [1]. Repeated administration of the amphetamines leads to the development of tolerance to many of its effects on behavior although the degree of tolerance depends to a large extent on the frequency of administration, the dose, the route of administration and the behavior in question. Several investigators have presented evidence that amphetamines exert their effects upon behavior by increasing the concentration of catecholamines at the synaptic cleft by blocking re-uptake and/or promoting release.

The overall purpose of this investigation was to determine the effect of long-term repeated intravenous administration of methamphetamine in increasing doses to rhesus monkeys. This study was part of a larger research program designed to determine if long-term administration of methamphetamine results in irreversible effects in the rhesus monkey. To this end, measures were made on behavior, neuropathology and brain catecholamines. In this paper we shall report both short and long-term changes in brain catecholamines that were caused by prolonged administration of methamphetamine. It was found that methamphetamine induced changes in regional brain catecholamines, some of which persisted even after the drug was discontinued.

Rhesus monkeys were injected intravenously 8 times daily. The dose of methamphetamine was increased until a final dose was reached which could be tolerated by the particular monkey involved. Tolerance to a particular dose was determined through observing diminished food intake and weight loss. Doses were incremented only when signs of behavioral tolerance appeared.

Two studies were carried out: in the first, monkeys were treated for 3 - 6 months (final dose 3.0 - 6.5 mg/kg/inj) eight times per day. Monkeys were either killed 24 hours or 3 - 6 months after the last injection. In the

TABLE 1

NE - 8 Injections of methamphetamine (meth.) daily for several months

Treatment	Pons-medulla	Mid-brain	Hypothalamus	Frontal cortex
Control $N = 12$	0.45 ± 0.04	0.59 ± 0.03	1.8 ± 0.26	0.21 ± 0.03
Chronic meth. 24 h post meth. $N = 5$	0.23 ± 0.07 (51%) $p < 0.05$	0.40 ± 0.06 (67%) $p < 0.05$	0.76 ± 0.18 (42%) $p < 0.05$	0.08 ± 0.23 (38%) $p < 0.05$
Chronic meth. 3 - 6 months post meth. $N = 6$	0.31 ± 0.08	0.39 ± 0.07 (66%) $p < 0.05$	1.4 ± 0.43	0.10 ± 0.01 (48%) $p < 0.05$

NE - 8 Injections of methamphetamine (meth.) for a period of two weeks

Treatment	Pons-medulla	Mid-brain	Hypothalamus	Frontal cortex
Chronic meth. 24 h post meth. $N = 3$	0.21 ± 0.11 (48%)	0.41 ± 0.12 (69%)	1.77 ± 0.23 (98%)	0.12 ± 0.04 (57%)
Chronic meth. 2 weeks post meth. $N = 3$	0.40 ± 0.01 (88%)	0.48 ± 0.02 (81%)	1.4 ± 0.34 (77%)	0.11 ± 0.01 (52%)

second study, monkeys were treated with methamphetamine for a period of two weeks (final dose 2 - 3 mg/kg/inj) eight times per day. These monkeys were killed either 24 hours or two weeks after the last methamphetamine injection.

On the final day of the experiment the monkeys were anesthetized with pentobarbital and killed by exsanguination after severing the ascending carotid arteries. The brain was rapidly removed (3 min) and taken into a cold room where it was dissected and frozen in liquid nitrogen until assay. Norepinephrine (NE) was assayed by the method of Bertler *et al.* [2] and dopamine (DA) was assayed by the method of Carlsson and Waldeck [3].

Monkeys killed after 3 - 6 months of continued daily (8 times/day) methamphetamine administration exhibited fairly uniform depletion of NE in all brain areas 24 hours after the last methamphetamine injection (Table 1). These data show the brain areas affected including pons-medulla, the midbrain, the hypothalamus and frontal cortex. A 24 hour post injection depletion of NE is consistent with the view that methamphetamine causes blockade of

TABLE 2

DA - 8 Injections of methamphetamine (meth.) daily for several months

Treatment	Pons-medulla	Mid-brain	Hypothalamus	Caudate	Frontal cortex
Control $N = 12$	0.16 ± 0.01	0.51 ± 0.04	0.83 ± 0.12	10.1 ± 0.57	0.09 ± 0.01
Chronic meth. 24 h post meth. $N = 5$	0.51 ± 0.20 ns	0.61 ± 0.11 ns	1.33 ± 0.30 ns	2.0 ± 1.0 (19%) $p < 0.001$	0.19 ± 0.04 ns
Chronic meth. 3 - 6 months post meth. $N = 6$	0.13 ± 0.03 ns	0.33 ± 0.07 ns	0.82 ± 0.17 ns	3.15 ± 0.64 (31%) $p < 0.001$	0.13 ± 0.05 ns

DA - 8 Injections of methamphetamine (meth.) for a period of two weeks

Treatment	Pons-medulla	Mid-brain	Hypothalamus	Caudate	Frontal cortex
Chronic meth. 24 h post meth. $N = 3$	0.19 ± 0.01	0.43 ± 0.07	1.14 ± 0.53	3.67 ± 0.50 (36%)	0.15 ± 0.02
Chronic meth 2 weeks post meth. $N = 3$	0.53 ± 0.98	0.76 ± 0.14	1.20 ± 0.45	2.3 ± 0.37 (23%)	0.30 ± 0.01

re-uptake and/or direct release of norepinephrine from nerve terminals. In the monkeys that were killed 3 - 6 months after the last methamphetamine injection, the levels of NE remained decreased in the mid-brain and frontal cortex, but returned to normal in the pons-medulla and the hypothalamus. The monkeys treated with methamphetamine for a two week period showed decreased NE levels 24 hours after the last injection, but there appears to be a trend to return to normal values two weeks after methamphetamine treatment (see Table 2). However, the N is not large enough for statistical testing.

The most striking change occurred in dopamine in the caudate nucleus (Table 2). There was approximately 70 to 80% reduction of caudate dopamine. This depletion occurred in both groups of monkeys that were treated for a 3 - 6 month period and appeared permanent in so far as there was no difference between monkeys that were killed 24 hours after the last injection and monkeys that were killed 3 - 6 months after the last injection.

Tonge [4] has reported depletion of norepinephrine and serotonin in rat brain in several brain areas which appears to last for up to 36 hours after chronic oral intake of *d*-amphetamine. These changes are consistent with some of the changes we observed in monkeys. Harris and Baldessarini [5] have demonstrated that amphetamine inhibits tyrosine hydroxylase in the corpus striatum of rat brain but not in other areas. They propose that the inhibition of tyrosine hydroxylase is due to the activation of dopamine receptors in the corpus-striatum by accumulation of DA released by amphetamine; the increase in DA leads to feedback inhibition of DA synthesis, possibly by a GABA neuron in the striatum. This mechanism conceivably can account for the effects of methamphetamine on DA seen in animals killed 24 hours after the last injection of methamphetamine. It is difficult, however, to explain the prolonged depletion of DA in these terms. One would have to assume that the feedback "tone" changes permanently as a result of prolonged administration of methamphetamine. While this appears unlikely in the absence of structural changes in the caudate, it is a possibility that must be considered.

The monkeys treated daily (8 times/day) on methamphetamine for 3 - 6 months exhibited tolerance to the disruptive effects of the drug on behavior maintained by a fixed ratio (FR) 10 or a differential reinforcement of low rate (DRL) schedule of food reinforcement [6]. In fact, monkeys responding on the DRL schedule showed an apparently irreversible tolerance to the response suppressant effects of methamphetamine as measured by acute injection of drug 3 - 6 months after cessation of methamphetamine maintenance [7]. This irreversible effect is correlated with permanent changes in the CA content of certain brain areas. Monkeys treated with methamphetamine for a prolonged period and killed 3 - 6 months after discontinuing the drug showed a 70% loss of caudate DA, a 33% loss in midbrain NE and 52% loss of NE in the frontal cortex. The largest effect on brain CAs appears to occur in caudate DA with smaller effects occurring in the NE system. Which, if any, of these changes are functionally related to the apparently irreversible behavioral tolerance seen in these same monkeys can only be determined by further research.

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