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Effects of Adult or Neonatal Treatment With 6-Hydroxydopamine or 5,7-Dihydroxytryptamine on Locomotor Activity, Monoamine Levels, and Response to Caffeine

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ERINOFF, L AND S R. SNODGRASS *Effects of adult or neonatal treatment with 6-hydroxydopamine or 5,7-dihydroxytryptamine on locomotor activity, monoamine levels, and response to caffeine.* PHARMACOL BIOCHEM BEHAV 24(4) 1039–1045, 1986 —Rats were treated as neonates or adults with desmethylimipramine (DMI) followed by intraventricular 6-hydroxydopamine (6-HDA) or 5,7 dihydroxytryptamine (5,7-DHT) Locomotor activity of treated rats was measured in photocell cages Neonatal treatment with 5,7-DHT produced hypoactivity during development while neonatal 6-HDA led to hyperactivity Treatment of adult rats with 5,7-DHT or 6-HDA, while resulting in equivalent monoamine depletions, was without effect on locomotor activity The dose response function for caffeine was determined in these rats. Depletion of dopamine by either neonatal or adult treatment with 6-HDA decreased caffeine stimulation of locomotor activity The adenosine receptor agonist l-phenylisopropyladenosine (L-PIA) decreased locomotor activity in all rats in a dose-dependent fashion

Locomotor activity 6-Hydroxydopamine 5,7-Dihydroxytryptamine Caffeine Development Serotonin Dopamine L-Phenylisopropyladenosine

THE development of locomotor activity following neonatal depletion of brain dopamine (DA) by 6-hydroxydopamine (6-HDA) has been studied extensively and most reports have described a period of hyperactivity [7, 20, 21, 24, 28]. Similar treatment of adult animals does not cause hyperactivity [7]. The effects of depletion of serotonin (5HT) on the development of locomotor behavior have not been as well characterized. A role for 5HT in the suppression of locomotor activity that occurs with development was proposed by Mabry and Campbell from their studies of rats treated with the 5HT synthesis inhibitor, p-chlorophenylalanine (PCPA) [19] Lucot *et al.* demonstrated that administration of p-chloroamphetamine (PCA) caused age dependent effects on locomotor activity, with increased activity seen in neonates, and decreased activity in adults [17] Serotonin depletion produced in neonatal rats by intracisternal or intraventricular 5,7-dihydroxytryptamine (5,7-DHT) has been reported to delay the normal suppression of locomotor activity seen with development in rats tested in groups of three in a photocell device [3], to heighten and delay the peak in locomotor activity in rats tested in isolation in stabilimeter cages [18], or to decrease open field locomotor activity [14].

The present study was designed to examine the effects of neonatal and adult treatment with intraventricular 5,7-DHT on locomotor activity In addition to control animals, 6-HDA treated rats were tested along with the 5,7-DHT animals to serve as positive controls We also examined the effects of caffeine administration on locomotor activity in these animals. Previous reports had shown that the locomotor stimulatory effect of caffeine is blocked by alpha-methyltyrosine (AMT) [32] or pimozide [8,31] or neonatal 6-HDA [5], all treatments which interfere with dopaminergic transmission In contrast, inhibition of 5HT synthesis by PCPA treatment has been reported to enhance caffeine induced activity [32]. Since caffeine stimulation of locomotor activity has been postulated to be mediated by A1 adenosine receptors [26], we administered the A1 adenosine agonist 1-phenylisopropyladenosine (L-PIA) to adult and neonatally

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TABLE 1

EFFECT OF TREATMENT WITH 6-HDA OR 5,7-DHT ON REGIONAL DOPAMINE CONTENT

	Dopamine (ng/g wet weight ± SEM)				
	Nuc Accumbens	Striatum	Olfactory Tub	Brainstem	Hypothalamus
Adult Treated					
Control (n=8)	3481 ± 359	6151 ± 537	1816 ± 127	74 ± 5	318 ± 35
5,7-DHT (n=6)	3188 ± 519	6201 ± 681	1948 ± 212	79 ± 2	289 ± 31
6-HDA (n=5)	1002 ± 317*	1494 ± 468*	723 ± 99*	48 ± 3*	195 ± 11*
Neonatally Treated					
Control (n=5)	3426 ± 391	5815 ± 666	2069 ± 224	69 ± 5	368 ± 47
5,7-DHT (n=10)	3176 ± 319	7135 ± 334	2308 ± 198	78 ± 4	352 ± 35
6-HDA (n=6)	576 ± 179*	501 ± 205*	678 ± 135*	51 ± 4*	296 ± 20

*$p<0.05$ vs respective control

TABLE 2

EFFECT OF TREATMENT WITH 6-HDA OR 5,7-DHT ON REGIONAL SEROTONIN CONTENT

	Serotonin (ng/g wet weight ± SEM)							
	Nuc Accumbens	Striatum	Hippocampus	Brainstem	Olfactory Tub	Cerebellum	Hypothalamus	Cortex
Adult Treated								
Control (n=8)	529 ± 60	606 ± 62	550 ± 41	970 ± 50	688 ± 109	93 ± 9	1077 ± 92	594 ± 42
5,7-DHT (n=6)	311 ± 30*	373 ± 51*	55 ± 9*	1109 ± 122	259 ± 53*	49 ± 11*	1069 ± 105	143 ± 19*
6-HDA (n=5)	554 ± 124	709 ± 110	477 ± 65	979 ± 74	663 ± 73	83 ± 11	1019 ± 61	591 ± 63
Neonatally Treated								
Control (n=5)	554 ± 34	582 ± 65	502 ± 42	1029 ± 78	911 ± 76	100 ± 7	1158 ± 59	604 ± 10
5,7-DHT (n=10)	262 ± 48*	351 ± 54*	88 ± 17*	1165 ± 115	219 ± 77*	74 ± 9*	1292 ± 220	109 ± 28*
6-HDA (n=6)	598 ± 96	907 ± 78*	489 ± 64	1128 ± 111	936 ± 114	89 ± 4	1210 ± 70	637 ± 31

*$p<0.05$ vs respective control

treated rats in doses previously reported to depress locomotor activity in mice [26,30]

METHOD

All rats were provided with a diurnal lighting cycle (0600, on, 1800, off) and ad lib access to food and water. Adult rats (Hilltop Lab Animals) were housed 5 per cage by treatment groups in large metal cages Adult male rats (270 g) were injected with DMI 20 mg/kg IP. One hour later, rats were anesthetized with ether and injected intraventricularly (1 5 mm posterior to bregma, 2.5 mm lateral to the midline, and 4.0 mm ventral to the surface of the skull) with saline (0 9%) ascorbate (0 1%), 6-HDA 250 μg/20 μl, or 5,7-DHT, 150 μg/20 μl. Three days later this procedure was repeated except that the opposite lateral ventricle was injected A smaller volume and dose of 5,7-DHT (75 μg/10 μl) was administered because repetition of the 150 μg dose induced convulsions

Pregnant rats were obtained at 14 days gestation (Hilltop Lab Animals) and housed individually in large metal cages Three litters of 8–10 male pups were formed The 6-HDA treatment group was born 1 day after the vehicle and DHT groups. At 3 days of age pups were injected with 20 mg/kg DMI subcutaneously, followed in one hour by ether anesthesia and the intraventricular (0.5 mm posterior to bregma, 1 5 mm lateral to the midline, and 2 5 mm ventral to the surface of the skull) injection of saline (0 9%) ascorbate (0.1%), 6-HDA 100 μg/10 μl, or 5,7-DHT 50 μg/10 μl On day 6 the procedure was repeated, using the opposite lateral ventricle. Pups were weaned at 30 days of age and continued to be group caged

To measure locomotor activity, rats were placed in individual wire cages (40×24×18 cm high) with 3 solid walls and a wire mesh wall and floor, mounted in a standard rack The cages were equipped with two light-emitting diodes, 2 4 cm above the floor of the cage and 12 cm from each end, aimed at oppositely situated photodetectors, so that their infrared beams crossed the long axis of the cage. Beam interruptions per 10-minute period were automatically recorded by a modified (G and B Electronics, Royston, U K.) AIM 65 (Rockwell) microcomputer For testing rats less than 18 days old a 1 3 cm thick wooden floor was placed over the wire mesh floor, and wooden inserts were used to decrease the

TABLE 3

EFFECT OF TREATMENT WITH 6-HDA OR 5,7-DHT ON REGIONAL NOREPINEPHRINE CONTENT

	Norepinephrine (ng/g wet weight ± SEM)							
	Nuc Accumbens	Striatum	Hippocampus	Brainstem	Olfactory Tub	Cerebellum	Hypothalamus	Cortex
Adult Treated								
Control (n=8)	195 ± 32	156 ± 18	376 ± 21	430 ± 17	140 ± 12	215 ± 6	942 ± 44	291 ± 12
5,7-DHT (n=6)	139 ± 16	152 ± 11	327 ± 20	417 ± 15	129 ± 18	201 ± 7	929 ± 54	309 ± 26
6-HDA (n=5)	127 ± 15	123 ± 11	242 ± 40*	315 ± 24*	133 ± 14	96 ± 13*	823 ± 37	253 ± 22
Neonatally Treated								
Control (n=5)	179 ± 11	159 ± 33	382 ± 4	449 ± 14	201 ± 17	193 ± 10	964 ± 64	284 ± 10
5,7-DHT (n=10)	197 ± 11	213 ± 14	384 ± 13	497 ± 12	236 ± 17	231 ± 10	1065 ± 34	295 ± 8
6-HDA (n=6)	190 ± 20	181 ± 30	337 ± 47	478 ± 15	243 ± 34	232 ± 20	1026 ± 96	325 ± 14

*$p<0.05$ vs respective control

size of the cage to 24×24×18 cm high, with one light beam crossing the center of the cage To prevent pups from climbing the wire mesh wall, sheets of acetate were placed over the mesh.

Testing of adult treated rats was begun 2 weeks after the second intraventricular injection. Rats were tested in 2 one-hour sessions starting at 0800 and 0900 with rats from each treatment group in each session. Activity was assessed three times a week. The study of caffeine and L-PIA effects on locomotor activity was begun after 4 sessions of baseline activity were recorded.

Neonatal rats were tested in 2 sessions that began at 0800 and 0900. All 3 treatment groups were tested in each session and activity was measured three times a week. The development of locomotor activity was monitored from days 10 through 46 for vehicle and DHT groups, and 9 to 45 for the 6-HDA group. Following this, the effects of caffeine and L-PIA on locomotor activity were assessed

Drug Testing

Caffeine (Sigma) dissolved in warm saline was administered IP 20 minutes before activity testing. L-PIA (Boehringer-Manheim) was dissolved in warm propylene glycol and injected IP 20 minutes before activity testing. When both drugs were given, L-PIA was given 20 minutes before testing followed by caffeine 10 minutes later, thus maintaining the time from initial drug injection constant. Drugs were administered in volumes of 1 ml/kg body weight.

Both adult and neonatally treated rats were killed 3 months after their last drug administration Neonatally treated rats were 6 months old at the time of sacrifice. The rats were killed by decapitation and their brains dissected on an ice-cold glass plate [11,15]. The following regions were studied: olfactory tubercle, nucleus accumbens, caudate nucleus, hypothalamus, hippocampus, cerebral cortex, cerebellum and brainstem. Brain regions were wrapped in aluminum foil and immediately frozen in liquid nitrogen. Monoamines were extracted by a modification of the method of Shellenberger and Gordon [25] and assayed by high pressure liquid chromatography using electrochemical detection [4]

RESULTS

Effects on Regional Monoamine Content

The effects on regional monoamine content of intraventricular injection of 6-HDA or 5,7-DHT in DMI pretreated adult and neonatal rats are given in Tables 1–3. Depletions of DA and 5HT were comparable in neonatally and adult treated animals. In 6-HDA treated rats, nucleus accumbens dopamine content was reduced by 71% in adult treated rats and 83% in neonatally treated rats. The reductions in dopamine levels were 76% and 91% in striatum, and 60% and 67% in olfactory tubercle. Hypothalamic dopamine content was reduced 38% in adult-treated rats. 5,7-DHT induced serotonin depletions of 90%, 82% (adult, neonatal) in hippocampus, 76%, 82% in cortex; 41%, 53% in nucleus accumbens; 38%, 40% in striatum; 62%, 76% in olfactory tubercle; and 47%, 26% in cerebellum. 6-HDA treatment produced decreases in regional norepinephrine content in adult but not neonatally treated rats. Norepinephrine concentration was reduced 36% in hippocampus, 27% in brain stem, and 55% in cerebellum. Treatment with 5,7-DHT had no effect on catecholamine concentrations in any brain region in adult or neonatally treated rats. However, 6-HDA treatment of neonatal rats, but not adults, produced a 56% increase in the striatal serotonin content. No other changes in regional serotonin content were found.

Effects on Locomotor Activity

Neonatal treatment with 6-HDA or 5,7-DHT altered the development of spontaneous locomotor activity in the rat as can be seen in Fig. 1 (2-way ANOVA, repeated measures on 1 factor, effect of treatment, $F(2,18)=15.76$, $p<0.001$, effect of age, $F(16,288)=11.02$, $p<0.001$, effect of treatment × age, $F(32,288)=3.88$, $p<0.001$). Neonatal treatment with 6-HDA resulted in increased locomotor activity in the third and fourth weeks of life while 5,7-DHT treatment produced hypoactivity during this same period. Rats treated with 6-HDA differed from controls on days 16–34, rats treated with 5,7-DHT differed from controls on days 16–23, and rats treated with 6-HDA differed from those treated with 5,7-DHT on days 16–34 ($p<0.05$, Duncan's multiple range test)

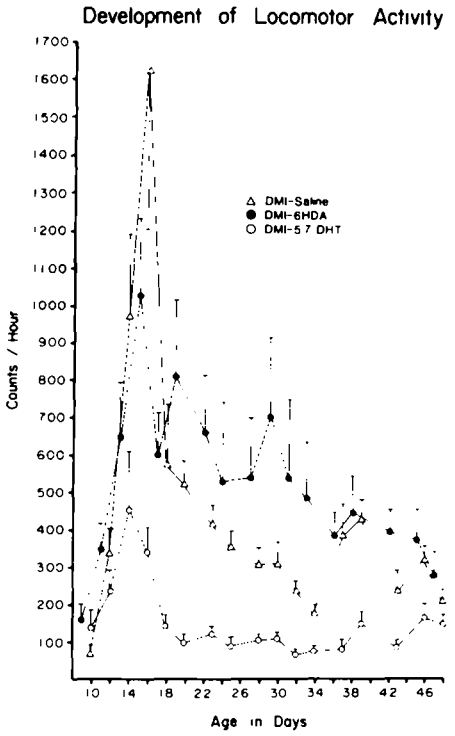

FIG 1 The mean activity in counts per hour ±SEM plotted against age in days of rats treated with DMI followed by intraventricular 6-HDA, 5,7-DHT, or vehicle on days 3 and 6 Significant differences from control were seen on days 16–34 in 6-HDA treated rats and days 16–23 in 5,7-DHT treated rats

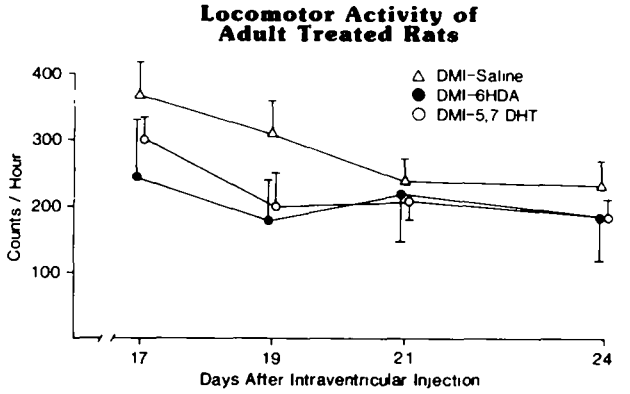

FIG 2 The mean activity in counts per hour ±SEM of adult rats treated with DMI followed by intraventricular 6-HDA, 5,7-DHT or vehicle There were no significant differences between any of the groups

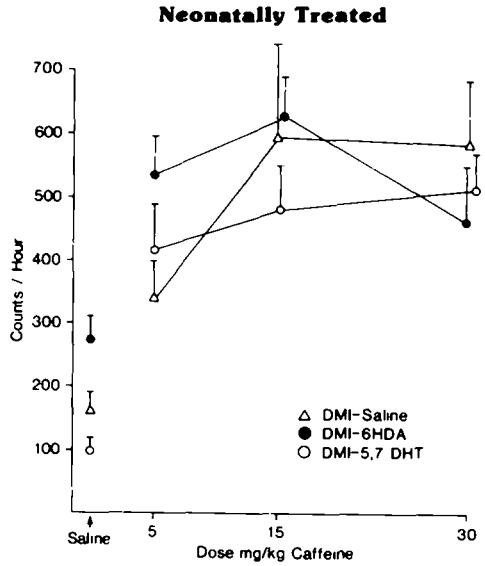

FIG 3 Dose response function for caffeine in rats treated with neurotoxins neonatally All 3 groups differed from each other, 6-HDA treated rats had the highest mean overall activity, 5,7-DHT treated rats the lowest, while vehicle control rats were intermediate

Despite these changes in activity levels, neither 6-HDA nor 5,7-DHT treatment affected the age at which peak locomotor activity occurred Although 6-HDA and 5,7-DHT treatments had opposite effects on locomotor activity, they produced similar decreases in body weight (20%) relative to controls (2-way ANOVA, $F(2,18)=4.62$, $p<0.01$, 6-HDA and 5,7-DHT different from control but not each other, Duncan's multiple range test, $p<0.05$) As can be seen in Fig 2, administration of 6-HDA or 5,7-DHT to adult rats had no effect on spontaneous locomotor activity measured in the third week following intraventricular drug injection (2-way ANOVA, $F(2,16)=1.74$, n s.)

Effects of Caffeine and L-PIA on Neonatal and Adult Treated Rats

The effects of caffeine on locomotor activity in rats treated with neurotoxins as neonates is presented in Fig 3 All three groups, control, 6-HDA, and 5,7-DHT, differed from each other (2-way ANOVA, repeated measures on 1 factor on log transformed data, $F(2,18)=17.67$, followed by Newman-Keuls' range test, $p<0.05$) The antilog of the average of the overall activity counts for each group was 436 for the 6-HDA treated group, 342 for the control group, and 282 for the 5,7-DHT treated rats When the dose response function was analyzed as the log transformation of the data expressed as a percent of saline vehicle activity scores, significant differences were again found in the three groups, $F(2,18)=9.29$, $p<0.001$, followed by Newman-Keuls' range test, $p<0.05$ The 5,7-DHT treated rats exhibited the greatest percent increase in locomotor activity (antilog of the average percent increase=475%), the 6-HDA treated rats had the least (198%) increase, and the control group was intermediate (271% increase) Table 4 presents the mean activity data presented in Fig 3 expressed as percent of saline vehicle

The dose effects function for caffeine on locomotor activity in adult treated rats is shown in Fig. 4 Analysis of the log transformation of the activity data in counts per hour of the three adult treated groups indicated that these groups did not differ significantly from each other, $F(2,16)=0.57$, n s When the data were expressed as percent of saline vehicle, the

TABLE 4

CAFFEINE EFFECTS ON LOCOMOTOR ACTIVITY AS PERCENT OF SALINE VEHICLE

	5 mg/kg	15 mg/kg	30 mg/kg
Adult Treated			
Control n=8	237 ± 20	323 ± 18	277 ± 27
5,7-DHT n=6	188 ± 37	272 ± 31	248 ± 26
6-HDA n=5	158 ± 50	153 ± 62	187 ± 28
Neonatally Treated			
Control n=5	206 ± 41	361 ± 54	353 ± 37
5,7-DHT n=10	423 ± 54	494 ± 40	518 ± 39
6-HDA n=6	198 ± 25	232 ± 26	172 ± 43

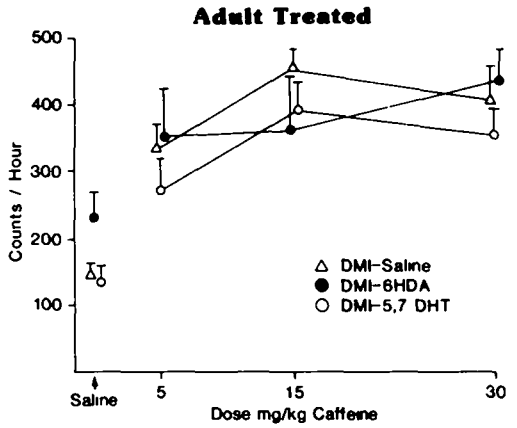

FIG 4 Dose response function for caffeine in rats treated with neurotoxins as adults No differences were seen in the groups

TABLE 5

EFFECT OF L-PIA ON LOCOMOTOR ACTIVITY

	Vehicle	0 05 mg/kg	0 1 mg/kg	0 2 mg/kg	0 1 mg/kg and 5 mg/kg Caffeine
Adult Treated					
Control	240 ± 37*	86 ± 31	58 ± 16	18 ± 4	198 ± 35
n=8	(100 ± 15)†	(36 ± 13)	(24 ± 7)	(8 ± 2)	(82 ± 14)
5,7-DHT	159 ± 22	66 ± 13	43 ± 14	18 ± 5	159 ± 34
n=6	(100 ± 14)	(42 ± 8)	(27 ± 9)	(11 ± 3)	(100 ± 21)
6-HDA	236 ± 29	119 ± 47	63 ± 40	34 ± 24	282 ± 46
n=5	(100 ± 12)	(50 ± 20)	(27 ± 17)	(14 ± 10)	(119 ± 19)
Neonatally Treated					
Control	188 ± 29	84 ± 23	46 ± 13	24 ± 3	155 ± 40
n=5	(100 ± 15)	(45 ± 12)	(24 ± 7)	(13 ± 2)	(82 ± 21)
5,7-DHT	176 ± 24	72 ± 16	40 ± 5	31 ± 5	148 ± 20
n=10	(100 ± 14)	(40 ± 9)	(23 ± 3)	(18 ± 3)	(84 ± 11)
6-HDA	268 ± 52	124 ± 16	43 ± 13	34 ± 19	183 ± 56
n=6	(100 ± 19)	(46 ± 6)	(16 ± 5)	(13 ± 7)	(68 ± 20)

*counts/hour ± SEM
†% of vehicle activity

6-HDA treated group was found to differ significantly from the 5,7-DHT and control groups (2-way ANOVA on log transformed data, $F(2,16)=4.40$, $p<0.05$, followed by Newman-Keuls' range test, $p<0.05$). The overall mean of the percent increases for each of the three groups (antilog of the means from the log transformed data) was 281% for the control group, 172% for the 6-HDA group, and 232% for the 5,7-DHT group The data from Fig 4 expressed as percent of saline vehicle are given in Table 4.

The effect of L-PIA on locomotor activity in both adult and neonatally treated rats is given in Table 5. L-PIA reduced locomotor activity in both adult and neonatally treated animals in a dose-related fashion When these means are expressed as a percent of the activity seen following propylene glycol vehicle, no differences are found in the effects of L-PIA on any of the treatment groups Five mg/kg caffeine completely antagonized the locomotor depression seen following 0 1 mg/kg L-PIA

DISCUSSION

Neonatal 6-HDA treated rats showed initial hyperactivity followed by a return to control levels of activity. This pattern of transient hyperactivity has been reported to correlate with

the degree of brain dopamine depletion [20], and our data agree with reports on rats with similar degrees of dopamine depletion [20,24] The lack of effect of adult 6-HDA treatment also confirms previous findings [7,20] Depletion of brain serotonin by neonatal treatment of rat pups with 5,7-DHT produced marked decreases in locomotor activity during development, while similar depletions in regional brain serotonin content produced by 5,7-DHT treatment of adult rats had no effect on locomotor activity The hypoactivity which we observed after neonatal DHT treatment is in agreement with the results of Hard *et al* [14] but contrasts with those of Breese *et al* [3] and Lucot and Seiden [18] The reasons for these disparate findings are not clear, although the 5,7-DHT dosing regimes and routes of administration (intracisternal or intraventricular injection) varied and may have produced differences in the degree or pattern of regional serotonin depletion Differences in the methodologies employed for assessing locomotor activity in each of these studies might also account for these differing results since measurements of locomotor activity are very much dependent on the type of apparatus used and the conditions of testing [22,23] Nonetheless, the present report demonstrates that depletion of serotonin in early life produces different effects on locomotor activity than equivalent depletions obtained in adult animals which is quite analogous to the differential effects on locomotor activity of dopamine depletion in adult and neonatal rats [7,20]

In the present study, serotonin content of the striatum was found to be increased after neonatal 6-HDA treatment Although the magnitude of the serotonin increase we report is less, our data support those of Stachowiak *et al* [27], who proposed that sprouting of striatal 5HT terminals occurs after neonatal 6-HDA induced dopamine depletion In agreement with Breese *et al* [2], we find that this increase in serotonin is limited to the striatum since the nucleus accumbens and olfactory tubercle, while similar to the striatum in their large dopamine depletions, showed no changes in serotonin content This increase in striatal serotonin content following neonatal 6-HDA treatment seems to be transmitter as well as region specific since the activities of choline acetyl transferase and gamma-aminobutyric acid decarboxylase, markers for cholinergic and GABAergic neurons, were unchanged in rats depleted of DA by neonatal 6-HDA treatment [5]

Caffeine characteristically yields an inverted U shaped dose-response function for the stimulation of locomotor activity in the rat [29,32], and this pattern was observed in all of the treatment groups in this study Relative to control animals, both adult and neonatally treated 6-HDA rats exhibited decreased caffeine stimulation expressed as percent increase over saline vehicle even though neonatally treated rats had elevated saline activity while adult treated rats did not differ from control Thus, it does not seem that this decreased response to caffeine can be explained by rate dependency Furthermore, it is unlikely that the decreased response to caffeine is due to a ceiling effect since, in a previous study, rats treated neonatally with 6-HDA who showed no response to caffeine did respond to other drugs with increased locomotor activity [6] These data showing decreased caffeine stimulation in rats depleted of DA as adults or neonates lend support to the idea that dopaminergic transmission is involved in the mediation of the effects of caffeine [8, 10, 12, 13, 31, 32, 33] The nature of the interaction between methylxanthines such as caffeine and dopaminergic systems has not been fully elucidated, in particular, it is not clear to what extent blockade of adenosine receptors accounts for this interaction (for discussion see [10,33])

Although caffeine has been shown to affect brain serotonin levels [1,9] and inhibition of serotonin synthesis with PCPA has been reported to augment caffeine stimulation of locomotor activity [32], the evidence for a link between the effects of caffeine and serotonin is not as strong as for dopamine Adult treatment with 5,7-DHT did not affect caffeine stimulation of locomotor activity The percent increase over saline in animals treated with 5,7-DHT as neonates was much greater than that seen in controls, however, these rats had lower saline activity so this greater percent increase may be a function of this initial low rate

Locomotor activity of all rats was decreased in a dose dependent manner by L-PIA, an A1 adenosine receptor agonist This decrease was antagonized by caffeine There were no differences in the percent decrease in locomotor activity of any of the treatment groups in response to L-PIA, in contrast to the data for caffeine stimulation of locomotor activity This suggests that the mechanism of caffeine stimulation of locomotor activity and L-PIA depression may be different Katims *et al* [16] have noted that caffeine is 5 times more potent at reversing L-PIA depression of locomotor activity than at stimulating basal locomotor activity and that methylxanthine reversal of L-PIA depression correlates better with affinity for adenosine binding sites than with stimulant effects

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