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Physiological and Behavioral Effects of Centrally-Administered 6-Hydroxydopamine in Cats[1]

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HOWARD, J. L. AND G. R. BREESE. *Physiological and behavioral effects of centrally-administered 6-hydroxydopamine in cats.* PHARMAC. BIOCHEM. BEHAV. 2(5) 651–661, 1974. – Intraventricular injections of 6-hydroxydopamine (6-OHDA) into cats produced a greater reduction of brain norepinephrine than dopamine content while producing no change in brain serotonin. The effects immediately after the first injection of 6-OHDA included hypothermia, increase in respiratory rate, and in the presence of pargyline, sham rage. Following the first injection, a prolonged period of hypophagia and hypodipsia occurred. Subsequent injections produced less hypothermia and greater increases in respiratory rate. Measurement of sleep-wake ratios indicated that paradoxical sleep was initially reduced by 6-OHDA administration, but eventually recovered to occupy its normal percentage of the cycle. However, wake time was increased and slow-wave sleep time decreased. Animals treated with 6-OHDA had lower baseline heart rates and showed no evidence for acquiring a conditioned heart rate response, although heart rate response to shock was potentiated.

6-Hydroxydopamine Temperature Central catecholamines Respiration Consummatory behavior Sleep Cardiovascular system Autonomic conditioning

FOLLOWING cerebroventricular administration, 6-hydroxydopamine (6-OHDA) destroys fibers rich in catecholamine-containing terminals producing a reduction in brain catecholamine content and tyrosine hydroxylase activity [8]. These biochemical changes have not been without effect on behavior. Immediately following 6-OHDA injection into brain, there are acute changes, e.g., hypothermia, which have been attributed to a release of brain catecholamines [5]. Chronic effects of central 6-OHDA injection include decrements in T-maze behavior [15], electrical self-stimulation and active avoidance behavior [4], all of which have been attributed to destruction of central catecholamine terminals in brain.

With the exception of a few reports (e.g., [6, 21, 22, 23]), most studies with 6-OHDA have been performed in rodents. The purpose of the present study was to examine the acute and chronic effects of administering 6-OHDA intraventricularly to a higher mammal – the cat. Of particular interest were the acute and chronic effects of 6-OHDA on temperature, respiration, and resting cardiovascular function. Cardiovascular function was also examined in a classical aversive conditioning paradigm. In view of the controversy concerning the role of catecholamine-containing fibers in sleep [13, 18, 19], sleep-wake cycles were also examined following 6-OHDA administration.

METHOD

Animals

Cats were obtained from the Laboratory Animal Facility of the University of North Carolina and were of unknown background. Upon arrival, cats were treated for feline distemper and intestinal worms and were housed individually with ad lib food and water.

Surgery

The animals used in these studies were implanted under sodium pentobarbital anesthesia (35 mg/kg) with a cannula aimed for the lateral ventricle close to the Foramen of Monroe. Cannulas were 18 or 19 ga needle stock. The stereotaxic coordinates, derived from Snider and Niemar [25], were anterior +12.5, lateral 3.0, and vertical +7.0.

The 8 cats used for sleep recordings were implanted with electrodes for measuring eye movements, nuchal EMG,

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cortical EEG and in some cases, lateral geniculate spike activity. All leads were brought to a nine-pin Amphenol plug embedded in dental acrylic on the surface of the skull.

Drug Injections

The 6-OHDA was injected by dissolving 2.4, 4.8 or 9.6 mg (calculated as the free base) in 0.3 cc of saline containing 0.5% ascorbic acid. Saline (0.1 cc) was initially injected to flush the catheter followed by the 0.3 cc 6-OHDA solution followed by an additional 0.1 cc saline wash. Control injections of saline were performed in the same way except no 6-OHDA was present in the saline-ascorbic acid vehicle. When multiple injections of 6-OHDA were given, animals were allowed to recover their ability to eat and drink and maintain body weight before receiving a second injection (14–35 days). Subsequent injections were given weekly. When intraventricular injections were preceded by pargyline, 50 mg/kg of this monoamine oxidase inhibitor were injected i.p. 30 min before the central injection of 6-OHDA. Central injections of other drugs followed the same format. Norepinephrine (NE) was injected by dissolving 200 μg in 0.3 cc of saline and α-methyl-norepinephrine (αCH_3NE) was injected by dissolving 1 mg in 0.3 cc of saline. Reserpine treatment was begun by injecting 0.5 mg/kg i.p. 48 hr before 6-OHDA and 0.1 mg/kg i.p. 24 hr before 6-OHDA followed by 50 mg/kg 1-α-methyltyrosine (αMT) 2–3 hr before 6-OHDA administration.

Measurement and Quantification

Following central injection of drugs, body temperature, respiration and in some cases heart rate, EEG and general activity was recorded for 3 to 4 hr. Measurement of acute reactions to central injections took place in an 18 x 18 in. enclosure. The animals were unrestrained except for lead wires necessary to measure various physiological activities. Lead wires exited through the top of the recording cage.

Body temperature was measured every 30 min by inserting a rectal temperature probe (Yellow Springs Instrument Co.). In about 30% of the cases the probe was taped in place so that the animal would not have to be handled. Respiratory rate was obtained by quantifying movements produced in the pressure sensitive floor of the recording cage. The validity of this measure was checked by placing a rubber bellows around several animals and measuring pressure changes in the bellows via a Statham pressure transducer. Heart rate was determined from an EKG obtained from taping Ag/AgCl electrodes to the thorax of the animal.

Sleep-stage data was obtained on a 24 hr basis. Recording cages had a 3 x 3 ft. floor area and were 6 ft high. Leads for EEG, EMG, eye movements and lateral geniculate activity were brought to a mercury commutator. Recordings were made inside a shielded room and a Grass EEG machine was used to record the data. The usual procedure was to habituate cats to living in the cages for approximately one week following which three or four 24 hr periods of recording were obtained prior to any treatment. Animals lived continuously in the sleep cages except for periods of acute recording following injection. Sleep records were recorded at 10 mm/second paper speed and were scored during each 30 sec epoch by usual criteria into slow wave sleep (SWS), paradoxical sleep (PS) and wake (W).

Food and water consumption were measured on a daily basis following 6-OHDA treatment in some animals and are reported to the nearest gram.

Classical Conditioning

Conditioning was carried out in an 18 x 18 in. wooden enclosure with a pressure-sensitive floor. Two speakers in the top of the enlosure were driven by tone generators set to deliver 500 and 1000 Hz tones, respectively, at an intensity clearly audible over background noise. The unconditioned stimulus (UCS) was produced by a 60 Hz constant current shock source and was delivered to the animal through two electrodes taped to the left foreleg. The EKG was converted to beats-per-minute by a Beckman cardiotachometer and all measures were recorded on a Beckman Dynograph.

Conditioning was performed using a method similar to the one suggested by Seligman [24] to provide within-subject control for sensitization effects. Two stimuli are presented to the animal, one of which (CS+) is consistently associated with the UCS. The other stimulus (CS–) is followed by shock only when it occurs in conjunction with CS+. The CS+ tone was presented with an average intertrial interval of 70 sec (range 50–90 sec). The same number of CS– tones were similarly distributed without regard to the CS+ tones. All tones were 10 sec in duration. The initial period of trials on each day, during which no UCS was presented, consisted of 12 CS+, 12CS–, and 3 CS+/CS– tone presentations. This period was immediately followed by a 40-min conditioning session in which 30 CS+, 30 CS–, and 5CS+/CS– tone presentations were administered. The animals received 3 days of conditioning 3 or 4 days apart.

The conditioning results were quantified by averaging heart rate 5 sec prior to CS onset (Preperiod) and on a second-by-second basis for 15 sec following CS onset. CS+/CS– trials were not analyzed. On each trial the difference between the average heart rate value of the Preperiod and the average heart rate value for each second of the 15 sec following onset of CS presentation was obtained. The difference scores for each second following CS onset were summed separately over all CS+ trials and all CS– trials giving 15 data points for each CS type on each day for each animal for the initial non-shock trials and conditioning.

Catecholamine Analysis

In order to obtain the brain for biochemical analysis, animals were injected with sodium pentobarbital and then the skin, muscles and skull covering the brain were removed with care taken to leave the blood supply intact. When the brain had been dissected free, the blood supply and spinal cord were severed, the brains were immediately rinsed in cold water, and each half of the brain was dissected over ice into the following pieces: brain stem, caudate, diencephalon, cortex and rest of brain. Brain parts were weighed and immediately homogenized in 10 ml of ice-cold 0.4 N perchloric acid. The homogenate was analyzed immediately or frozen (–20°C) and analyzed within 24 hours. After thawing (if necessary), the homogenate was spun in a refrigerated centrifuge for 15 min, and an aliquot of the supernatant was stirred with alumina and transferred to a column [8]. Catecholamines were eluted with 7 ml of 0.2 N acetic acid and were assayed spectrofluorometrically [1,2]. In some of the animals, half of the brain was used to determine tyrosine hydroxylase activity

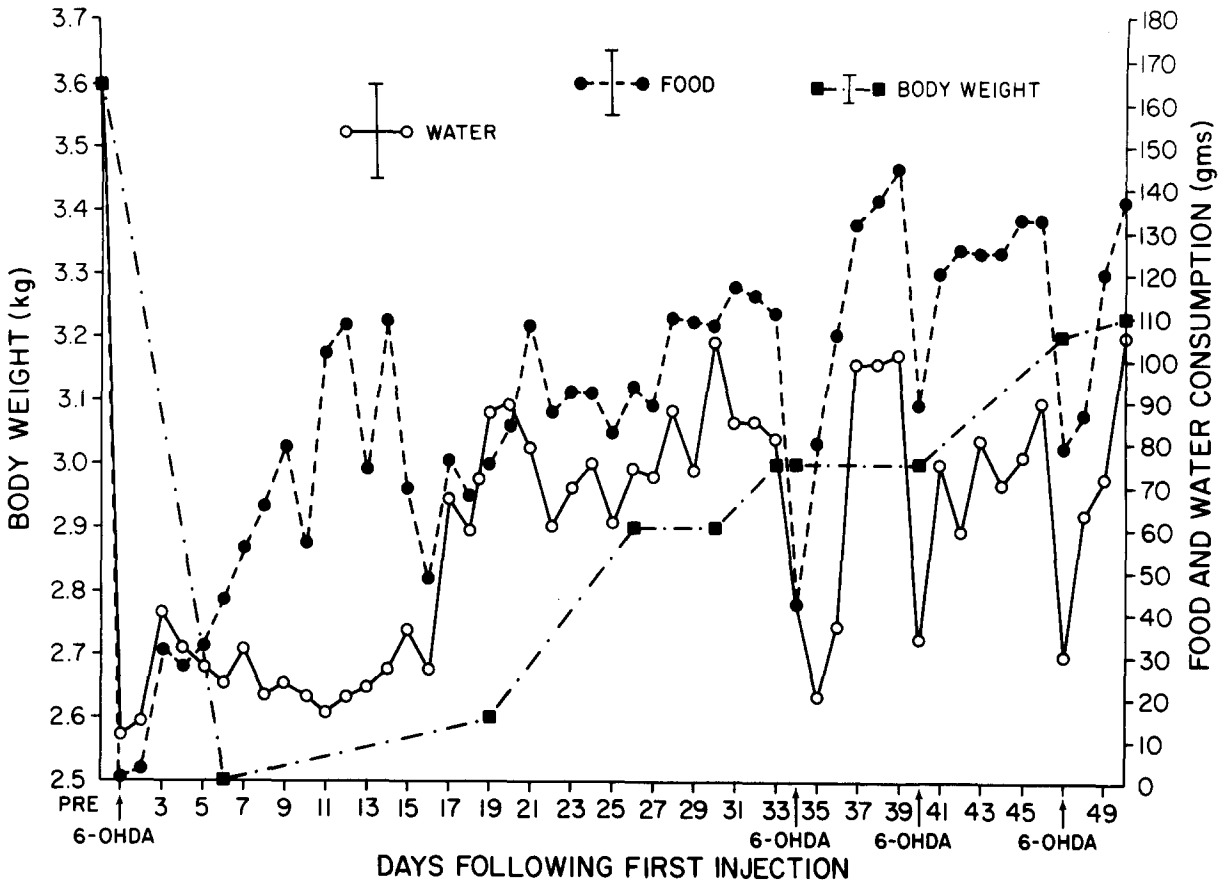

FIG. 1. Body weight, food and water consumption following 4 injections of 2.4 mg 6-OHDA (mean of 7 animals). Line identifiers give the mean and standard error of 5 control animals over the same time period.

[7,8]. For the measurement of brain serotonin, brains were homogenized in 0.1 N HCl containing 0.5% ascorbic acid. After centrifugation, serotonin was extracted from an aliquot of the supernatant according to the method of Bogdanski *et al.* [3], and serotonin concentrations were determined spectrofluorometrically from native fluorescence [3].

RESULTS

General Observations Following 6-OHDA Injection

Following intraventricular injections of saline, the animals appeared normal and did not exhibit any signs of excitation or depression. After intraventricular 6-OHDA, within 1 or 2 min, the animals became restless and within 5 min defecated, urinated, and in about 75% of the cases vomited. There followed a period of intense agitation lasting about 15 min which was greater and lasted longer following 6-OHDA in the presence of pargyline. Within about 5 min, the animals began to salivate copiously and displayed extreme pupillary dilation. After this period of intense agitation, the animals appeared sedated and usually put their heads into a corner of the cage displaying an obstinate progression. In pargyline-pretreated animals, even though the animals appeared sedated, they often gave periodic vocalizations and any stimulus would provoke an intense rage. Over the first 5 hr after 6-OHDA, heart rate dropped progressively and usually stabilized at 50–60 beats per min. For long periods of time, one heart beat would occur for each 3 to 5 breaths. Cortical EEG was usually synchronized during this period.

The chronic 6-OHDA treated animal did not eat or drink as much as normal animals, lost weight (see Fig. 1), and did not keep themselves as clean. After several small injections or after one large injection, animals would spend a great deal of time lying on one side making walking motions. However, neurological examination did not reveal any gross abnormalities.

Effects of 6-OHDA on Temperature

Table 1 shows the change in body temperature at various time periods after various central injections. Injection of saline vehicle with or without pargyline pretreatment did not produce any change in rectal temperature. Intraventricular injection of 6-OHDA was followed by a progressive hypothermia over a 3 hour period. An injection of 2.4 mg of 6-OHDA produced as large a response as did 4.8 mg and pretreatment with pargyline not only did not potentiate the response as had been observed in rats [5], but reduced it. A second injection of 6-OHDA caused less hypothermia and, when a fourth injection of 2.4 mg

TABLE 1

MEANS AND STANDARD ERRORS (IN PARENTHESES) OF CHANGE IN BODY TEMPERATURE (IN C°) FOLLOWING VARIOUS TREATMENTS

Treatment	Number of Animals	Minutes Following Injection 30	60	90	120	150	180
Vehicle	4	0.0 (0.1)	0.0 (0.2)	0.0 (0.1)	+0.2 (0.3)	−0.1 (0.2)	0.0 (0.2)
Vehicle + Pargyline (P)	4	−0.3 (0.1)	−0.5 (0.4)	−0.5 (0.3)	−0.3 (0.3)	−0.2 (0.2)	−0.2 (0.2)
First 2.4 mg 6-OHDA	7	−1.6* (0.5)	−3.8* (0.3)	−5.4* (0.3)	−7.0* (0.6)	−7.1* (0.6)	−9.2* (0.6)
Second 2.4 mg 6-OHDA	4	−0.9 (0.8)	−1.6 (1.2)	−1.8 (1.9)	−2.1 (2.3)	−2.2 (2.8)	−1.7† (3.2)
Third 2.4 mg 6-OHDA	4	−0.9 (0.5)	−1.1† (1.0)	−0.3† (0.9)	−0.6† (1.6)	−0.1† (0.8)	−0.1† (1.1)
Fourth 2.4 mg 6-OHDA	4	+1.5*† (0.6)	+3.3*† (1.3)	+3.8† (1.7)	+3.8† (1.6)	+3.7*† (1.4)	+3.6† (1.6)
First 2.4 mg 6-OHDA + P	3	−0.9* (0.2)	−1.4*† (0.5)	−3.0*† (0.5)	−4.1*† (0.1)	−4.5*† (0.1)	−4.8*† (0.3)
Second 2.4 mg 6-OHDA + P	3	−0.7 (0.6)	−1.6 (1.0)	−2.0 (1.5)	−2.1 (1.7)	−2.3 (2.3)	−2.5 (1.6)
Third 2.4 mg 6-OHDA + P	3	+1.0 (0.7)	+0.8 (0.8)	+0.9 (0.6)	+1.1 (0.8)	+0.6 (0.9)	+0.2 (0.7)
Fourth 2.4 mg 6-OHDA + P	3	+0.7 (0.4)	+1.1 (0.8)	+1.6 (0.7)	+2.2* (0.7)	+2.5 (1.1)	+2.4 (1.4)
First 4.8 mg 6-OHDA	2	−2.1* (0.3)	−3.9* (0.2)	−5.0* (0.2)	−6.3* (0.4)	−7.1* (0.3)	−8.2* (0.3)
Second 4.8 mg 6-OHDA	2	−2.0* (0.6)	−3.5* (0.9)	−4.0* (1.1)	−4.5* (1.2)	−5.0* (1.5)	−5.1* (1.3)
2.4 mg 6-OHDA + Reserpine and αCH_3 Tyrosine	6	−1.0* (0.3)	−1.8*† (0.4)	−2.9*† (0.6)	−3.8*† (0.7)	−4.4*† (0.2)	−6.0*† (0.9)

An * beside a mean indicates a difference from saline injection by two-tailed *t*-test beyond the 0.05 level.
A † beside a mean indicates a difference from 2.4 mg 6-OHDA injection by two-tailed *t*-test beyond the 0.05 level.

6-OHDA was administered, the temperature response became hyperthermic.

In an effort to show that the hypothermia was related to a catecholamine mechanism, animals were pretreated with reserpine and α-MT. Injection of 2.4 mg of 6-OHDA into these animals produced a hypothermia, but one which was significantly less than the one produced without this pretreatment. NE (200 μg) injected intraventricularly did not produce as large a response as did 2.4 mg 6-OHDA; however, αCH_3NE (1 mg) produced a slightly greater hypothermia. Since Breese *et al.* [5] showed that the hypothermic response of rats to 6-OHDA was potentiated by placing the animals in a cold environment, this same procedure was used in cats. Figure 2 shows that cats also show a potentiated response to 6-OHDA when placed in a cold environment (0° C).

Respiratory Rate Following 6-OHDA

Table 2 shows respiratory rate at various time periods following intraventricular injection of 6-OHDA. Saline injection with or without pargyline pretreatment, did not produce any significant change in respiratory rate. Administration of 6-OHDA at a dose of 2.4 mg produced a threefold increase in respiratory rate. Subsequent injections without pargyline produced increasingly greater changes in respiratory rate which by the fourth injection was 10 times resting level 30 to 40 min after injection. In combination

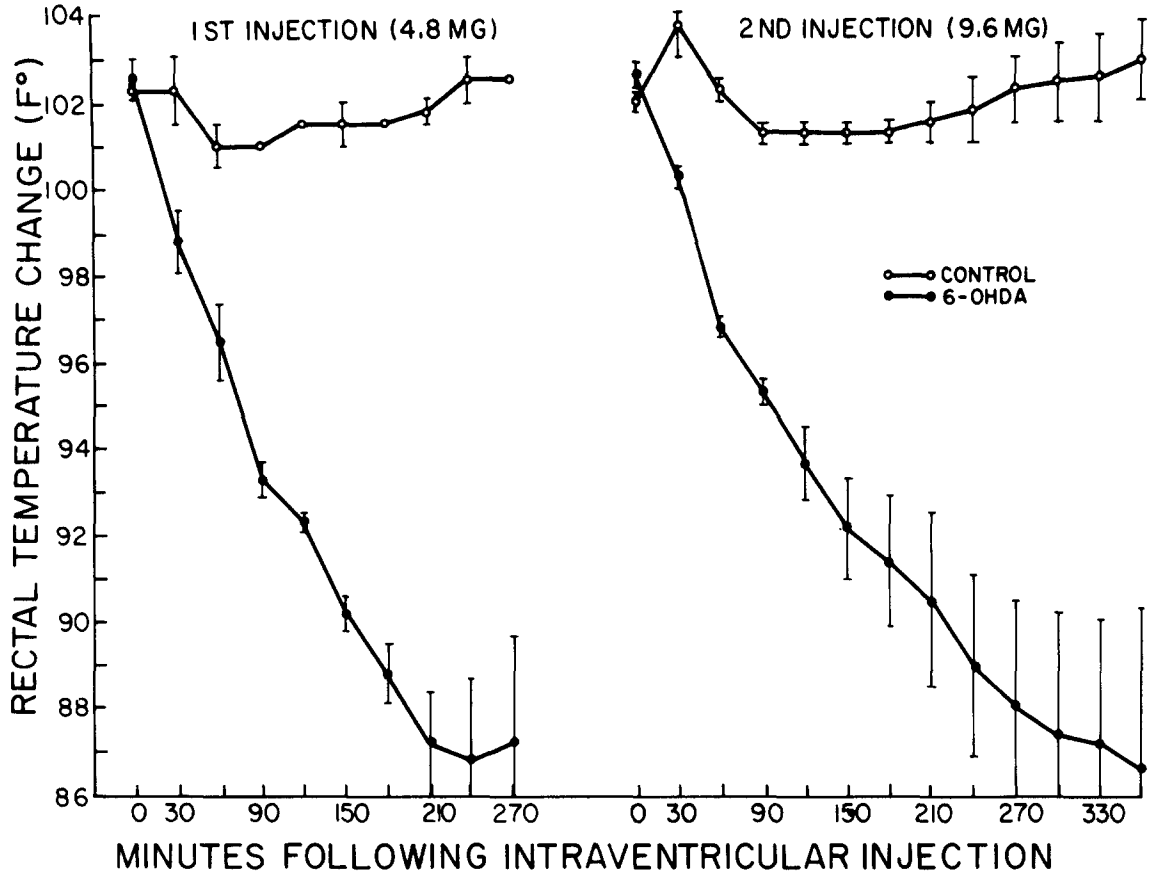

FIG. 2. Rectal temperature change in a 0°C environment following injection of 6-OHDA (3 animals) or saline (2 animals) on two occasions. Points represent means and standard errors. Note that the ordinate is given in °F.

with pargyline, a second treatment with 6-OHDA produced a greater respiratory response but the third and fourth injections in the presence of pargyline produced a lower peak response although the response was considerably prolonged. In contrast to its effect on body temperature, doubling the dose of 6-OHDA to 4.8 potentiated the respiration rate increase, and a second 4.8 mg injection produced the highest rate seen.

Pretreating the animals with reserpine and α-MT before giving 6-OHDA did not reduce the peak effect on respiratory rate; however, it did significantly reduce the length of the effect. The intraventricular injection of 200 μg NE or 1 mg αCH_3NE produced approximately the same increase in respiratory rate as did 2.4 mg 6-OHDA.

Effect of 6-OHDA on Sleep-Wake Cycles

Saline vehicle injection did not alter the percentage of time the animals spent in each phase of the cycle nor did it alter the number of PS episodes (Table 3). In the 24 hours immediately following 2.4 mg 6-OHDA, there was little change in sleep-wake ratios. Six days later, wake time was significantly increased and SWS and PS were significantly decreased. The decrease in PS was due to a decrease in the number of episodes not to a decrease in the length of each. A second injection of 6-OHDA produced a virtually complete suppression of PS; but 6 days later PS had recovered to occupying 8% of the cycle and the length of the episodes were not changed. Six days after a third 6-OHDA injection, the percent of PS was normal although the number of PS episodes were reduced. At this point there was a significant increase in wake time and a significant decrease in SWS.

Effect of 6-OHDA on Classical Aversive Conditioning

In accord with previous reports [17], base level heart rate was found to be lower in the 6-OHDA treated animals during conditioning than in control cats (control – 200 beats/min; 6-OHDA – 169 beats/min; $F(1,6) = 7.80$, $p<0.05$. Figure 3 displays the conditioned heart rate responses to CS+ and CS– over the 3 conditioning days for control and 6-OHDA treated animals. Control animals developed a clear deceleration to CS+, the tone followed by shock, whereas the 6-OHDA-treated animals showed a much smaller response. This pattern of results gave a Drug treatment × Days × CS type × Seconds interaction, $F(18,108) = 1.91$, $p<0.05$. Subsequent analyses, gave CS type × Seconds interactions on all three conditioning days for the control group (Day 1 – $F(9,27) = 3.67$, $p<0.01$/ Day 2 – $F(9,27) = 2.39$, $p<0.05$/Day 3 – $F(9,27) = 6.02$, $p<0.01$), but not in the 6-OHDA-treated group.

TABLE 2

MEANS AND STANDARD ERRORS (IN PARENTHESES) OF CHANGE IN RESPIRATORY RATE (IN BREATHES/MIN) FOLLOWING VARIOUS TREATMENTS

Treatment	Number of Animals	Minutes Following Injection 10	20	30	45	60	120
Vehicle	4	0 (0.7)	0 (1.1)	−1 (2.3)	−6 (2.2)	0 (1.7)	−2 (2.0)
Vehicle + Pargyline (P)	4	−1 (2.1)	+4 (1.6)	+3 (2.5)	+5 (6.2)	+7 (5.6)	−7 (6.1)
First 2.4 mg 6-OHDA	7	+70* (7.1)	+69* (7.4)	+77* (11.2)	+81* (6.4)	+74* (5.5)	+36* (4.9)
Second 2.4 mg 6-OHDA	4	+132*† (8.7)	+191*† (17.2)	+171*† (16.4)	+119*† (14.3)	+65* (19.8)	+14*† (7.3)
Third 2.4 mg 6-OHDA	4	+106*† (12.1)	+182*† (16.8)	+166*† (14.2)	+100* (12.7)	+53* (7.9)	+12† (3.0)
Fourth 2.4 mg 6-OHDA	4	+157*† (21.6)	+295*† (27.8)	+305*† (41.1)	+305*† (32.7)	+215*† (27.4)	+55* (7.3)
First 2.4 mg 6-OHDA + P	3	+56* (5.9)	+53* (7.4)	+82* (11.0)	+96* (6.4)	+90* (5.5)	+74*† (15.1)
Second 2.4 mg 6-OHDA + P	3	+144* (5.3)	+214* (11.4)	+122* (34.0)	+79* (19.4)	+65 (28.0)	+55* (21.6)
Third 2.4 mg 6-OHDA + P	3	+130* (16.2)	+162* (23.7)	+140* (21.5)	+76* (7.0)	+51* (9.4)	+26* (5.3)
Fourth 2.4 mg 6-OHDA + P	3	+79* (11.2)	+75* (9.7)	+33* (6.2)	+25* (7.8)	+29* (5.1)	+79* (16.0)
First 4.8 mg 6-OHDA	2	+178*† (5.0)	+191*† (37.0)	+215*† (41.5)	+196*† (35.02)	+198*† (26.0)	+138*† (22.5)
Second 4.8 mg 6-OHDA	2	+244* (43.5)	+308* (50.0)	+220* (37.0)	+228* (34.0)	+144* (28.0)	+152* (37.0)
2.4 mg 6-OHDA + Reserpine and αCH_3 Tyrosine	6	+89*† (3.1)	+69* (3.2)	+50 (22.2)	+15† (19.4)	+17*† (4.7)	+4† (2.5)

An * beside a mean indicates a difference from saline injection by two-tailed t-test beyond the 0.05 level.

A † beside a mean indicates a difference from 2.4 mg 6-OHDA injection by two-tailed t-test beyond the 0.05 level.

Figure 4 shows the heart rate response of the two groups to shock. The 6-OHDA-treated group accelerated heart rates more on early trials on Day 1 than did control animals and furthermore did not show the usual habituation to shock over days. The greater heart rate acceleration to shock by the 6-OHDA-treated cats was confirmed by the Drug treatment x Days x Blocks x Seconds interaction, $F(4,24) = 3.24$, $p<0.05$). Subsequent analyses showed that 6-OHDA-treated cats accelerated on both blocks on all days to shock ($ps<0.05$), but that the control animals accelerated reliably only on Day 1 Block 1 ($p<0.05$).

Effect of 6-OHDA on Central Catecholamine Levels

NE, dopamine (DA), tyrosine hydroxylase (THO) and serotonin content in 6-OHDA-treated animals that received four doses of 2.4 mg or two doses of 4.8 and 9.6 mg are shown in Table 4. Four injections of 2.4 mg of 6-OHDA reduced NE levels to 9% of control values in whole brain with the brain stem being least affected and cortex and rest of brain being most affected. In whole brain, DA was reduced by 56%. In general, the reduced THO activity supports the position that the reduction in catecholamines

TABLE 3

MEANS AND STANDARD ERRORS (IN PARENTHESES) OF PERCENTAGE OF 24-HR SPENT IN STAGES OF SLEEP–WAKE CYCLE FOLLOWING 6-OHDA ADMINISTRATION

	Number of Animals	Wake	Slow-wave Sleep	Paradoxical Sleep (PS)	Length of PS Episodes	Number of PS Episodes
Control	8	33.3 (3.0)	55.4 (5.7)	11.3 (1.9)	7.0	22.7 (2.4)
Saline	8	32.0 (6.4)	55.3 (9.1)	12.7 (2.3)	6.0	26.2 (1.2)
6-OHDA	6	57.3 (16.5)	39.0 (14.5)	3.7* (2.4)	7.8	14.7 (6.7)
1 Week	8	55.8 (11.9)	38.0 (11.5)	6.2* (0.8)	7.4	7.8* (2.2)
6-OHDA	5	73.7* (1.3)	25.0* (1.7)	1.3* (0.9)	9.6	1.5* (1.5)
1 Week	5	63.5* (12.3)	29.0* (9.0)	7.5 (3.5)	7.2	16.1 (6.7)
6-OHDA	5	–	–	–	–	–
1 Week	4	47.5* (0.6)	42.0 (2.2)	10.5 (1.4)	8.9	17.8 (5.1)
6-OHDA	4	–	–	–	–	–
1 Week	4	62.5 (15.7)	29.0 (15.1)	8.5 (0.6)	7.8	15.8 (6.2)

*Indicates difference from control by two-tailed *t*-test beyond the 0.05 level.

was due to a destruction of catecholamine fibers. Giving 2 larger doses of 6-OHDA did not produce as great a reduction in brain catecholamines as did 4 smaller doses. Brain serotonin content was unaffected by 6-OHDA administration.

DISCUSSION

The hypothermia found following injection of 6-OHDA in cats has been previously reported [16,20], and is consistent with the hypothesis of Feldberg and Myers [11] which holds that release of catecholamines in the hypothalamus leads to heat loss if it is assumed that 6-OHDA acts in some manner to cause catecholamine release. This mechanism has been proposed to account for the hypothermia in the rat following 6-OHDA administration [5]. In the present study, it would also appear that the hypothermia is related to the release of endogenous catecholamines based upon the observations that (1) the hypothermia becomes progressively less with subsequent injections, (2) pretreatment with reserpine and α-MT reduces the amount of hypothermia, and (3) NE and αCH_3NE, a potent false transmitter, also produce hypothermia.

In contrast to its action on body temperature, the effect of 6-OHDA injection on respiratory rate did not seem to be due directly to a release of catecholamines since the respiratory rate increases become progressively greater with subsequent injections. However, other evidence would point to central adrenergic systems being involved. Toda *et al.* [26] found an increase in respiratory rate following central injection of NE and we also observed this. Also pretreatment with reserpine and α-MT caused a decrement in the rate increase. Therefore, it is possible that as many as three mechanisms may be involved in causing the effect on respiration: First, release of catecholamines by 6-OHDA via a false transmitter effect; second, a direct effect of 6-OHDA on receptors and/or third, an effect of 6-OHDA to act through a temperature control mechanism. The second mechanism is supported by the observation that doubling the amount of 6-OHDA injected, enhances the respiratory rate increase but does not affect the hypothermia. The third mechanism is supported by the observation that as the effect of 6-OHDA changes from causing a hypothermia to causing a hyperthermia, the respiratory rate increase becomes greater.

In general, the effects of 6-OHDA on sleep-wake ratios give equivocal support to Jouvet's [18] monoaminergic theory of sleep. Jouvet postulated that NE release under

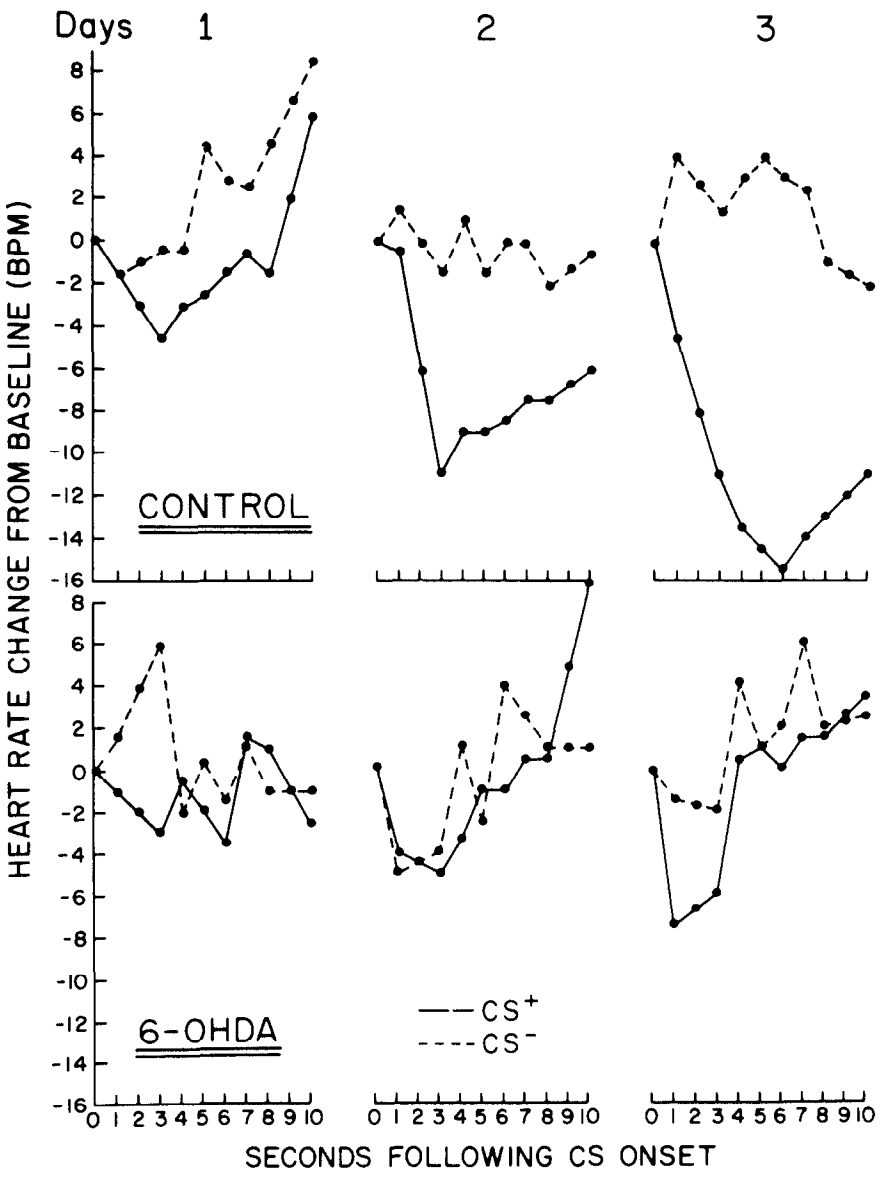

FIG. 3. Heart rate change from baseline during the 10 sec CS+ and CS− tone interval for saline and 6-OHDA injected cats on three conditioning sessions. Each point represents the mean of 4 animals.

the proper conditions was responsible for the occurrence of PS. However, although reduced early, normal amounts of PS were observed 6 days after the third injection and fourth injection (Table 3) of 6-OHDA, which reduced central NE content by approximately 90% (Table 4). It can be argued that the amount of NE remaining in the brain stem (33% of control) is high enough to maintain PS, especially if a receptor supersensitivity develops [5,27]. Another laboratory has recently described a decreased PS after administering 6-OHDA into dorsolateral pontine tegmentum or after destruction of the ventral noradrenergic pathway [21,29]. On the other hand, other treatments which reduce central catecholamines have been observed to produce no change or an increase in PS in rats and cats [13, 14, 19]. In contrast to the effects of chronic 6-OHDA treatment on PS, the animals show increase wake and lesser amounts of SWS. This according to Jouvet's [18] hypothesis would most probably be due to a decrease in serotonin levels following 6-OHDA treatment. However, in our experiments, no reduction in serotonin levels occurred following central administration of 6-OHDA reducing the likelihood for an involvement of serotoninergic fibers in our observations. Petitjean *et al.* [22] and Zolovick *et al.* [29] have in fact showed that central 6-OHDA decreased central serotonin levels in their cat studies. Presently, an adequate explanation for this difference is not apparent. However, such paradoxical findings concerning the effects of 6-OHDA on serotonin content have also been reported in rats [8,10]. In view of the apparent differences in findings, additional work will clearly be necessary to define the role of monoamines in sleep mechanisms.

The data obtained from classical conditioning in the

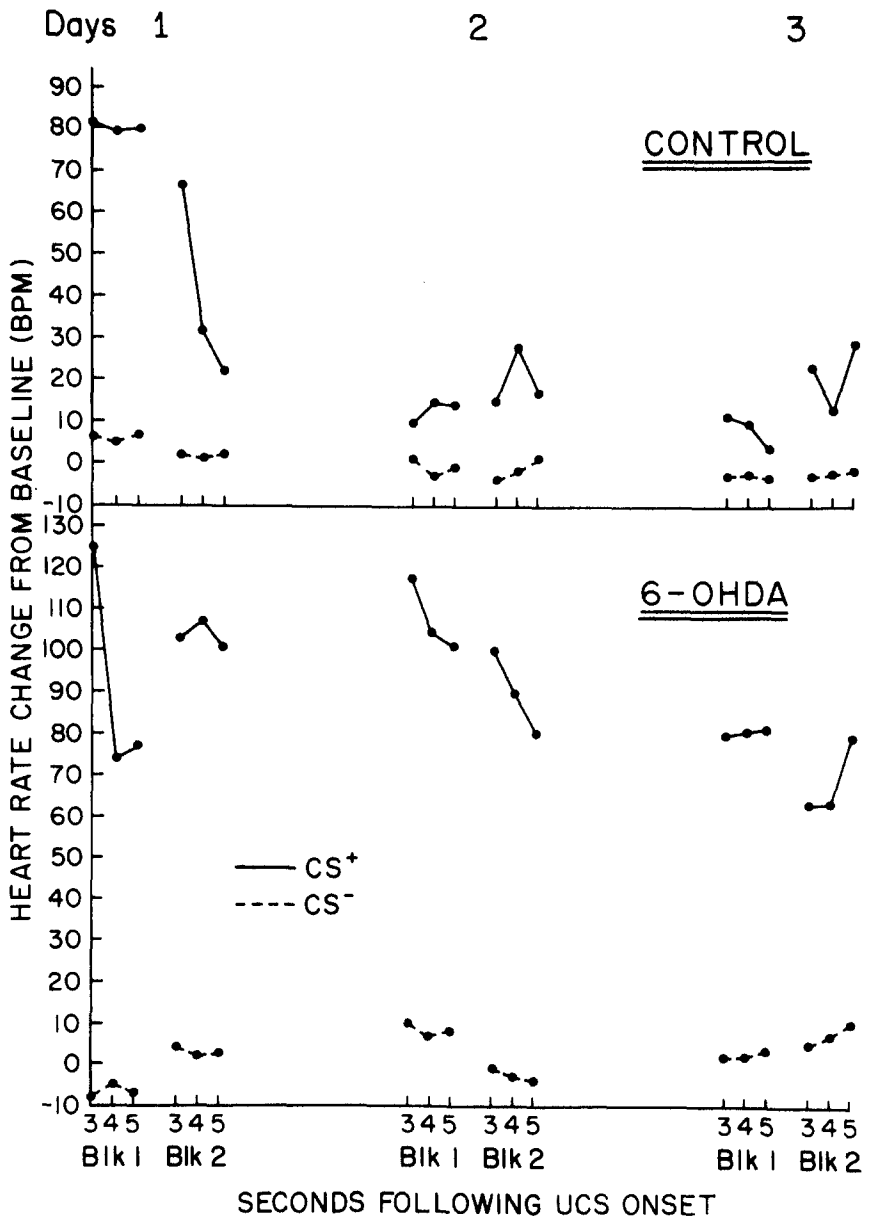

FIG. 4. Heart rate change from baseline during the 3 sec immediately following the 2 sec shock-CS+ (or comparable seconds not following shock-CS−) during the early or late block of trials on three conditioning days. Each point represents the mean of 4 saline or 6-OHDA injected animals. Shock levels were 30% lower for 6-OHDA injected animals.

6-OHDA-treated cat are similar to that reported in the rat [17]. The 6-OHDA-treated animal shows lower baseline heart rates, lack of conditioned heart rate changes, and a potentiated response to shock. The acute and chronic decrease in base level heart has been previously observed in the cat and rabbit [9,12], thus suggesting a role for central catecholamine-containing neurons in the control of cardiovascular function. Accompanying the lowered heart rate, lower blood pressures have been reported [17], although other investigators have found blood pressure unchanged [28].

The lack of conditioned heart rate change in 6-OHDA-treated animals may be due to the lack of ability to learn as has been reported in several operant paradigms following 6-OHDA [4,15]. The efferent control of the cardiovascular system is probably not responsible since the heart rate response to the shock is potentiated.

TABLE 4

MEANS AND STANDARD ERRORS (IN PARENTHESES) OF BRAIN AMINES FOLLOWING VARIOUS TREATMENTS

Treatment	Brain Region					
	Brain Stem	Caudate	Cortex	Diencephalon	Rest of Brain	Total Brain
Norepinephrine (ng/g)						
Control (7)	280.1 (40.0)	98.8 (22.9)	117.6 (40.0)	1341.1 (156.6)	176.5 (50.6)	197.9 (35.5)
4 X 2.4 mg 6-OHDA (5)	119.1* (21.2)	22.9* (15.8)	23.6 (22.1)	178.8* (14.0)	13.5* (3.4)	26.7* (3.6)
2 X 4.8 mg 6-OHDA (4)	156.8* (14.2)	55.0 (7.8)	41.7 (11.6)	330.0* (45.3)	21.5* (4.4)	51.5* (4.3)
Dopamine (ng/g)						
Control	63.8 (14.3)	6832.7 (1063.8)	184.2 (66.2)	1052.7 (262.7)	169.2 (30.9)	315.3 (42.3)
4 X 2.4 mg 6-OHDA	116.9 (59.1)	2849.1* (176.9)	278.4 (109.0)	503.8 (135.6)	104.5 (22.9)	186.6* (29.2)
2 X 4.8 mg 6-OHDA	37.5 (13.1)	4834.8 (850.3)	208.3 (62.7)	817.0 (69.9)	60.0* (9.6)	187.2* (29.2)
Tyrosine hydroxylase (counts/mg/hr)						
Control	17.3 (7.4)	309.7 (79.3)	5.2 (0.6)	44.9 (18.8)	9.9 (7.8)	22.5 (8.5)
4 X 2.4 mg 6-OHDA	4.1 (1.3)	51.4* (8.6)	2.4* (0.7)	11.3* (1.5)	0.4 (0.1)	2.6* (0.2)
2 X 4.8 mg 6-OHDA	7.5 (3.3)	208.8 (73.1)	4.3 (0.4)	24.1 (2.5)	5.3 (4.9)	12.9 (3.4)
Serotonin (ng/g)						
Control	3105.8 (129.7)	4456.2 (159.2)	1402.3 (360.0)	14464.3 (696.4)	1322.7 (232.2)	1612.5 (279.9)
4 X 2.4 mg 6-OHDA	3320.6 (807.0)	4934.2 (327.6)	1520.7 (193.2)	14637.5 (514.3)	1007.4 (171.6)	1726.4 (233.7)

*Indicates significant difference from control animals given either 2 or 4 injections of saline-ascorbic acid vehicle by a two-tailed *t*-test beyond the 0.05 level.

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