*Pharmacology Biochemistry and Behavior*, Vol. 3, pp. 325–329. ANKHO International Inc., 1975. Printed in the U.S.A.

# Effects of 6-Hydroxydopamine and Alpha-Methyl-Para-Tyrosine on the Acoustic Startle Response in Rats[1]

CHARLES A. SORENSON[2]

*Amherst College, Department of Psychology*

AND

MICHAEL DAVIS

*Yale University, School of Medicine and Connecticut Mental Health Center*

(Received 15 May 1974)

SORENSON, C. A. AND M. DAVIS. *The effects of 6-hydroxydopamine and alpha-methyl-para-tyrosine on the acoustic startle response in rats.* PHARMAC. BIOCHEM. BEHAV. 3(3) 325–329, 1975. – The acoustic startle response was measured in rats after depletion of central catecholamines either chronically (through intraventricular injection of 6-hydroxydopamine) or acutely (through intraperitoneal injections of alpha-methyl-para-tyrosine). Chronic depletion resulted in an augmented startle response which could not be attributed to a failure of habituation or enhanced sensitization, while acute depletion depressed startle amplitude. The results were interpreted as evidence that catecholamines normally exert a facilitatory influence on the startle response and that the enhanced response seen in the chronically lesioned animal reflects the potentiation of the role of catecholamine-containing neurons through the development of denervation supersensitivity. This interpretation is consistent with other observations which suggest that catecholamines play a general role in modulating thresholds to aversive events.

6-Hydroxydopamine Alpha-methyl-para-tyrosine Startle Habituation Sensitization Supersensitivity

A VARIETY of studies have shown that animals receiving daily injections of p-chloro-phenylalanine (PCPA), which depletes central 5-hydroxytryptamine (5-HT), and animals receiving moderate intraventricular injections of 6-OHDA, which partially depletes central catecholamines (CAs), display similar behavioral syndromes characterized by hyperreactivity to external stimulation. Animals treated with these drugs become hyperaggressive toward other animals [9, 21, 23], show reduced activity in the open field under some conditions [2,23], and drink less quinine solution compared to normal rats [2,23].

Closer analysis of the exact details of the PCPA and 6-OHDA syndromes does reveal, however, important differences between the two syndromes as well. Thus, 6-OHDA treatments have been reported to increase shock-elicited aggression [9,23] but to have no effect on predatory aggression [9], while PCPA treatments have the reverse effect [9]. In the open field test in the presence of a continuous bright light, 6-OHDA-treated animals show less ambulation and more defecation than controls, indicating increased emotionality under these conditions [23]. In contrast, PCPA-treated animals under similar conditions show more ambulation and hence less emotionality by this measure [2]. In order to further differentiate the effect of PCPA and 6-OHDA, additional indices of reactivity to external stimulation would be useful.

An extremely sensitive index of reactivity to external stimulation is the acoustic startle response. Startle amplitude is highly dependent on the parameters of the eliciting stimulus [11] and can be altered by very small changes in the surrounding acoustic [13] or visual environment [15]. In addition, startle is sensitive to the emotional state of the

[1] This research was supported by National Science Foundation Grant GB-23685 and the State of Connecticut. Our thanks to Lee Schulof for her assistance in data collection and analysis.

[2] Reprint requests should be sent to C. A. Sorenson, Department of Psychology, Amherst College, Amherst, Massachusetts 01002, U.S.A.

animal at the time the reflex is elicited. Thus startle amplitude can be altered by cues that have previously been paired with shocks [3] or rewards [1] or even the absence of reward [27].

Recent studies have shown that PCPA does not affect the amplitude of the startle response [4,6] but does affect the rate of response decrement after several acoustic stimuli have been presented repetitively. Instead of altering startle directly (e.g. by changing startle thresholds) PCPA must enhance sensitization or perhaps reduce rate of habituation. A comparable study has not been done with 6-OHDA. The purpose of the present study, therefore, was to evaluate whether 6-OHDA would alter startle amplitude and/or influence startle habituation or sensitization. If 6-OHDA were to affect startle amplitude without altering habituation or sensitization, this would provide another important difference between the effects of PCPA and 6-OHDA.

Since some behavioral effects of 6-OHDA may reflect the development of denervation supersensitivity [10, 17, 18, 19, 23, 26], alpha-methyl-para-tyrosine was also used to evaluate startle after acute CA depletion.

## EXPERIMENT 1

The purpose of Experiment 1 was to evaluate the effects of 6-OHDA treatment on startle amplitude and the rate of startle habituation over trials and over days.

### METHOD

*Animals*

Sixty-six male albino Sprague-Dawley rats, initially weighing between 300–350 g were used.

*Apparatus*

The apparatus to measure startle amplitude has been described in detail elsewhere [7]. Briefly, it consisted of 5 identical cages (9 X 15 X 15 cm) suspended within a wooden frame. Cage movement resulted in displacement of an accelerometer, the output of which was proportionate to the velocity of displacement. The amplified signal was then fed to a specially designed 5 channel circuit which sampled the peak accelerometer voltage of each of the 5 cages that occurred during a 200 msec time band, 15 msec after the startle-eliciting stimulus. Immediately prior to this sample period, each channel was discharged so that any spontaneous activity occurring between stimulus exposures was erased. The amplitude of the startle response was defined as mm of galvanometer deflection recorded on a Honeywell Visicorder. The 5 cages were housed in a dark, sound-attenuated chamber (Industrial Acoustics Company – IAC) and were placed in a semicircle 45 in. from a high-frequency speaker. The startle-eliciting stimulus was a 4000 Hz., 90 msec, 120 db tone, shaped through an electronic switch to have a rise–decay time of 5 msec. Throughout all phases of the experiments a background level of 60 db white noise was maintained. Sound intensities were measured with a General Radio Model 1551-C sound level meter at the 20 kHz setting.

*Procedure*

To reduce random variability between groups, the 66 rats were divided into 2 matched groups based on their mean response to ten 120 db tones presented at a 30 sec interstimulus interval (ISI). On the following day all animals were anesthetized with chloral hydrate (7%), and 37 animals (the 6-OHDA group) were injected in the lateral ventricle with 200 μg of 6-OHDA HBr (calculated as the base) dissolved in 20 μl of saline solution (with 0.1 mg/ml ascorbic acid added to retard auto-oxidation). The other 29 animals were injected with the vehicle solution alone. The extra animals were included in the 6-OHDA group because pilot studies suggested a somewhat higher death rate in this group.

During testing, each rat was placed in a stabilimeter and after 5 min a total of 50 tones were presented at a 30 sec ISI on each of 4 consecutive days. Testing began about 3 months after lesioning, at which time thirty-one 6-OHDA-treated animals and 27 controls were still living.

*Biochemical Assay*

Brain levels of CAs were estimated by assaying whole brains of seventeen 6-OHDA-treated animals and 14 controls selected randomly from the larger groups. NE and DA levels were determined fluorometrically according to the methodology of Walters and Roth [28].

### RESULTS AND DISCUSSION

The results of the biochemical assays are presented in Table 1. 6-OHDA produced a highly reliable depletion of NE (67.2 percent) but failed to produce a statistically significant reduction in DA.

TABLE 1

EFFECT OF 6-OHDA AND AMPT ON THE LEVELS OF WHOLE BRAIN CATECHOLAMINES

| Treatment | N | NE Level | DA Level |
|---|---|---|---|
| 6-OHDA Controls | 14 | 309 ± 23 | 548 ± 62 |
| 6-OHDA | 17 | 101 ± 15* | 429 ± 65 |
| | | (32.8%) | (78%) |
| AMPT Controls | 8 | 377 ± 43 | 627 ± 62 |
| AMPT | 8 | 74 ± 12* | 179 ± 23* |
| | | (19.6%) | (28.6%) |

Values shown are means in ng/g ± s.e.m. Each value was compared to its control using a 2-tailed *t*-test. Numbers in parentheses are percent of control.

*$p<0.001$

The results of the startle experiment are shown in Fig. 1. The major effect of 6-OHDA was to enhance startle amplitude, and the overall difference in startle between the groups was highly reliable ($t = 2.69$, $df = 56$, $p<0.01$). This difference was evident on the very first tone presented ($t = 2.24$, $df = 56$, $p<0.05$) and cannot be attributed, therefore, to a faster rate of sensitization in the 6-OHDA animals. 6-OHDA did not interfere with either within or between session habituation. In fact, the rate of between session

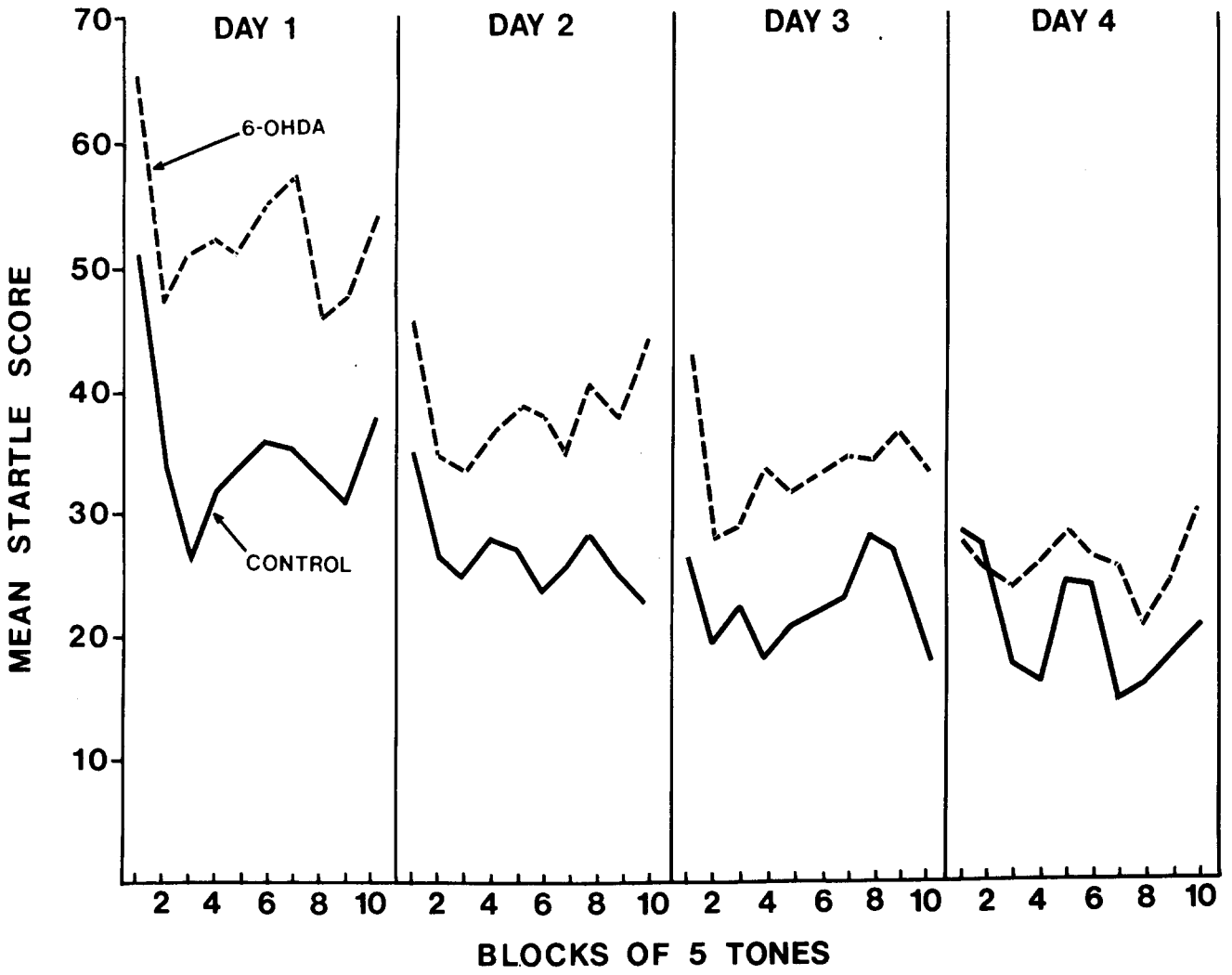


FIG. 1. Mean startle amplitude over blocks of 5 tones on each of the 4 days for the 6-OHDA and the control groups.

response decrement was actually greater in the 6-OHDA group. This was supported by a reliable Days X Groups interaction, $F(3,68) = 4.80$, $p<0.005$. As shown in Fig. 1, the difference between the 2 groups had disappeared by Day 4 ($t = 0.9$, $df = 56$, n.s.) in contrast to the large and highly significant difference on Day 1 ($t = 3.57$, $df = 56$, $p<0.001$) and the smaller, though significant, differences on Day 2 ($t = 2.24$, $df = 56$, $p<0.05$) and Day 3 ($t = 2.34$, $df = 56$, $p<0.05$). While this may indicate greater habituation in the 6-OHDA animals, it must be viewed with some caution given the difference in initial startle amplitude and the possibility of a floor effect in the control group. Nonetheless, it does represent the first example of a physiological manipulation showing a tendency to enhance between session startle habituation.

Enhanced startle in the 6-OHDA group is consistent with the hypothesis that moderate doses of 6-OHDA produce a general syndrome of hyperreactivity to external stimuli. It is important to note, however, that the way in which 6-OHDA brought about a change in startle is considerably different from the way in which depletion of 5HT after PCPA or lesions of the midbrain raphe nuclei change startle. After 6-OHDA startle was augmented on the very first tone presentation, and 6-OHDA did not interfere with startle sensitization or habituation. In contrast, both PCPA [4,6] and lesions of the midbrain raphe nuclei [8] do not alter startle on the first tone presentation but only after several tones have been presented. CA depletion, therefore, seems to have a more direct effect on startle *per se* than 5-HT depletion does.

## EXPERIMENT 2

One interpretation of the facilitated startle seen in Experiment 1 is that the CAs normally exert a suppressing effect on startle, and the increase in startle following 6-OHDA represents a release phenomenon. However, it now appears that some aspects of the behavioral syndrome of the animal receiving moderate doses of 6-OHDA might be attributed to the development of denervation supersensitivity. This has been inferred from studies in which d-amphetamine, which releases central CAs, is found to be more potent following partial destruction of CA systems but less potent after more extensive damage. Specifically, animals receiving either three daily 25 μg injections [23] or a single 100 μg injection [10] of 6-OHDA show an enhanced potentiation of activity following d-ampheta-

mine, while animals receiving either 6 daily 25 μg injections [23] or two 250 μg injections of 6-OHDA spaced 48 hr apart [10] show a subnormal response to d-amphetamine. Perhaps the most direct evidence to date for central supersensitivity after 6-OHDA treatment is that this treatment greatly augments activity to intraventricularly infused NE [20]. One way to test these alternate hypotheses would be to acutely deplete central CAs using alpha-methyl-p-tyrosine (AMPT), since acute depletion of CAs does not appear to result in the development of supersensitivity, as there is no enhanced response to ventricularly infused NE after this manipulation, in contrast to the potentiation which occurs after chronic treatment with AMPT [12]. In addition, since AMPT inhibits the synthesis of CAs, which is the mechanism thought to be responsible for the maintenance of the small functional pool of transmitter [22], AMPT appears to temporarily suppress the activity of CA neurons. If startle were still enhanced following acute depletion of CAs, this would support a disinhibition hypothesis for the effect of 6-OHDA. If startle were depressed, this would support a supersensitivity hypothesis.

## METHOD

### *Animals*

Twenty male Sprague-Dawley rats weighing between 400–500 g were used in this experiment.

### *Apparatus*

All features were identical to those in Experiment 1.

### *Procedure*

On the first experimental day the 20 rats were given ten 120 db tones at a 30 sec ISI and divided into 2 matched groups of 10 rats each, based on their average startle amplitude across the 10 tones.

Twenty-four hours later, one of the groups was given two 50 mg/kg IP injections of alpha-methyl-para-tyrosine HCl methyl ester (AMPT), calculated as the base, dissolved in 1 ml of saline. The other groups received 2 equivalent volumes of saline. Each pair of injections was spaced 3 hr apart. Three hours after the last injection the rats were returned to the test cages and after 5 min presented with ten 120 db tones at a 30 sec ISI. The injection and testing schedules were arranged so that across all animals the average time of day in which testing occurred was similar for both conditions. Forty-eight hr later the above injection and test procedures were repeated. All conditions were identical except that rats which had previously been given AMPT were now given saline and vice-versa, so that each animal served as his own control.

### *Biochemical Assays*

Estimates of the depletion of CAs by AMPT were made by running a parallel series of 16 animals, half of which received AMPT on the identical schedule as the animals that were used in the startle experiment and half of which served as saline-injected controls. These animals were sacrificed 6 hr after their initial injection and their brains prepared for assay. NE and DA levels were determined flurometrically according to the method of Walters and Roth [28].

## RESULTS AND DISCUSSION

AMPT resulted in a significant depletion of both NE (80.4 percent) and DA (71.4 percent) 3 hr after the second injection (Table 1). When startle was tested at this time, it was significantly depressed. Startle amplitude following AMPT was 30.22 compared to 39.85 after saline, and this difference was statistically reliable ($t$ = 2.98, $df$ = 19, $p<0.01$). Further analysis revealed that the difference was apparent on the first tone presented ($t$ = 2.40, $df$ = 19, $p<0.05$).

These results are consistent with the hypothesis that CAs normally exert a facilitatory influence on the startle response and that a denervation supersensitivity has developed in the animals treated with 6-OHDA but not in those treated with AMPT. This interpretation is supported by the finding that amphetamine augments the startle response [5, 14, 16]. However, it should be clear that using the notion of supersensitivity to account for the enhanced startle observed in the 6-OHDA group is highly speculative. The evidence that supersensitivity exists in the CNS is indirect. And there is no logical necessity for believing that a neural system shown to be supersensitive using pharmacological procedures must be responsible for enhanced behavioral responses to environmental stimulation. However, a connection is suggested by the finding that hyperaggressiveness, which has been tied to heightened activity in CA systems [9], only occurs in those 6-OHDA-treated animals that hyperreact to amphetamine [9]. The validity of the postulated supersensitivity must ultimately await studies on the effect of iontophoretically-applied CAs on behavioral and CNS single cell responses.

The fact that 6-OHDA reliably depleted only NE, whereas AMPT depleted both CAs, weakens a supersensitivity interpretation of these data. It is possible that the different startle amplitudes observed could be a function of the difference in the ratios of CAs in the different groups. This hypothesis has been offered previously to account for the enhanced effect of d-amphetamine after a moderate dose of 6-OHDA, which depleted NE substantially more than DA, as opposed to the subnormal response to d-amphetamine occurring after a large dose of 6-OHDA, which depleted both CAs about the same [10].

## GENERAL DISCUSSION

The results of the present study extend the hyperreactivity syndrome of the animal receiving moderate doses of 6-OHDA to include an augmented startle response to a loud tone. When viewed with the literature on the effects of PCPA on startle, they also provide another example of the difference between the effects of PCPA and 6-OHDA. 6-OHDA altered startle amplitude but did not alter startle sensitization or habituation, whereas PCPA has just the reverse effect. Moreover, if heightened startle amplitude reflects heightened emotionality [3], these data provide another example of increased emotionality in the 6-OHDA-treated animal.

One consistent pattern that emerges is that in each instance where 6-OHDA-treated animals have been found to be hyperreactive, the applied stimulus or set of stimuli have an aversive quality. These stimuli include an opposing male rat plus footshock [9,23], quinine solutions [24], novel boxes [23], open fields with bright lights [23], and loud

tones. On the other hand, non-aversive stimuli (e.g. sucrose [24]) do not appear to elicit enhanced responses.

A workable hypothesis is that the animal receiving moderate doses of 6-OHDA has a reduced threshold for responding only to those stimuli normally eliciting withdrawal responses (or perhaps fixed action patterns). This would predict that any stimulus conditions normally aversive to animals would elicit augmented behavior in these 6-OHDA-treated animals. This prediction has been affirmatively tested [25].

## REFERENCES


1. Armus, H. L., K. R. Carlson, J. F. Guinan and R. A. Crowell. Effect of secondary reinforcement stimulus on the auditory startle response. *Psychol. Rep.* **14:** 535–540, 1964.
2. Brody, J. F. Behavioral effects of serotonin depletion and of p-chlorophenylalanine (a serotonin depletor). *Psychopharmacologia* **17:** 14–33, 1970.
3. Brown, J. S., H. I. Kalish and I. E. Farber. Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. *J. exp. Psychol.* **41:** 317–327, 1951.
4. Carlton, P. L. and C. Advokat. Attenuated habituation due to parachlorophenylalanine. *Pharmac. Biochem. Behav.* **1:** 657–663, 1973.
5. Cladel, C. E., M. H. Cho and R. D. MacDonald. Effect of amphetamine and catecholamines on startle response and general motor activity of albino rats. *Nature* **210:** 864–865, 1966.
6. Conner, R. L., J. M. Stolk, J. D. Barchas and S. Levine. Parachlorophenylalanine and habituation to repetitive auditory startle stimulus in rats. *Physiol. Behav.* **5:** 1215–1219, 1970.
7. Davis, M. Differential retention of sensitization and habituation of the startle response in the rat. *J. comp. physiol. Psychol.* **78:** 260–267, 1972.
8. Davis, M. and M. H. Sheard. Habituation and sensitization of the rat startle response: Effects of raphe lesions. *Physiol. Behav.* **12:** 425–431, 1974.
9. Eichelman, B. S. and N. B. Thoa. The aggressive monoamines. *Biol. Psychiat.* **6:** 143–164, 1973.
10. Evetts, K. D., N. J. Uretsky, L. L. Iversen and S. D. Iversen. Effects of 6-hydroxydopamine on CNS catecholamines, spontaneous motor activity, and amphetamine induced hyperactivity in rats. *Nature* **225:** 961–962, 1970.
11. Fleshler, M. Adequate acoustic stimulus for startle reaction in the rat. *J. comp. physiol. Psychol.* **60:** 200–207, 1965.
12. Geyer, M. A. and D. S. Segal. Differential effects of reserpine and alpha-methyl-p-tyrosine on norepinephrine and dopamine induced behavioral activity. *Psychopharmacologia* **29:** 131–140, 1973.
13. Hoffman, H. S. and B. L. Wible. Role of weak signals in acoustic startle. *J. acoust. Soc. Amer.* **47:** 489–497, 1970.
14. Horlington, M. Startle response circadian rhythm in rats: Lack of correlation with motor activity. *Physiol. Behav.* **5:** 49–53, 1970.
15. Ison, J. and G. Hammond. Modification of the startle reflex in the rat by changes in the auditory and visual environment. *J. comp. physiol. Psychol.* **75:** 435–452, 1971.
16. Kirkby, R. J., D. S. Bell and A. C. Preston. The effects of methylamphetamine on stereotyped behavior, activity, startle, and orienting response. *Psychopharmacologia* **25:** 41–48, 1972.
17. Nakamura, L. and H. Thoenen. Increased irritability: a permanent behavior change induced in the rat by intraventricular administration of 6-hydroxy-dopamine. *Psychopharmacologia* **24:** 359–372, 1972.
18. Schoenfeld, R. I. and N. J. Uretsky. Operant behavior and catecholamine-containing neurons: prolonged increase in lever-pressing after 6-hydroxy-dopamine. *Eur. J. Pharmac.* **20:** 357–363, 1972.
19. Schoenfeld, R. I. and J. J. Uretsky. Enhancement by 6-hydroxydopamine of the effects of dopa upon the motor activity of rats. *J. Pharmac. exp. Ther.* **186:** 616–624, 1973.
20. Segal, D. S., C. McAllister and M. A. Geyer. Ventricular infusion of norepinephrine and amphetamine: direct versus indirect action. *Pharmac. Biochem. Behav.* **2:** 79–86, 1974.
21. Sheard, M. H. The effect of p-chlorophenylalanine on behavior in rats: relation to brain serotonin and 5-hydroxyindoleacetic acid. *Brain Res.* **15:** 524–528, 1969.
22. Shore, P. A. Transport and storage of biogenic amines. *A. Rev. Pharmacol.* **12:** 209–226, 1972.
23. Sorenson, C. A. and G. D. Ellison. Nonlinear changes in activity and emotional reactivity scores following central noradrenergic lesions in rats. *Psychopharmacologia* **32:** 313–325, 1973.
24. Sorenson, C. A., G. D. Ellison and D. Masuoka. Changes in fluid intake suggesting depressed appetities in rats with central catecholaminergic lesions. *Nature New Biol.* **237:** 279–281, 1972.
25. Sorenson, C. A. and M. Gordon. Effects of 6-hydroxydopamine on shock-elicited aggression, emotionality, and maternal behavior in female rats. *Pharmac. Biochem. Behav.* **3:** 331–335, 1975.
26. Thoa, N. B., B. Eichelman, J. Richardson and D. Jacobowitz. 6-Hydroxydopa depletion of brain norepinephrine and the facilitation of aggressive behavior. *Science* **178:** 75–77, 1972.
27. Wagner, A. R. Conditioned frustration as a learned drive. *J. exp. Psychol.* **66:** 142–148, 1963.
28. Walters, J. R. and R. H. Roth. Effect of gamma-hydroxybutyrate on dopamine and dopamine metabolites in the rat striatum. *Biochem. Pharmac.* **21:** 2111–2121, 1972.