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The Effect of 6-Hydroxydopamine on Habituation of Activity in the Developing Rat Pup[1]

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SHAYWITZ, B. A., J. W. GORDON, J. H. KLOPPER, AND D. A. ZELTERMAN. *The effect of 6-hydroxydopamine on habituation of activity in the developing rat pup.* PHARMAC. BIOCHEM. BEHAV. 6(4) 391–396, 1977. The role of brain catecholaminergic mechanisms in habituation of activity was investigated in rat pups treated with 6-hydroxydopamine (6-OHDA). Intracisternal administration of this agent in the neonatal period resulted in a permanent and significant depletion of brain dopamine to 35.5% of controls while brain norepinephrine remained unchanged. Activity levels in normal developing rat pups increased rapidly between 15 22 days, then declined at maturity (26 days), while activity in 6-OHDA treated animals during this peak period of behavioral arousal increased to a significantly greater degree than that of their littermate controls. Habituation of activity, defined as the decrement of spontaneous activity, was calculated by regression over the first 30 min of observation. At both 5 and 8 days of age 6-OHDA and control rat pups exhibited low levels of activity whose decrease with time did not differ significantly and this pattern continued through 12 days of age. However, by 15 days of age activity in control animals declined by 19% each 10 min period compared to only a 10% decline found in 6-OHDA animals. At 19 days normal rat pups declined by 10% compared to a significantly reduced decrement of 3% found in treated animals, but these differences were no longer apparent by 22, 26, or 29 days of age. Our results are consistent with the notion that habituation of activity is a complex phenomenon mediated in part by catecholaminergic systems.

6-Hydroxydopamine Dopamine Norepinephrine Catecholamines Activity Habituation Neonatal Developing rat

HABITUATION is generally defined as a response decrement following repeated or continous presentation of an eliciting stimulus. Although this phenomenon has been reported to occur in a wide variety of organisms under many diverse experimental situations [23], recent evidence suggests that habituation is not a unitary process but that separate classes of habituation each mediated by a distinct neuropharmacological mechanism may be discerned. Evidence from several lines of investigations provides support for such a notion. Thus, administration of the anticholinergic agent scopolamine significantly retards the habituation of exploratory activity in both rats and mice [2,14]. Such data lends support to Carlton's proposal that central muscarinic cholinergic mechanisms are involved in behavioral inhibition in general, and in habituation of activity in particular [3]. In contrast to the behaviorally more complex parameter of habituation of exploratory activity, habituation of the elicited startle response does not seem to depend on cholinergic influences, for startle response is unaffected by scopolamine [35]. Several studies however, have demonstrated that administration of parachlorophenylalanine (PCPA) a competitive inhibitor of tryptophanhydroxylase (and thus an inhibitor of serotonin formation) will significantly attenuate habituation of startle response [4,5]. Moreover, recent studies indicate that catecholaminergic mechanisms may also be important in the modulation of the startle response [11,32]. Such investigations suggest that the response decrement of reflexive behavior as exemplified by the startle response is mediated via monoaminergic mechanisms while the decrement in evoked exploratory behavior depends on cholinergic influences.

Catecholaminergic systems play an important role in the modulation of activity [24,25], but their effects on habituation of exploratory activity remain largely unexplored. The relatively recent technique of pharma-

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cological destruction of central catecholaminergic systems by administration of 6-hydroxydopamine (6-OHDA) has provided a means to clarify the relationship between catecholamines and a variety of behavioral parameters. In order to examine the relationship between catecholamines and habituation, we have studied the effects of 6-OHDA induced depletion of brain dopamine on the habituation of activity in developing rat pups. Our results support a role for central catecholaminergic mechanisms in this form of habituation.

METHOD

Animals

Litters of Sprague-Dawley strain (Charles River Company) rat pups and mothers were obtained at one day of age and culled to eight pups with approximately equal numbers of male and female. At 5 days of age the rat pups were numbered by toe punch and randomly allocated to control or treated groups. Results reported here encompassed 40 control and 40 treated rat pups, comprising 10 litters.

Procedure

Treated rat pups received desmethylimipramine (DMI 25 mg/kg intraperitoneally) 1 hr prior to the intracisternal administration of 6-hydroxydopamine. This was accomplished by an injection of 100 μg 6-OHDA (given as hydrobromide in 25 μl of solution and calculated as free base). Ascorbic acid at a concentration of 0.5 mg/m was added as an antioxidant. Littermate controls received DMI then intracisternal saline 25 μl. The 6-OHDA solution was prepared immediately before use and the compound was shielded from light and kept on ice to prevent autoxidation.

Activity Measurements

Activity was determined on 8 occasions during the first month of life: at 5, 8, 12, 15, 22, 26, and 29 days of age, always between 1300–1500 hr to minimize the variation due to circadian periodicity. The rat pups were removed from their mother and immediately placed in clear plastic cages, 46 × 24 × 14.5 cm deep, in a previously prepared random arrangement. Activity was determined by a time sample measure of activity [15,27] by an observer seated 8 ft from the cages. Each minute the cages were scanned and a record made of the rat pup's activity at that particular instant using one of the following mutually exclusive parameters: sleeping: lying motionless, body resting on floor, head often tucked under body; inactive: standing or sitting motionless; ambulating: walking or running about cage; climbing: forepaws climbing on side of cage or about water bottle; rearing: both forepaws clear of floor; eating: gnawing at food in hopper or at pieces on cage floor or holding food in forepaws; drinking: mouth contact with nozzle of water bottle; sniffing: sniffing at any part of cage or in the air; grooming: any self-washing or licking movement; scratching: scratching body with hind legs. Each parameter of activity was calculated separately; in addition, various parameters were combined to determine total inactivity (sleeping and inactivity); total very active behavior (ambulating, climbing, eating, rearing, drinking); total slight activity (sniffing, grooming, scratching); and total activity (ambulating, climbing, eating, rearing, drinking, sniffing, grooming). Each litter was observed for 1 hr and 60 measures were thus made for each animal on any one day.

Biochemical Determinations

At the conclusion of the experiment, animals were sacrificed by decapitation and brains rapidly removed and frozen on dry ice for subsequent determination of dopamine and norepinephrine. Catecholamines were determined within 2 weeks utilizing fluorometric techniques described elsewhere [1,26].

Statistical Methods

Measurements of each activity were calculated as a percentage of observations for 1 hr. Activity measurements were then grouped into 10 min epochs and analyzed by least squares regression. In order to minimize the confounding of habituation with effector fatigue, habituation of activity was calculated for the first 30 min in the environment. Data for the complete 60 min observation period is, however shown in the figures and tables. Catecholamine concentrations between control and 6-OHDA rats were compared by *t*-test [31].

RESULTS

1. Development of Activity in the Rat Pup

The activity patterns in developing rat pups are similar to those reported by us in previous investigations [29,30] and are detailed in Table 1. For the first week of life the normal rat pup moves very little. Beginning at 12 days and continuing throughout the next 10 days his total activity increases dramatically, so that at 22 days it is two to three times the activity at Day 8, comprising 68% of his time. By 26 days, total activity (ambulation, climbing, rearing, eating, drinking, sniffing, grooming, scratching) has declined to the level it was prior to the increase. Very active behavior, (comprising ambulation, climbing, rearing, eating and drinking) and slight activity (sniffing, grooming, scratching) follow a similar developmental pattern.

Those rat pups depleted of brain dopamine by the neonatal administration of 6-OHDA also demonstrate this pattern of activity as they mature. However, 6-OHDA treated rat pups appear to develop increased activity earlier and to a significantly greater degree than do their littermate controls. Initially, treated rat pups are less active than controls. We believe that this reflects the acute effects of the treatment, since total activity at 5 days was measured just 2 hr after the intracisternal injection of 6-OHDA. By 15 days total activity of treated rat pups is significantly greater than that of controls, and these differences continue at 19 days and 22 days. At 26 days, total activity in treated rats has decreased to levels comparable to those of controls and remains similar to those of controls thereafter. This increased total activity found in dopamine depleted animals appears to reflect increases in very active behavior occurring between 15 and 22 days of age, and for the most part represents ambulatory activity.

2. Habituation to Novel Environment in Normal and 6-OHDA Rat Pups

Figure 1 represents the mean total activity for groups of 6-OHDA treated and control rat pups plotted as mean

TABLE 1

ONTOGENY OF ACTIVITY IN DEVELOPING RAT PUPS

	Age (Days)						
	5	8	12	15	19	22	26-29
Slightly Active							
C	19.8 ± 2.35	13.6 ± 1.65	21.2 ± 1.64	33.3 ± 2.02	26.1 ± 1.92	30.4 ± 2.35	21.8 ± 2.50
6-OHDA	15.7 ± 2.36	17.1 ± 1.60	23.1 ± 1.92	27.7 ± 1.71	36.3 ± 1.87	37.3 ± 1.81	23.3 ± 2.64
Very Active							
C	4.76 ± 1.14	10.9 ± 1.98	16.7 ± 1.67	24.6 ± 2.22	33.7 ± 2.60	37.5 ± 3.16	26.9 ± 3.44
6-OHDA	2.33 ± 0.645	14.7 ± 2.25	21.4 ± 2.35	42.0 ± 2.52	43.9 ± 2.23	42.8 ± 2.28	30.2 ± 3.41
Total Activity							
C	26.7 ± 3.02	24.5 ± 2.57	38.0 ± 2.61	58.4 ± 3.02	59.9 ± 3.43	67.9 ± 3.91	48.7 ± 4.72
6-OHDA	18.0 ± 2.36	31.8 ± 2.65	44.5 ± 3.30	70.7 ± 3.00	80.1 ± 2.56	80.0 ± 3.72	53.5 ± 4.64

Activity as percentage ± SEM of observations for one hour.

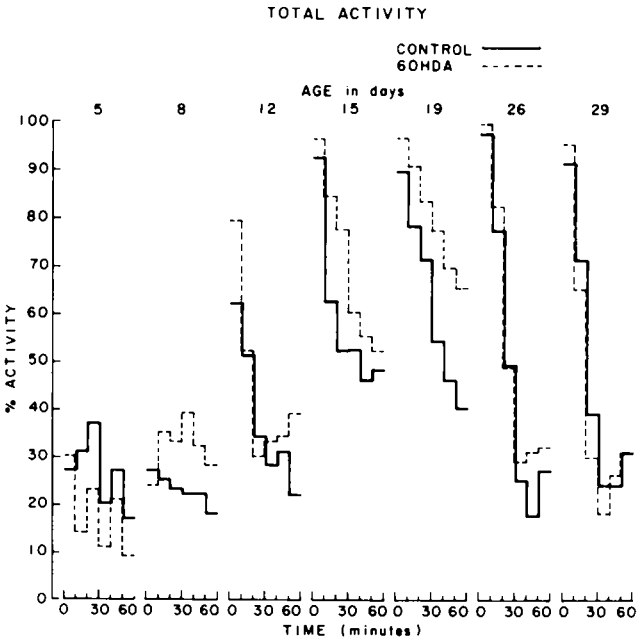

FIG. 1. Habituation of total activity in rat pups treated with 6-OHDA at 5 days of age compared to littermate controls. Total activity is represented as the percentage of observations during 1 hr. Activity for 8 different hour sessions on days 5 - 29 is shown as the mean for each 10 min epoch.

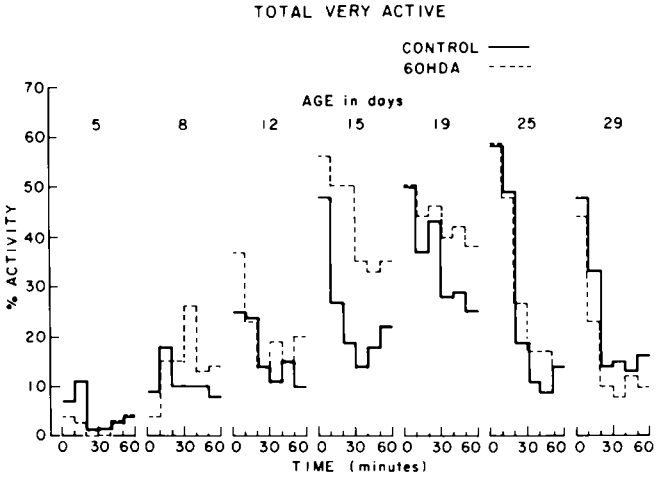

FIG. 2. Habituation of total very active behavior. This comprises ambulation, rearing, climbing, eating, and drinking. Activity measures as in Fig. 1.

percentage of total activity in each 10 min epoch for the full 60 min of observation. The decrement in spontaneous activity was calculated as a regression over the first 30 min of observation. At both 5 and 8 days of age 6-hydroxydopamine treated and control rat pups exhibit low levels of activity which did not decrease significantly over the time periods. By 12 days of age, 6-hydroxydopamine treated animals are beginning to exhibit more activity than controls, but decline at the same rate as controls. However, by 15 days 6-OHDA treated rat pups are significantly more active than controls, and at this point their activity decrement was significantly less than controls, $F(1,992) = 17.2$, $p < 0.001$. At 15 days, the 6-OHDA treated rat pups declined 10% each 10 min period compared to a 19% decline for a similar period in the controls. At 19 days of age activity of dopamine depleted rat pups declined 3% each 10 min interval compared to a 10% decline activity in controls, again a significant difference, $F(1,992) = 10.0$, $p < 0.001$. Decline in activity in both control and treated rat pups was similar at 22, 26 and 29 days of age. Decrement in total very active behavior (including ambulation, climbing, rearing, eating, drinking), exhibited similar patterns with maturation (Fig. 2). Thus, at 15 days, control rat pups declined 13% compared to a decline of just 3% each 10 min period in 6-OHDA treated animals, a significant difference, $F(1,992) = 10.7$, $p < 0.001$. By 19 days, 6-OHDA treated rat pups increased activity by 1% each period while activity in control pups decreased by 2% over the same interval, $F(1,992) = 3.1$, $p < 0.05$. There were no differences apparent in decrement of slight activity which includes sniffing, grooming and scratching. (Fig. 3).

Biochemical Analysis

As shown in Table 2, concentrations of brain dopamine in 6-OHDA treated rat pups is markedly reduced compared to their littermate controls. Thus, the dopamine in the treated animals averaged 35.5% of controls, ($p < 0.001$) a

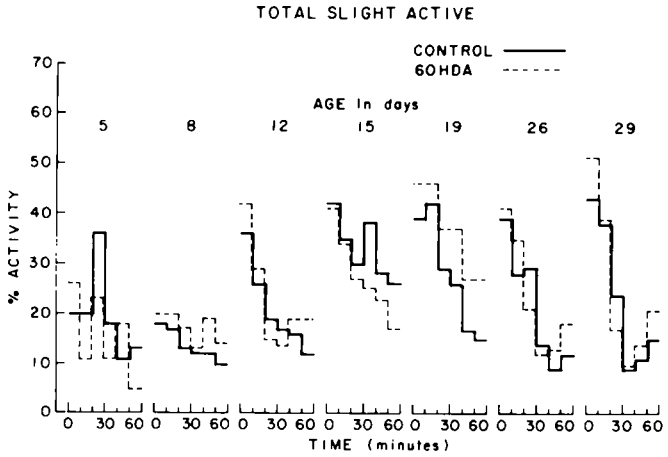

FIG. 3. Habituation of slight activity, comprised of sniffing, grooming, and scratching.

TABLE 2

CONCENTRATIONS OF BRAIN CATECHOLAMINES IN CONTROL AND 6-OHDA TREATED RAT PUPS

	Dopamine ng/g	Norepinephrine ng/g
Controls (40)	541 ± 19.9	285 ± 20.6
6-OHDA (38)	192 ± 39.1	266 ± 17.8
6-OHDA/Controls	35.5%	93.3%

Dopamine corrected for 80% recovery.
Norepinephrine corrected for 72% recovery.
( ) = n.
Concentrations ± SEM.
6-OHDA rat pups treated at 5 days with DMI (desmethylimipramine) followed in one hour by 100μg 6-OHDA-HBr in 25μl intracisternally.

highly significant difference. However, concentration of brain norepinephrine averaged 93.3% of controls, a difference that did not reach significance ($p > 0.05$).

DISCUSSION

Habituation is generally defined as the response decrement following repeated or continuous stimulation provided factors such as effector fatigue and sensory adaptation are satisfactorly excluded. The phrase short-term habituation is often defined as a decrement in response with a time course in the order of seconds, while long-term habituation implies a response decrement of longer duration. The precise differentiation between these terms is not universally agreed upon however. For example, File [13] describes experiments by Grant [14] as demonstrating "short-term" habituation when effects were observed over a time course of minutes. In the present investigation we have utilized the definitions suggested by Davis [8] and considered that habituation of activity is a measure of long term habituation occurring over minutes rather than seconds. Methodological difficulties also must be carefully described since mathematically the determination of habituation may be represented in at least 3 ways: (1) duration of stimulus before the response disappears (2) absolute decrease in response strength (3) relative decrease in response rate compared to the initial response [20]. In the latter method the rate of habituation does not depend on amplitude of the initial response and is the procedure utilized in the present investigation.

Another difficulty presented by measurements of habituation is the not infrequent occurrence of fluctuations in response that may under certain circumstances indicate increases rather than decreases in response rate following repeated or continuous stimulation. This phenomenon led Groves and Thompson [16] to suggest that repetitive stimuli exposure is most parsimoniously viewed as a dual mechanism with habituation producing a decremental response and sensitization contributing an incremental effect. Example of such dual effects are reviewed by Hinde [20] and techniques to clarify the relative effects of habituation and sensitization have been described by Davis [9,10].

Recent investigations have centered on the neurochemical basis of habituation. Carlton [2,3] and Grant [14] noted that the anticholinergic agent scoplolamine antagonized habituation supporting the view that a limbic based cholinergic system may modulate a non-specific facilitory system. Conner and associates [5] as well as Carlton and Advokat [4] provided evidence that central serotonergic mechanisms may also play a role in habituation. Thus reduction in brain serotonin by administration of parachlorophenylalanine (PCPA) attenuated the rate of response decrement of the acoustic startle response. Administration of PCPA, however, proved to have no effect on habituation of activity while scopolamine did not affect habituation of startle response yet blocked habituation of activity. Such findings led Williams and associates [35] to suggest that habituation could be dissociated both pharmacologically and anatomically into at least two types: (1) habituation of exploratory behavior in a novel environment, an operant response mediated primarily by cholingergic influences and (2) habituation of the acoustic startle response, an elicited response, dependent in part on serotoninergic mechanisms. Such a view may require revision, in light of recent investigations. Thus, File [13] noted that scopolamine prevented habituation of one measure of exploration in a complex test situation. However, she was unable to demonstrate that two other muscarinic antagonists, atropine and benzhexol, had any effect on habituation of exploration as measured by head dips in rats, suggesting that the impairment of habituation of an exploratory response could not be ascribed solely to involvement of central cholinergic pathways.

While central catecholaminergic mechanisms are clearly related to activity, their relationships to habituation have not been explored in detail except in relation to the acoustic startle response. Sorenson and Davis [32] noted potentiation of the startle response following the intraventricular administration of 6-OHDA. Alphamethylparatyrosine (AMPT) a competitive inhibitor of tyrosine hydroxylase, and an inhibitor of catecholamine formation, also reduced the startle response but again had no effect on habituation of the response.

Because amphetamine is believed to exert its central nervous system actions via central catecholaminergic mechanisms, the effect of this agent may illuminate a relationship between brain monoamines and the acoustic startle response. Amphetamines increase release of catecholamines from neuronal terminals, inhibit their reuptake by

the neuronal membrane and in high concentrations inhibit degradation by inhibiting monoamine oxidase-all actions that serve to increase the amount of catecholamines available at the synaptic cleft [6, 19, 21]. Davis and associates [11] investigated the effects of d-and l-amphetamine on acoustic startle. Both isomers augmented the response although the d-isomer was clearly more potent than l-amphetamine.

Considerable controversy exists over the relative capacities of d- and l-amphetamine to affect neuronal reuptake of the catecholamine. Some observers consider the two isomers to exert equal effect on dopamine accumulation in the striatum with the d-isomer 10 times more effective than the l-isomer on norepinephrine in cortex [7]. Others have noted that the d-isomer is more potent in inhibiting dopamine in neostriatum and that both isomers are equipotent in inhibiting norepinephrine in cortex [18]. A more recent investigation suggests that d-amphetamine is 5 times more potent than the l-isomer in inhibiting dopamine release from neostriatum and 2 times more potent in inhibiting uptake of dopamine, norepinephrine and serotonin from cortex [21]. In view of these studies, Davis and associates [11] interpreted their findings as indicating that the sensitivity of the startle response is mediated by catecholaminergic (primarily dopaminergic) influences. However, habituation of the startle response does not appear to be affected by alterations in brain catecholamines.

The relationships between catecholaminergic mechanisms and habituation of activity has received limited attention. Swonger and Rech [33] observed that doses of amphetamine large enough to increase activity but small enough so that sterotypies were not produced, disrupted habituation of exploratory activity in the adult rat. File, [12] studying habituation of the orienting response, found that amphetamine significantly impaired habituation. As discussed above, amphetamine is believed to mediate its effects via central catecholaminergic mechanisms and disruption of habituation by amphetamine suggests that catecholamines do indeed play a role in habituation of activity.

Results of our investigations add further support to such a concept. Rats selectively depleted of brain dopamine by the intracisternal administration of 6-OHDA in the neonatal period, demonstrated significant impairment in the habituation of spontaneous activity between 15 and 19 days of age. This time period corresponds to that of behavioral arousal in normal rat pups, and is believed to be related to the increase in central catecholamines occurring rapidly during this period of brain development.

We suggest that habituation of exploratory activity is a complex phenomenon mediated by catecholaminergic as well as other (presumably cholinergic) neurotransmitter mechanisms. According to this concept habiutation of activity is dependent in part on a normally functioning neostriatal dopaminergic system. Administration of amphetamine results in an increase in catecholamines with a resultant disruption of habituation. The apparent paradox of a similar impairment in habituation arising from a reduction in brain dopamine after 6-OHDA is most parsimoniously explained by considering the concept of denervation supersensitivity.

Supersensitivity of central catecholaminergic neurons is a phenomenon whose pharmacological and behavioral correlates have been well described [28,36]. We speculate that the 6-OHDA induced reduction of brain dopamine is accompanied by supersensitivity of the postsynaptic receptors in the remaining dopaminergic nrurons. Thus, despite a reduction of brain dopamine the net effect of 6-OHDA is an increase of postsynaptic receptor sensitivity resulting in behavioral effects similar to those observed after amphetamine. Such a hypothesis would also explain the paradoxical reduction of activity in 6-OHDA treated rat pups noted by us after amphetamine [30] administration.

It is also particularly intriguing that dopamine agonists such as d-amphetamine and apomorphine increase acetylcholine concentrations within the striatum while agents which act to block postsynaptic dopaminergic receptors such as haloperidol and chlorpromazine result in reduction of acetylcholine [17,22]. Such investigations suggest that the apparent influences from both catecholaminergic and cholinergic mechanisms on habituation of activity may be a behavioral correlate of the neuropharmacological link exisiting between cholinergic and catecholaminergic mechanisms in brain.

REFERENCES

1. Boadle-Biber, M., J. Highes and R. H. Roth. Acceleration of noradrenaline biosynthesis in the guinea pig vas deferens by potassium. *Br. J. Pharmac.* **40:** 702–718, 1970.
2. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. *Psychol. Rev.* **70:** 19–39, 1963.
3. Carlton, P. L. Brain acetylcholine and inhibition. In: *Reinforcement: Current Research and Theories*, edited by J. T. Tapp. New York: Academic Press, 1969.
4. Carlton, P. L. and C. Advokat. Attenuated habituation due to parachlorophenylalanine. *Pharmac. Biochem. Behav.* **1:** 657–663, 1973.
5. Conner, R. L., J. M. Stalk, J. D. Barchas and S. Levine. Parachlorophenylalanine and habituation to repetitive auditory startle stimuli in rats. *Physiol. Behav.* **5:** 1215–1219, 1970.
6. Costa, E. and S. Garattimi, Editors. *International Symposium on Amphetamines and Related Compounds.* New York: Raven Press, 1970.
7. Coyle, J. T. and S. H. Snyder. Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different areas. *J. Pharmac. exp. Ther.* **170:** 221–231, 1969.
8. Davis, M. Effects of interstimulus interval length and variability on startle-response habituation in the rat. *J. comp. physiol. Psychol.* **72:** 177–192, 1970.
9. Davis, M. Differential retention of sensitization and habituation of the startle response in the rat. *J. comp. physiol. Psychol.* **78:** 260–267, 1972.
10. Davis, M. Sensitization of the rat startle response by noise. *J. comp. physiol. Psychol.* **87:** 571–581, 1974.
11. Davis, M., T. H. Svensson and G. K. Aghajanian. Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psycholpharmacologia* **43:** 1–11, 1975.
12. File, S. E. Effects of parachlorophenylalanine and amphetamine on habituation of orienting. *Pharmac. Biochem. Behav.* **3:** 979–983, 1975.
13. File, S. E. Are central cholinergic paths involved in habituation of exploration and distraction? *Pharmac. Biochem. Behav.* **4:** 695–702, 1976.
14. Grant, M. Cholinergic influences on habituation of exploratory activity in mice. *J. comp. physiol. Psychol.* **86:** 853–857, 1974.

15. Gray, J. A time-sample study of the components of general activity in selected strains of rats. *Can. J. Psychol.* **19:** 74–82, 1965.
16. Groves, P. M. and R. F. Thompson. Habituation: A dual process theory. *Psychol. Rev.* **77:** 419–450, 1970.
17. 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 neo-striatal cholinergic system. *Brain Res.* **84:** 227–244, 1975.
18. Harris, J. E. and R. J. Baldessarini. Uptake of ^{3}H-catecholamines by homogenates of rat corpus striatum and cerebral cortex: Effects of amphetamine analogues. *Neuropharmac.* **12:** 669–679, 1973.
19. Heikkila, R. E., H. Orlansky, C. Mytilneou and G. Cohen. Amphetamine: Evaluation of d and l-isomers as releasing agents and uptake inhibitors for ^{3}H-dopamine and ^{3}H-norepinephrine in studies of rat neostriatum and cerebral cortex. *J. Pharmac. exp. Ther.* **194:** 47–56, 1975.
20. Hinde, R. A. Short-term changes in neural activity and behavior. In: *Behavioral Habituation*, edited by G. Horn and R. A. Hinde. Cambridge: Cambridge University Press, pp. 3–40, 1970.
21. Holmes, J. C. and C. O. Rutledge. Effects of the d- and l-isomers of amphetamine on uptake, release and catabolism of norepinephrine, dopamine and 5-hydroxytryptamine in several regions of rat brain. *Biochem. Pharmac.* **25:** 447–451, 1976.
22. Ladinsky, H., S. Consolo, S. Bianchi, R. Samanin and R. Ghezzi. Cholinergic-dopaminergic interaction in the striatum: The effect of 6-hydroxydopamine or pimozide treatment on the increased striatal acetylcholine levels induced by apomorphine, piribedil and d-amphetamine. *Brain Res.* **84:** 221–226, 1975.
23. Liebrecht, B. C. Habituation 1940-1970: Bibliography and key word index. *Psychonomic Monograph Suppl.* **4:**(11), No. 59, 1972.
24. Pijnenburg, A. J. J., W. M. M. Honig, J. A. M. Vander Heyden and J. M. Van Rossum. Effects of chemical stimulation of the mesolimbic dopaminergic system upon locomotor activity. *Eur. J. Pharmac.* **35:** 45–58, 1976.
25. Roberts, D. C. S., A. P. Zis and H. C. Fibiger. Ascending catecholamine pathways and amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. *Brain Res.* **93:** 441–454, 1975.
26. Roth, R. M. and E. A. Stone. The action of reserpine on noradrenergic biosynthesis in sympathetic nerve tissue. *Biochem. Pharmac.* **17:** 1581–1590, 1968.
27. Russel, P. A. Sex differences in rats' stationery cage activity measured by observation and automatic recording. *Anim. Learn. Behav.* **1:** 278–282, 1973.
28. Sharpless, S. K. Supersensitivity-like phenomenon in the central nervous system. *Fedn Proc.* **34:** 1990–1997, 1975.
29. Shaywitz, B. A., R. D. Yager and J. H. Klopper. Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. *Science* **191:** 305–308, 1976.
30. Shaywitz, B. A., J. H. Klopper, R. D. Yager and J. W. Gordon. Paradoxical response to amphetamine in developing rats treated with 6-hydroxydopamine. *Nature* **261:** 153–155, 1976.
31. Snedecor, G. W. and W. G. Cochran. In: *Statistical Methods*, 6th edition. Ames: Iowa University Press, 1967.
32. Sorenson, C. A. and M. Davis. Effects of 6-hydroxydopamine and alphamethylparatyrosine on the acoustic startle response in rats. *Pharmac. Biochem. Behav.* **3:** 325–329, 1975.
33. Swonger, A. K. and R. H. Rech. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y maze. *J. comp. physiol. Psychol.* **81:** 509–522, 1972.
34. Thompson, R. F. and W. A. Spencer. Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73:** 16–43, 1966.
35. Williams, J. M., L. W. Hamilton and P. L. Carlton. Pharmacological and anatomical dissociation of two types of habituation. *J. comp. physiol. Psychol.* **87:** 724–732, 1974.
36. Yarbrough, G. G. and J. W. Phillis. Supersensitivity of central neurons—a brief review of an emerging concept. *Can. J. Neurol. Sci.* **2:** 146–152, 1975.