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Developmental Changes in Amphetamine-Induced Taste Aversions[1]

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INFURNA, R. N. AND L. P. SPEAR. *Developmental changes in amphetamine-induced taste aversion.* PHARMAC. BIOCHEM. BEHAV. 11(1) 31–35, 1979.—In this study a conditioned taste aversion paradigm was employed to examine the ontogenetic trend in psychopharmacological responsiveness to amphetamine among infant (18 days of age), periadolescent (35 days of age), and young adult (52 days of age) rats. The ability of amphetamine to alter taste preference increased with dosage level and this effect interacted with age. Infant rats demonstrated greater sensitivity to the taste aversion inducing properties of amphetamine than either periadolescent or young adult animals. In contrast, periadolescent animals demonstrated a marked resistance to amphetamine's taste aversion inducing properties when compared with either infant or young adult animals. This developmental pattern in amphetamine drug responsiveness seen utilizing the taste aversion paradigm parallels the previously examined ontogenetic trend in amphetamine response using locomotor activity as a response measure.

Developmental psychopharmacology Drug responsiveness Amphetamine Taste aversion learning Behavioral toxicology

EVIDENCE has accumulated over the past few years to suggest that periadolescent rats exhibit a greatly attenuated response to amphetamine. While infant (10–25 days of age) and adult rats markedly increase locomotor activity in response to amphetamine [10], periadolescent animals (35–42 days of age) exhibit very little amphetamine-induced increase in locomotor activity [5,22]. A similar pattern of reduced responsiveness among periadolescent animals has been reported using other catecholaminergic agonists such as apomorphine and clonidine [28, 29]. However, as all of these experiments have employed locomotor activity as the response measure, developmental changes in baseline locomotor activity could conceivably mediate this developmental trend in drug responsivity. For example, a recent study [30,31] reported that periadolescent rats (35–42 days of age) showed not only an attenuated response to amphetamine but also greater levels of baseline locomotor activity when compared with weanling (23–30 days of age) and young adult (47–54 days of age) animals (also see [22]). Therefore, it appears critical to examine the ontogenetic pattern of amphetamine responsiveness employing a completely different response measure.

In this study, the ontogenetic pattern of amphetamine-induced taste aversions was examined. Taste aversion learning and its retention enable animals to avoid the debilitating consequences of toxic foods and, as might be expected, this capacity emerges relatively early in the developing rat. Taste aversions can be established in one trial and retained over long retention intervals in preweanling rats [1, 8, 19, 20]. Although the taste aversion paradigm has been employed extensively to investigate learning [4,25], it has more recently been used as a behavioral assay for determining the hedonic value of drugs [6, 12, 33]. For example, adult rats of both sexes exhibit decreased preference for a flavored solution when prior consumption was followed by amphetamine administration [14,15]. The acquired taste aversion presumably reflects the aversive properties of the drug experience as the magnitude of the aversion is positively related to amphetamine dose level.

The ontogenetic pattern of amphetamine-induced taste aversions has not been examined systematically. Therefore, in this study we evaluated amphetamine-induced taste aversions in preweanling (18 days of age), periadolescent (35 days of age), and young adult (52 days of age) rats of both sexes to determine if the ontogenetic pattern of amphetamine-induced taste aversions resembles that of amphetamine responsiveness seen when measuring locomotor activity; namely, attenuation of the effect of amphetamine in periadolescent animals when compared with weanling and young adult animals.

METHOD

Animals

One hundred and forty-four Sprague-Dawley derived rats of both sexes were used in this investigation. These animals

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were born in our colony at the State University of New York at Binghamton. All litters were culled to 10 animals at birth. The animals were maintained in a temperature and humidity controlled vivarium with a 16 hr light–8 hr dark illumination cycle. Light onset occurred at 1800 hr and the experiment was conducted between the hours of 1800 and 2000.

The preweanlings were conditioned on postnatal Day 18 and tested at 24–27 days of age (P18 group). The periadolescent animals were conditioned on postnatal Day 35 and tested at 41–44 days of age (P35 group). The young adult animals were conditioned on postnatal Day 52 and tested at 58–61 days of age (P52 group).

Animals in the P18 group were housed with their parents and conspecifics within maternity cages. Animals in the P35 and P52 groups were weaned on postnatal Day 21 and housed by sex in groups of 4–6 in wire mesh gang cages where they were maintained throughout the experiment. All animals were maintained on Purina rat chow and tap water ad lib unless noted otherwise.

Design and Procedure

Figure 1 depicts the experimental design employed in this study. The animals were 18, 35, or 52 days of age on conditioning day. An equal number of males and females of each age were employed. Four doses of amphetamine (0, 1, 4, 8 mg/kg) were used in conditioning. The factorial combination of age, sex, and amphetamine dose in conditioning generates 24 groups. Within each age group six animals were randomly assigned to each condition.

Conditioning and testing were as follows: After 24 hours of water deprivation (and the removal of both parents from each of the maternity cages in the P18 group) the animals were placed individually in wire mesh cages and allowed 1 hour access to a 15% sucrose solution. Measurements of sucrose intake were taken after 30 min and at the end of the 1 hr period. Immediately following this 1 hr period of sucrose access, each animal was weighed and given an intraperitoneal injection of either 0, 1, 4, or 8 mg/kg dl-amphetamine dissolved in a 0.9% saline solution. All animals were subsequently returned to their home cages and, for the 18-day old animals, the parents were replaced in the maternity cage. After a 5-day ad lib food and water recovery period, all animals were again water deprived for 24 hours (and the parents removed from the maternity cage of the P18 group). Animals were then placed for a 1-hr period into wire mesh cages with access to two bottles: one containing tap water and the other containing a 15% sucrose solution. Following the first two-bottle test, the animals were maintained on deprivation and tested in the same manner every 24 hours for a total of four two-bottle tests.

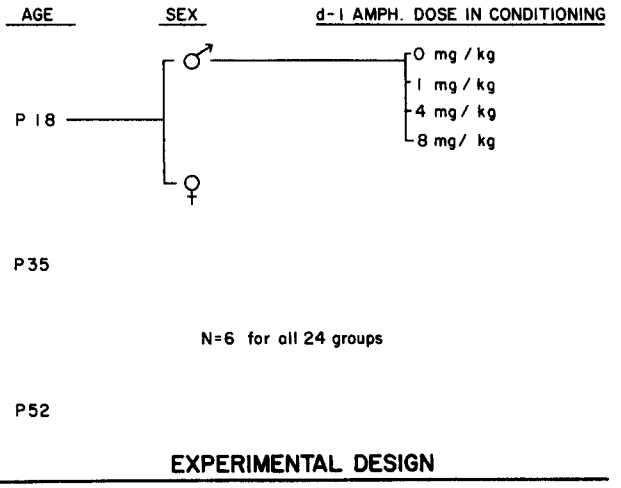

FIG. 1. The 3×2×4 factorial design with Test Days as the repeated measure (not shown).

RESULTS

Conditioning Day

As might be expected, the post-weanling animals consumed a greater absolute amount of sucrose on the conditioning day then the preweanlings (P18: mean=1.42 ml, SEM=0.06; P35: mean=8.33 ml; SEM=0.47; P52: mean=9.41, SEM=0.41). Within each age group, however, the amount of sucrose solution consumed prior to amphetamine injections on conditioning day did not differ across drug dose groups (all Fs<1). The temporal distribution of sucrose intake on the conditioning day was also analyzed by computing for each subject the proportion of total fluid consumed during the first half hour of the 1 hour access period. The ANOVA on these scores did not reveal any main effects of Age or Drug Dose, nor was the interaction significant (all Fs<1). The mean proportion consumed during the first 30 min of the 1 hr access period for each Age group was .62, .63, .72 for the P18, P35 and P52 animals, respectively. Therefore, the temporal pattern of sucrose intake on conditioning day, and thus the effective CS-US interval, did not differ across Age or Drug Dose groups.

Overall Analysis of Testing for Conditioned Aversion

A sucrose preference score was computed for each subject by dividing the amount of sucrose solution consumed by the total fluid (sucrose and water) ingested during each two-bottle test. These preference scores were analyzed by a mixed-model Analysis of Variance to assess the effects of Age, Sex, and Dose between groups and of Test Days within groups.

Because of the Analysis of Variance revealed that neither the main effect of Sex nor interactions involving this factor was significant (all F's<1), the sucrose preference scores were collapsed across Sex. The mean sucrose preference scores for all independent groups (n=12) across all four test days are presented in Fig. 2. The main effects of Age, $F(2,120)=4.07$, $p<0.025$, Dose, $F(3,120)=20.68$, $p<0.001$, and Test Days, $F(3,360)=44.27$, $p<0.001$, in addition to the Dose×Age, $F(6,120)=2.47$, $p<0.05$, Dose×Test Days, $F(9,360)=11.23$, $p<0.001$, and Age×Test Days, $F(6,360)=5.61$, $p<0.001$, interaction effects were all significant. However, all of these effects were tempered by the presence of a significant Age×Dose×Test Days interaction effect, $F(18,360)=1.62$, $p<0.05$. The locus of this three-way interaction was assessed by statistical procedures adequately described elsewhere [21].

Baseline Preference

Among the saline control groups, there were no differences due to Age or Test Days, nor any interaction involving these factors (all F's<1). Therefore, the sucrose preference of the saline control groups remained relatively stable across

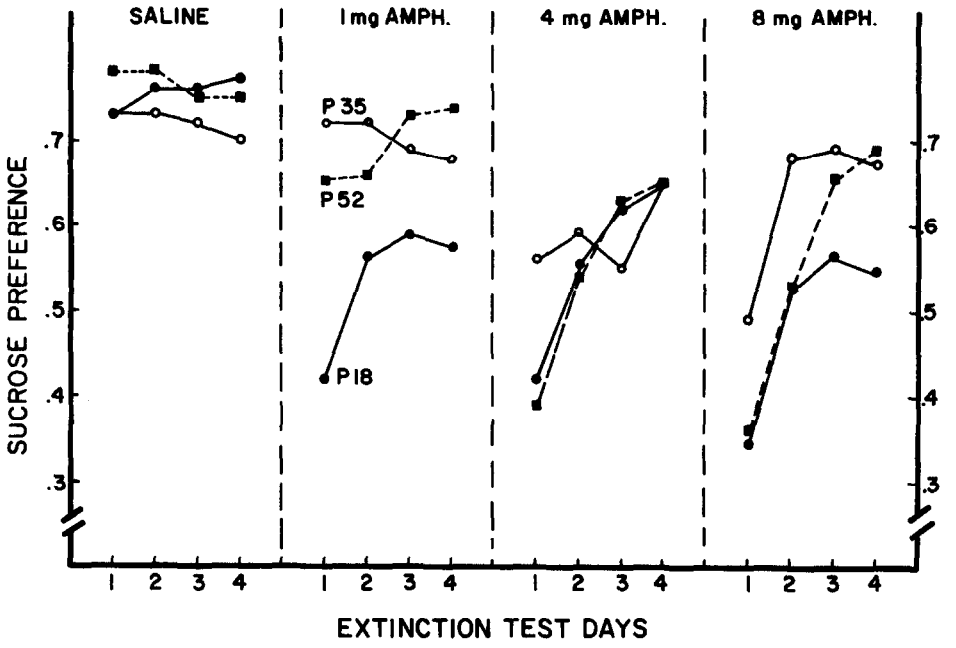

FIG. 2. The mean sucrose preference scores at each amphetamine dosage level for all age groups across extinction test days.

Age and Test Days. The absence of Age differences in baseline sucrose preference justifies the between age comparisons made below.

The Age×Amphetamine Dose Interaction on the Initial Test Day

Figure 3 depicts the mean sucrose preference scores for all groups (collapsing across Sex) for Test Day 1.

Among those animals administered 1 mg/kg amphetamine during conditioning, the P18 group exhibited significantly lower preference scores (i.e., greater taste aversions) than both the P35 and P52 groups (which did not differ from each other), $F(2,120)=6.96$, $p<0.01$.

Among those animals receiving 4 mg/kg amphetamine during conditioning, the P18 and P52 groups did not differ from each other but both displayed significantly greater taste aversions than the P35 animals, $F(2,120)=3.36$, $p<0.05$. This pattern is repeated at the 8 mg/kg amphetamine dose: P18 and P52 animals displayed equivalent taste aversions, and the magnitude of the taste aversion in both of these groups was significantly greater than the P35 animals.

Taste Aversion as a Function of Dose Within Each Age

In order to assess which amphetamine doses produced taste aversions, the dose effects within each age group were assessed by ANOVA and subsequent Duncan's Multiple Range Tests ($p<0.05$ criterion) on the sucrose preference scores for Test Day 1.

Within the P18 group the effect of amphetamine dose was reliable, $F(3,40)=15.13$, $p<0.001$. Subsequent comparisons revealed that P18 animals receiving the 1, 4 and 8 mg/kg doses displayed a significantly lower sucrose preference than their saline controls (0 mg/kg) but did not differ from each other. Therefore, significant taste aversions were induced by all amphetamine doses in P18 animals.

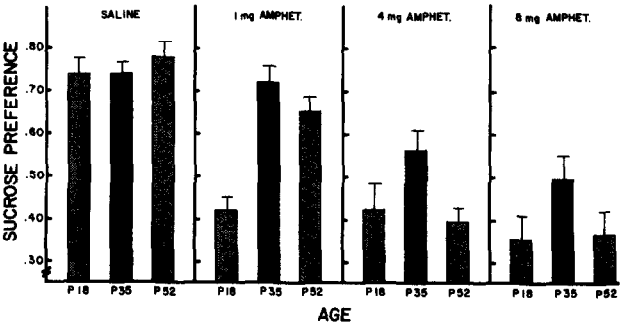

FIG. 3. The mean sucrose preference scores on Test Day 1 for each amphetamine dosage level and all age groups.

The analysis of the P35 group produced a significant effect of amphetamine dose, $F(3,40)=4.93$, $p<0.005$. Those animals receiving the 1 mg/kg dose did not differ from their saline controls, but those receiving 4 or 8 mg/kg, while not differing from each other, did exhibit preference scores that were reliably lower than saline controls. Therefore, in the P35 age group, taste aversions occurred only at the two high doses.

Within the P52 group the effect of dose was statistically reliable, $F(3,40)=27.18$, $p<0.001$. Animals receiving the 1 mg/kg dose evidenced reliably lower preference scores than their saline controls. The 4 mg/kg and 8 mg/kg groups, while not differing from each other, evidenced reliably lower sucrose preference scores than both the 1 mg/kg and saline control groups. Therefore, for the P52 animals, significant taste aversions were produced at all three amphetamine doses with greater aversions being displayed at the higher doses.

Extinction of the Taste Aversion Across Test Days: The Effects of Age at Each Amphetamine Dosage Level

At the 1 mg dose (see Fig. 2), the sucrose preference scores of P35 animals remained at the level of their saline controls across all four tests. The P52 displayed an increase in their sucrose preference (i.e., extinction of their taste aversion) over the same period reaching saline control levels by Test Day 3. The P18 animals, although displaying an increase in their sucrose preference over Test Days, never attained the level of their saline controls during the four days of testing. This pattern of results produced a significant Age×Test Days interaction effect, $F(6,360)=3.98$, $p<0.001$. Therefore, at the 1 mg/kg dose, the magnitude of the aversion exhibited by the P18 group was significantly greater than that for either the P35 or P52 groups.

At the 4 mg/kg dose, the difference between age groups (see Test Day 1 analysis above) decreased over days as all age groups increased their sucrose preference and reached the level of their saline controls on Test Day 4, $F(6,360)=3.66$, $p<0.005$. Therefore, at the 4 mg/kg dose all age groups demonstrated taste aversions. The magnitude of the aversion exhibited by the P18 and P52 animals was significantly greater than that exhibited by the P35 group only on Test Day 1.

Among those animals administered 8 mg/kg amphetamine during conditioning, the P35 animals attained the level of their saline controls by Test Day 2, the P52 animals reached their control levels on Test Day 3, and the P18 animals did not reach the sucrose preference level of their controls, $F(6,360)=2.44$, $p<0.005$. Therefore, the 8 mg/kg dose of amphetamine produced significant taste aversions in all age groups and the aversion was greatest and most durable among the P18 animals and least among P35 animals.

Summary

Figure 4 depicts the data for Test Day 1 graphed in an amphetamine dose sucrose preference response relationship across age. Relative to the older animals, the dose-response relationship is shifted to the left for the P18 group presumably reflecting their greater sensitivity to amphetamine. Conversely, the dose-response relationship for the P35 group is shifted to the right relative to the other age groups suggesting that these animals are resistant to amphetamine's taste aversion inducing properties.

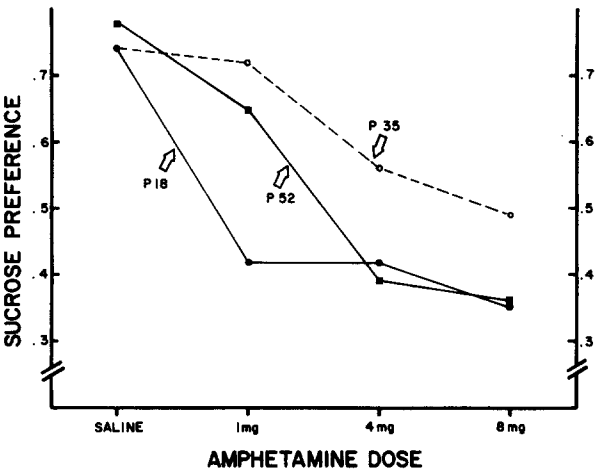

FIG. 4. Amphetamine dose-sucrose preference response relationship for each age group.

DISCUSSION

The results of this study demonstrate that the capacity of amphetamine to alter taste preferences varies with age. Although their greater retention capacity and resistance to extinction conventionally favors older animals [32], the weanlings in the present study exhibited stronger taste aversions than the periadolescent and young adult animals. This finding is consistent with previous research indicating that infants are more sensitive than adults to amphetamine when using locomotion as a response measure [10]. The relative resistance to amphetamine-induced taste aversions exhibited by the periadolescent animals parallels the resistance to amphetamine-induced locomotor activity previously reported in animals of this age group [5,22].

The periadolescent rat, in comparison with slightly younger and older animals, not only shows an attenuation to the psychopharmacological effects of amphetamine but also to other catecholamine agonists, such as clonidine [28] and apomorphine [29], as well. Conversely, animals of this age may also show an accentuated response to catecholamine antagonists as animals of this age are markedly more sensitive to haloperidol-induced catalepsy than younger or older animals [30,31]. Moreover, periadolescent animals, after chronic treatment with haloperidol [30,31], amphetamine [26], or 6-hydroxydopa [27] early in ontogeny, do not exhibit the characteristic alterations in behavior and psychopharmacological responsiveness seen in slightly younger or older animals. Animals of this age also vary behaviorally from younger or older animals. They are hyperactive and exhibit an increase in hole-poke behavior when compared with younger and older animals [30,31]. Additionally, rats of this age have greater difficulty learning discrimination tasks than younger or older animals [2], and female mice of this age exhibit more pup killing behavior than younger or older mice [17]. These alterations in behavioral and psychopharmacological responsiveness seen in periadolescent animals may be the result of pubertal hormonal activity or of some other developmental events occurring in the nervous system of animals of this age.

The greater sensitivity to amphetamine evidenced by the infants in this study may be due in part to the additional consequence of maternal deprivation. There is also evidence to suggest that the 24-hr fluid deprivation period prior to conditioning may have been more severe for the infants than the older animals [9]. In addition, amphetamine is less readily metabolized in the liver of the immature organism, so its pharmacological effects would be expected to endure longer [16,24]. Since the aversive properties of amphetamine and other drugs of abuse appear to be related to their duration of action [11], this might be an additional factor which could have resulted in the stronger taste aversions exhibited by the infants in this study.

There is a dearth of information on the detrimental effects of stimulant chemotherapy in children diagnosed with minimal brain damage or as hyperactive. The dangers of interpolating data gathered with rats to humans notwithstanding, the establishment of amphetamine-induced taste aversions in developing organisms may be of clinical importance as stimulant chemotherapy has been found to significantly suppress the growth of children [23]. It has traditionally been

thought that amphetamine and other stimulant drugs suppress long-term weight gain by suppressing appetite and subsequent food intake, however, tolerance to this anorexic drug effect develops rapidly with subsequent administration of the drug [13]. Perhaps food intake is also suppressed, and food preference altered, as a consequence of stimulant chemotherapy by the production of amphetamine-induced taste aversions. Not only will this effect increase in magnitude with repeated drug use, but also the alteration in feeding behavior would occur whether or not the drug is present. This possible side effect of stimulant chemotherapy is supported by the reports of taste aversions in humans [18]. Moreover, food aversions have been demonstrated in children receiving cancer chemotherapy which also has weight loss as one of its side effects [7]. Therefore, it is possible that alterations in food preferences and food intake due to the development of taste aversions may be a side effect of stimulant chemotherapy in children, and may be responsible, in part, for the significant suppression of long-term weight gain in these children. Collectively, these findings argue for stricter control over the temporal aspects of stimulant chemotherapy and more specifically argues against the recommendation [3] that stimulant drugs be administered after the morning meal on a daily basis.

REFERENCES

1. Ader, R. and J. Peck. Early learning and retention of a conditioned taste aversion. *Devl. Psychobiol.* **10:** 213–218, 1977.
2. Amsel, A. The ontogeny of appetitive learning and persistence in the rat. In: *Ontogeny of Learning and Memory,* edited by N. E. Spear and B. A. Campbell. Hillsdale, New Jersey: Earlbaum Associates, 1979.
3. Anders, T. A. and R. D. Ciaranello. Pharmacological treatment of minimal brain dysfunction syndrome. In: *Psychopharmacology From Theory to Practice,* edited by J. D. Barchas, P. A. Berger, R. D. Ciaranello and G. R. Elliot. New York: Oxford University Press, 1977.
4. Barker, L. M., M. R. Best and M. Domjan (Eds.). *Learning Mechanisms in Food Selection.* Waco, Texas: Baylor University Press, 1977.
5. Bauer, R. H. and D. L. Duncan. Differential effects of d-amphetamine in mature and immature rats. *Physiol. Psychol.* **3:** 312–316, 1975.
6. Berger, B. D. Conditioning of food aversions by injections of psychoactive drugs. *J. comp. physiol. Psychol.* **81:** 21–26, 1972.
7. Bernstein, I. L. Learned taste aversions in children receiving chemotherapy. *Science* **200:** 1302, 1978.
8. Campbell, B. A. and J. A. Alberts. Ontogeny of long-term memory for learned taste aversions. *Behav. Neural Biol.* **25:** 139–156, 1979.
9. Campbell, B. A. and G. A. Cicala. Studies of water deprivation in rats as a function of age. *J. comp. physiol. Psychol.* **55:** 763–768, 1962.
10. Campbell, B. A., L. D. Lytle and H. C. Fibiger. Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science* **166:** 635–636, 1969.
11. Cappell, H. and A. LeBlanc. Conditioned aversion by psychoactive drugs: Does it have significance for an understanding of drug dependence? *Addict. Behav.* **1:** 55, 1975.
12. Cappell, H. and A. E. LeBlanc. Gustatory avoidance conditioning by drugs of abuse: Relationships to general issues in research on drug dependence. In: *Food Aversion Learning,* edited by N. W. Milgram, L. Krames and T. Alloway. New York: Plenum Press, 1977.
13. Carey, R. J. A comparison of the food intake suppression produced by giving amphetamine as an aversion treatment versus as an anorexic treatment. *Psychopharmacology* **56:** 45–48, 1978.
14. Carey, R. J. and E. B. Goodall. Amphetamine-induced taste aversion: A comparison of d- versus l-amphetamine. *Pharmac. Biochem. Behav.* **2:** 325–330, 1974.
15. D'Mello, G. D., I. P. Stolerman, D. A. Booth and W. T. Pilcher. Factors influencing flavour aversions conditioned with amphetamine in rats. *Pharmac. Biochem. Behav.* **7:** 185–190, 1977.
16. Done, A. K. Developmental pharmacology. *Clin. Pharmac. Ther.* **5:** 432–478, 1964.
17. Gandelman, R. The ontogeny of maternal responsiveness in female Rockland-Swiss mice. *Hormones Behav.* **4:** 259–268, 1973.
18. Garb, J. L. and A. J. Stunkard. Taste aversions in man. *Am. J. Psychiat.* **131:** 1204–1207, 1974.
19. Gregg, B., E. M. W. Kittrell, M. Domjan and A. Amsel. Ingestional aversion learning in preweanling rats. *J. comp. physiol. Psychol.* **92:** 785–795, 1978.
20. Infurna, R. N., P. A. Steinert and N. E. Spear. Ontogenetic changes in the modulation of taste aversion learning by home environmental cues. *J. comp. physiol. Psychol.*, 1979, in press.
21. Keppel, G. *Design and Analysis: A Researcher's Handbook.* Englewood Cliffs, New Jersey: Prentice-Hall Inc., 1973.
22. Lanier, L. P. and R. L. Isaacson. Early developmental changes in the locomotor response to amphetamine and their relation to hippocampal function. *Brain Res.* **126:** 567–575, 1977.
23. Lipman, R. S. Stimulant medication and growth in hyperkinetic children. *Psychopharmac. Bull.* **14:** 61–62, 1978.
24. Lytle, L. D. and F. C. Keil. Brain and peripheral monoamines: Possible role in ontogenesis of normal and drug induced response in immature mammals. In: *Dynamics of Degeneration and Growth in Neurons,* edited by K. Fux, L. Olson and Y. Zotterman. New York: Pergamon Press, 1974.
25. Milgram, N. W., L. Krames and T. Alloway (Eds.). *Food Aversion Learning.* New York: Plenum Press, 1977.
26. Monder, H. Effects of prenatal amphetamine on the development of behavior in rats. Ph.D. dissertation, SUNY at Binghamton, 1978.
27. Nomura, Y. and T. Segawa. Apomorphine-induced locomotor stimulation in developing rats treated with 6-hydroxydopa. *Eur. J. Pharmac.* **50:** 153–156, 1978.
28. Reinstein, D. K. and R. Isaacson. Clonidine sensitivity in the developing rat. *Brain Res.* **135:** 378–382, 1977.
29. Reinstein, D., D. McClearn and R. Isaacson. The development of responsiveness to dopaminergic agonists. *Brain Res.* **150:** 216–223, 1978.
30. Shalaby, I., L. Spear and J. Brick. Long-term effects of haloperidol administration during development. *Soc. Neurosci. Abstr.* **8:** 502, 1978.
31. Spear, L., I. Shalaby and J. Brick. Chronic administration of haloperidol during development: Behavioral and psychopharmacological effects. Submitted for publication, 1979.
32. Spear, N. E. and B. A. Campbell (Eds.). *Ontogeny of Learning and Memory.* Hillside, New Jersey: Lawrence Erlbaum Associates, 1979.
33. Vogel, J. R. Conditioning of taste aversion by drugs of abuse. In: *Neurobiology of Drug Dependence, Volume 1, Behavioral Analysis of Drug Dependence,* edited by H. Lol and J. Singh. New York: Futura, 1975.