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Learned Taste Aversion to Saccharin Following Intraventricular or Intraperitoneal Administration of d,l-Amphetamine

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GREENSHAW, A. J. AND O. BUREŠOVÁ. *Learned taste aversion to saccharin following intraventricular or intraperitoneal administration of d,l-amphetamine.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1129–1133, 1982.—The effects of intracerebroventricular (ICV—160, 250, 500 μg) and intraperitoneal (IP—3,5 mg/kg) administration of d,l-amphetamine were compared using a multiple-bottle CTA procedure. After one conditioning trial animals receiving IP amphetamine exhibited a marked aversion to saccharin. This effect was dose-dependent. With cannulated animals receiving ICV saline the effectiveness of amphetamine at 5 mg/kg IP was equivalent to that of 3 mg/kg IP with unoperated rats. After one conditioning trial amphetamine at 160 μg ICV was ineffective in inducing an aversion to saccharin. Animals receiving 250 or 500 μg ICV exhibited a marked aversion to saccharin after one trial. The 160 μg ICV dose was effective after two conditioning trials. This differential potency of centrally and peripherally administered amphetamine after one conditioning trial indicates that the aversive stimulus properties of amphetamine may not simply be centrally mediated. It is proposed that both central and peripheral amphetamine effects may be necessary for the induction of a CTA with this drug.

Conditioned taste aversion Saccharin Amphetamine Route of administration

THE conditioned taste aversion (CTA), i.e. reduced intake of a flavoured fluid (CS) the ingestion of which has been followed by an aversive internal state (US), can be elicited by a wide spectrum of chemical stimuli. The bibliography of Riley and Clark [17] lists over 90 substances that have been used as the US in the CTA paradigm. While most of these drugs elicit poisoning accompanied by severe gastrointestinal distress (e.g. LiCl or $CuSO_4$) manifested by prostration, spasms of the abdominal wall, increased peristalsis and diarrhoea, some drugs (e.g., amphetamine or apomorphine) may act as a US by stimulating critical brain areas, without producing significant visceral symptoms.

The experimental evidence for the central mediation of CTA-eliciting effects of drugs is, however, indirect. Berger *et al.* [2] demonstrated that a peripherally acting drug, methylscopolamine, elicits a CTA in intact animals but not in rats with area postrema lesions. As the same damage did not reduce the CTA-eliciting potency of amphetamine, the authors hypothesised that the destruction of chemoreceptors in this region reduces the effectiveness of blood-borne toxins, but does not block that of centrally acting drugs penetrating the haemato-encephalic barrier. These results have been recently confirmed by McGlone *et al.* [13] who reported that lesioning of area postrema prevents the formation of a CTA with LiCl but not with amphetamine. Alternatively, the presence of the poison in the body can be detected not only by the chemoreceptors of area postrema but also through visceral afferents activated by the local irritation of intestinal mucosa. Coil *et al.* [5] have demonstrated that subdiaphragmatic vagotomy blocks a CTA elicited by intragastric or intraperitoneal injection of $CuSO_4$, a poison with predominantly visceral effects. The observation that vagotomy does not prevent the formation of a CTA with LiCl [10] is consonant with the view that this CTA eliciting agent may affect chemoreceptors in the area postrema region [13].

In the above studies the failure to block CTA formation either by the destruction of area postrema or by vagal deafferentation cannot be considered sufficient proof of the central mediation of a US effect. Even a combination of the above interventions would not rule out the possible participation of spinal visceral afferents in the detection of noxious stimuli. Central effects may be demonstrated more directly by intracerebral application of the US. The aim of the present paper is to test the putative central mediation of the

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aversive stimulus properties of amphetamine in CTA learning by a comparison of the effectiveness of systemic and intracerebroventricular (ICV) application of this drug.

METHOD

Subjects

Seventy male hooded rats (Druckray strain) aged between 2–3 months and weighing 200 g were used in the experiment. They were housed under natural lighting conditions in groups of not more than six animals per cage. Food was available ad lib in the home cages.

Surgery

Animals receiving ICV injections of amphetamine or saline were implanted with stainless steel guide cannulae (23 g) stereotaxically placed in the third ventricle by the method of Walls and Wishart [20]. With this procedure, withdrawal of CSF with a microinjection needle (30 g) connected to a Hamilton syringe was used as the criterion for correct placement. Surgery was conducted under pentobarbital anaesthesia (40–50 mg/kg IP). The guide cannulae were secured to the skull with anchoring bolts and dental acrylic. Seven recovery days were allowed before the operated rats were water-deprived.

Procedure

Fluid consumption test. As two-bottle choice procedures have been reported to be more sensitive tests of CTA acquisition [6,9], a choice procedure was used. The rats were individually exposed to a fourteen-bottle choice situation: with this procedure the rats had access to fourteen glass drinking-tubes placed along the wall of a drinking box (50×40×20 cm) in a line at 2 cm intervals 8 cm above the floor. Each tube contained 2.0 ml of fluid. On conditioning days all tubes were filled with a 0.1% saccharin solution and on retention days alternate tubes were filled with saccharin solution and tap water, respectively. The necessity to sample fluid from a number of tubes in order to achieve a normal level of fluid intake prevents initial persistent drinking at the first bottle chosen and ensures a valid assessment of preference without the need to counterbalance bottle position. An important feature of this test is that, although sampling is forced, an animal may consume a substantial volume of either fluid on a choice day.

Drug administration. d,l-Amphetamine sulphate (SPOFA) was used throughout the experiment. All doses are expressed as the salt.

Peripheral application: Animals receiving intraperitoneal (IP) amphetamine (3.0, 5.0 mg/kg) were injected immediately after saccharin consumption. The drug was dissolved in physiological saline and injected in a volume of 0.5 ml/kg. Control animals received injections of physiological saline in an equivalent volume.

Central application: Animals receiving ICV amphetamine (160, 250, 500 μg/rat) were microinjected with the drug immediately after saccharin consumption. The drug was applied manually in a volume of 10 μl over a period of two to three minutes. The 30 g microinjection needle protruded 0.5 to 1.0 mm below the tip of the guide cannula and was connected to a 10 μl Hamilton syringe with portex tubing (PE 10) during this procedure. Control animals received 10 μl injections of physiological saline by the same method.

Conditioning procedure. All animals received three daily sessions of exposure to the drinking apparatus to adapt them to drinking tap water from the sampling tubes. Initially the animals were 48 hr water-deprived and subsequently only had access to fluid in the test situation. Drinking sessions were of 15 minutes duration and fluid consumption was measured to the nearest 0.25 ml. Following the adaptation period the animals were exposed to saccharin alone paired with the appropriate injection treatment. Since after peripheral amphetamine injection strong CTA was observed already on the first choice day, extinction was monitored by repeating the choice tests also on subsequent two days. On the other hand, animals that received central amphetamine injections displayed weak CTA on the first choice day and were, therefore, exposed to another saccharin ICV amphetamine pairing followed by another choice day. Comparison of the IP versus ICV saccharin administration was based on the results of the first choice test.

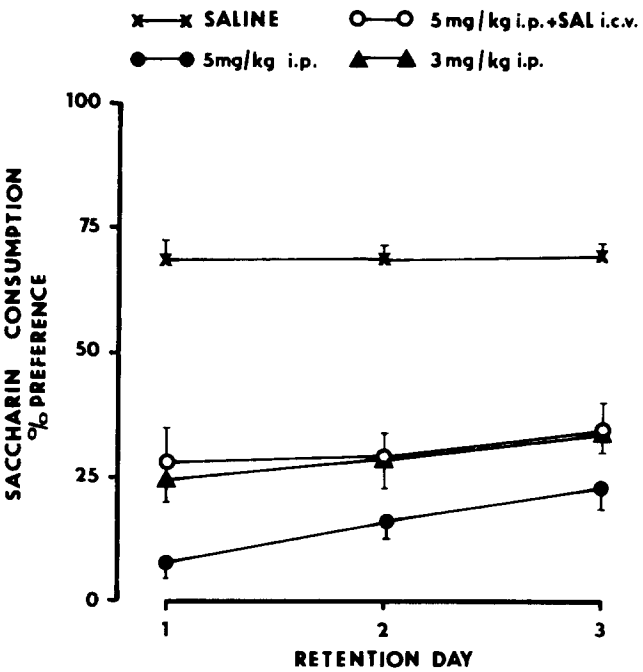

FIG. 1. Mean saccharin preference for each group on each of the three retention days following the conditioning procedure. The vertical bars indicate SEM values.

Control groups: Three control groups were included in this study. Two were conventional control groups receiving saline injections (IP and ICV, respectively), being included for an assessment of attenuation of neophobia and the development of a preference for saccharin against which to compare the effect of the drug treatments. The third control consisted of a group of animals receiving ICV saline and IP amphetamine (5 mg/kg). This group was included to determine the effects of the cannulation and microinjection procedures on the acquisition of an amphetamine-induced CTA.

RESULTS

Peripheral Amphetamine

The effects of a single pairing of amphetamine with saccharin are displayed in Fig. 1 for three consecutive retention days. This figure shows that peripherally administered amphetamine at 3 and 5 mg/kg resulted in a marked alteration of

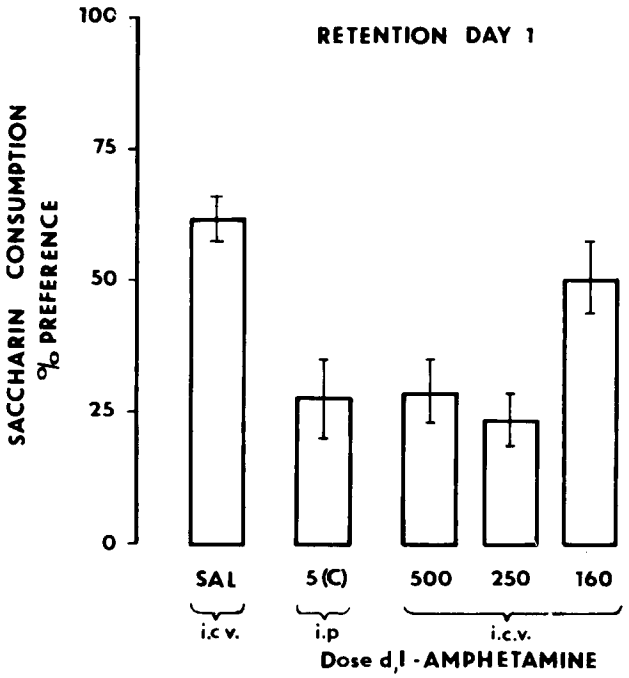

FIG. 2. Mean saccharin preference for each ICV amphetamine group and the ICV saline group on retention day 1. These data are displayed together with the first retention day data for the saline-treated cannulated group that received 5 mg/kg amphetamine IP on the conditioning day.

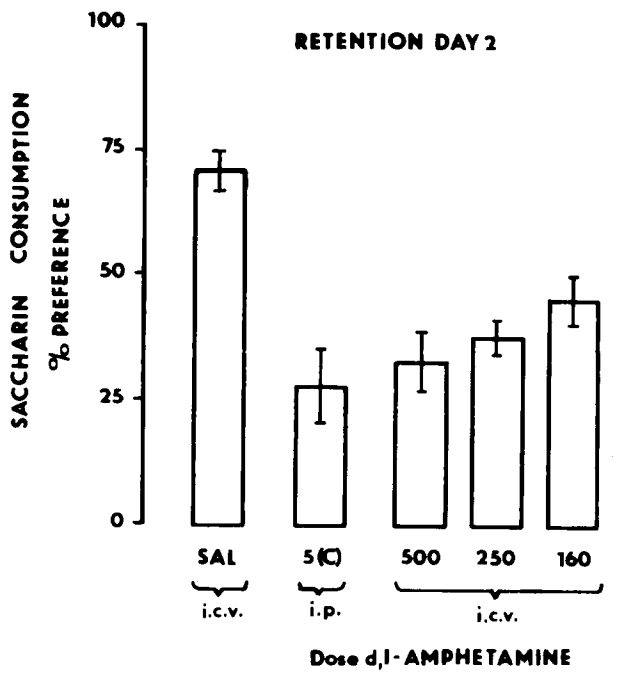

FIG. 3. Mean saccharin preference for each ICV amphetamine group and the ICV saline group on retention day 2. These data are displayed together with the first retention day data for the cannulated group treated with peripheral amphetamine as described for Fig. 2.

saccharin preference relative to the performance of the control group. The cannulated group of animals receiving 5 mg/kg of amphetamine IP exhibited less preference for saccharin than the unoperated controls, however, the mean preference scores for these animals were higher than those of the unoperated group receiving 5 mg/kg of amphetamine, being similar to the data for the 3 mg/kg group. A 2-way ANOVA with repeated measures (Groups × Retention Days) revealed a significant effect of amphetamine, $F(3,38)=41.17$, $p<0.001$, and of retention days, $F(2,56=6.34$, $p<0.005$. There was no significant interaction between these factors, $F(8,56)=0.68$, $p>0.25$. Newman-Keuls multiple-comparison between the mean preference scores of each group revealed significant differences between each drug group and the control group on each of the three test days. On test day one the unoperated 5 mg/kg group exhibited significantly less preference for saccharin than the 3 mg/kg group and the cannulated 5 mg/kg group.

Central Amphetamine

The effect of centrally administered amphetamine (160–500 μg/rat) on saccharin consumption after one and two drug-flavour pairings are displayed in Figs. 2 and 3, respectively. In both figures these effects of central amphetamine are displayed in relation to the performance of the cannulated group receiving ICV saline in combination with a 5 mg/kg IP injection of amphetamine after one drug-flavour pairing and that of the ICV saline control groups. From an inspection of both Figs. 2 and 3 it is apparent that central amphetamine administration resulted in an attenuation of saccharin preference relative to the saline control group. On test day 1 this effect of central amphetamine was greatest at higher (250, 500 μg) doses. One-way ANOVA's revealed significant effects of drug treatment on saccharin consumption on each test day, $F(4,38)=8.32$, $p<0.001$; 11.16, $p<0.001$, respectively. Newman-Keuls multiple comparisons between the mean preference scores of each group revealed no significant difference between the performance of the 160 μg and the saline groups on choice day 1, although this dose was effective on choice day 2.

Contrasting with the results of the saccharin-water free choice tests, saccharin consumption was not reduced during the second saccharin poisoning association. Average saccharin consumption in the pooled experimental groups ($n=30$) was 10.00 ± 0.3 and 10.1 ± 0.5 ml saccharin on the first and second CTA acquisition days, respectively. This finding confirms the differential sensitivity of the single bottle and two-bottle CTA testing procedures [6,9].

DISCUSSION

The belief that the amphetamine-induced CTA is centrally mediated [8] is largely based on the observations that amphetamine fails to elicit a CTA in 6-OHDA treated animals [18] and that a peripherally-acting amphetamine compound will only induce a CTA at a very high dose [3]. Contrary to expectation, the present data indicate that centrally applied amphetamine is a relatively ineffective US in the CTA paradigm. After one drug-flavour pairing, doses of 3 and 5 mg/kg of peripherally administered amphetamine induced a marked aversion to saccharin. According to Rech and Stolk [16], IP injection of 5 mg/kg of d-amphetamine increased the brain amphetamine level to 10 μg/g after a ten minute inter-

val, followed by a decrease to 6.2, 2.5, and 0.75 μg/g after intervals of 1, 2 and 4 hours, respectively. Approximately twice these levels were reported after the same IP dose of amphetamine at 2 and 4 hours after injection by Brodie *et al.* [4]. On the basis of the above pharmacokinetic studies brain amphetamine levels attained during the first 2 hr after IP administration of 3 and 5 mg/kg amphetamine were estimated as 3.6 and 6.0 μg/g, respectively. It was also assumed that ICV administered amphetamine is uniformly distributed in the approximately 1 g weight of rat brain. The estimated brain amphetamine levels (Fig. 4) after ICV administration of 160 μg of the drug exceed at least by an order of magnitude those attained after IP injection of 3 or 5 mg/kg amphetamine. Furthermore by the present ICV administration much higher amphetamine levels must have been attained in the brain tissue surrounding the third ventricle, an area which presumably plays an important role in CTA acquisition [7, 15, 21]. The effectiveness of the higher ICV doses (250 and 500 μg) in inducing a CTA after one drug-flavour pairing may possibly be due to the penetration of the centrally administered drug across the blood-brain barrier into peripheral circulation. With the 200 g rats used in this study, 500 μg per rat corresponds to a dose of 2.5 mg/kg which elicits a marked CTA with systemic application. Although the quantitative aspects of the above comparisons must be accepted with caution, the finding that CTA eliciting amphetamine dosages were almost the same with ICV and IP applications is incompatible with the predominantly central mediation of the drug effect.

Cannulation and the microinjection of saline into the third ventricle significantly attenuated the degree of aversion attained after IP application of 5 mg/kg of amphetamine to a level, exhibited by the unoperated group receiving 3 mg/kg IP. This result, together with the observation that the cannulated control animals showed a marked preference for saccharin rules out the possibility that the present data may be simply due to some feature of the surgical or microinjection procedures.

The possibility of inducing a CTA with centrally applied chemical stimuli has been demonstrated for injection of carbachol [14] into the medial septum and the lateral ventricle, of Δ^9THC [1] into the dorsal hippocampus and of 25% KCl [19] into the vestibular nuclei. At least some of the centrally administered drugs (e.g. carbachol: see [14]) may have caused peripheral effects either by the spread of the drug into the peripheral circulation, or by producing centrally-induced visceral responses constituting an aversive state.

There is an interesting parallel between the present findings and those of Martinez *et al.* [11,12]. These authors have demonstrated, using a one-trial passive avoidance procedure involving footshock, that centrally administered amphetamine (100 to 500 μg/rat) lacks the memory-enhancing actions of 1 mg/kg of the drug IP. The centrally applied amphetamine doses in the Martinez *et al.* studies were similar to those used in the present experiments, and are clearly in the range of doses that markedly affect general activity. Martinez *et al.* [11] reported dose-dependent increases in activity after 100 to 500 μg ICV applications of amphetamine [11]. Similarly, although no formal analysis of unconditioned effects was attempted, the drug-treated animals in the present study exhibited classical symptoms of amphetamine toxicosis, i.e. stereotyped behavior and brief catatonia (generally with 250 μg ICV).

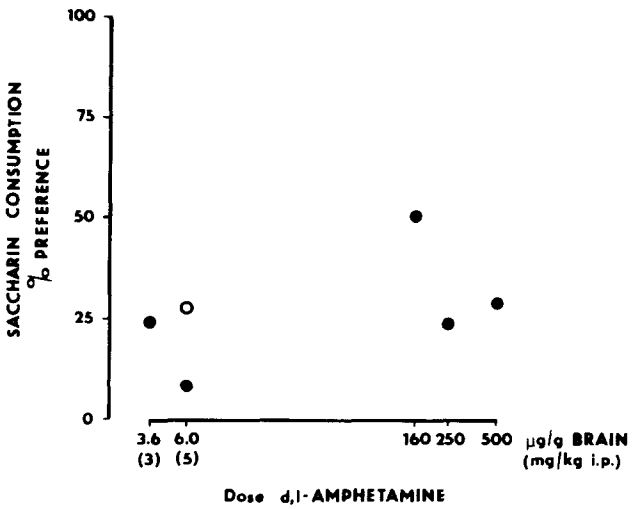

FIG. 4. Mean saccharin preference on the first retention day for each group receiving peripheral or central amphetamine on the first conditioning day, as a log function of estimated (IP) or applied (ICV) brain amphetamine levels. The dose-response function is markedly shifted to the right in animals receiving ICV amphetamine. The open circle denotes the effect of 5 mg/kg amphetamine in the cannulated animals.

The present results indicate that a single ICV application of amphetamine at a dose considerably higher than brain levels after peripheral administration is ineffective in inducing a CTA. However, the contribution of a central effect to the CTA-inducing properties of amphetamine cannot be ruled out. The failure to elicit a CTA with peripherally-acting hydroxy-amphetamine [3] or with amphetamine in 6-OHDA treated animals [18] indicates the importance of central mechanisms. It is possible that the central effects of amphetamine are a necessary but not sufficient condition for the efficacy of this drug in CTA learning. The aversion induced with the very high ICV doses of amphetamine in the present study after one drug-flavour pairing may be due to a combination of central and peripheral effects. Further work is needed in order to test this assumption experimentally, e.g. by simultaneous ICV application of amphetamine and IP administration of a peripherally acting compound such as hydroxy-amphetamine.

REFERENCES

1. Amit, Z., D. E. Levitan, L. V. Brown and F. Rogan. Possible involvement of central factors in the mediation of conditioned taste aversion. *Neuropharmacology* **16:** 121–124, 1977.
2. Berger, B. D., C. D. Wise and L. Stein. Area postrema damage and bait shyness. *J. comp. physiol. Psychol.* **82:** 475–479, 1973.
3. Booth, D. A., G. D. D'Mello, C. W. T. Pilcher and I. P. Stolerman. Comparative potencies of amphetamine, fenfluramine, and related compounds in taste aversion experiments in rats. *Br. J. Pharmac.* **61:** 669–677, 1977.

4. Brodie, B. B., A. K. Cho and G. L. Gessa. Possible role of hydroxynorephedrine in the depletion of norepinephrine induced by d-amphetamine and in tolerance to this drug. In: *Amphetamines and Related Compounds,* edited by E. Costa, S. Garatini. New York: Raven Press, 1970, pp. 63–73.
5. Coil, J. D., R. C. Rogers, J. Garcia and D. Novin. Conditioned taste aversions: Vagal and circulatory mediation of the toxic unconditioned stimulus. *Behav. Biol.* **24:** 509–519, 1978.
6. Dragoin, W., G. E. McCleary and P. McCleary. A comparison of two methods of measuring conditioned taste aversions. *Behav. Res. Meth. Instrum.* **3:** 309–310, 1971.
7. Gold, R. M. and D. M. Proulx. Bait shyness acquisition is impaired by VMH lesions that produce obesity. *J. comp. physiol. Psychol.* **84:** 488–495, 1973.
8. Goudie, A. J. Aversive stimulus properties of drugs. *Neuropharmacology* **18:** 971–979, 1979.
9. Grote, F. W., Jr. and R. T. Brown. Conditioned taste aversions: two stimulus tests are more sensitive than one stimulus test. *Behav. Res. Meth. Instrum.* **3:** 311–312, 1971.
10. Hartin, J. R., F. Y. Cheng and D. Novin. Acquisition of learned taste aversion following bilateral subdiaphragmatic vagotomy in rats. *Physiol. Behav.* **21:** 13–17, 1978.
11. Martinez, J. L., R. A. Jensen, R. B. Messing, B. J. Vasquez, B. Soumireu-Neurst, D. Geddes, K. C. Liang and J. L. McGaugh. Central and peripheral actions of amphetamine on memory storage. *Brain Res.* **182:** 157–206, 1980.
12. Martinez, J. L., B. J. Vasquez, H. Rigter, R. B. Messing, R. A. Jensen, K. C. Liang and J. L. McGaugh. Attenuation of amphetamine-induced enhancement of learning by adrenal demedullation. *Brain Res.* **195:** 433–443, 1980.
13. McGlone, J. J., S. Ritter and K. W. Kelley. The antiagressive effect of lithium is abolished by area postrema lesion. *Physiol. Behav.* **24:** 1095–1100, 1980.
14. Myers, R. H. and J. M. De Castro. Learned aversion to intracerebral carbachol. *Physiol. Behav.* **10:** 73–78, 1977.
15. Peters, R. H. and M. J. Reich. Effects of ventromedial hypothalamic lesions on conditioned sucrose aversions in rats. *J. comp. physiol. Psychol.* **84:** 502–506, 1973.
16. Rech, R. H. and J. M. Stolk. Amphetamine drug interactions that relate brain catecholamines to behavior. In: *Amphetamines and Related Compounds,* edited by E. Costa and S. Geratini. New York: Raven Press, 1970, pp. 385–413.
17. Riley, A. L. and C. M. Clarke. Conditioned taste aversions: A bibliography. In: *Learning Mechanisms in Food Selection,* edited by L. M. Barker, M. R. Best and M. Domjan. Waco: Baylor University Press, 1977, pp. 593–616.
18. Roberts, D. C. S. and R. C. Fibiger. Attenuation of amphetamine-induced conditioned taste aversion following intraventricular 6-hydroxydopamine. *Neurosci. Lett.* **1:** 343–347, 1975.
19. Semenov, L. A., O. Burešová and J. Bureš. Conditioned taste aversion induced in rats by unilateral chemical blockade or electrical stimulation of vestibular nuclei. *Physiologia bohemoslov.* **31:** 279–280, 1982.
20. Walle, K. K. and T. B. Wishart. Reliable method for cannulation of the third ventricle of the rat. *Physiol. Behav.* **19:** 171–173, 1977.
21. Weisman, R. N. and L. W. Hamilton. Increased conditioned gustatory aversion following VMH lesions in rats. *Physiol. Behav.* **9:** 801–804, 1972.
22. Winer, B. T. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1971.