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Differential Effects of d,l-Amphetamine on Licking Maintained by Electrical Hypothalamic Stimulation and/or Water in Rats

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KUNDU, S. N., A. J. GREENSHAW AND J. BUREŠ. *Differential effects of d,l-amphetamine on licking maintained by electrical hypothalamic stimulation and/or water in rats.* PHARMACOL BIOCHEM BEHAV 18(5) 663–668, 1983.—The role of the reinforcer as a determinant of the behavioural effects of amphetamine was assessed with a procedure under which response requirements were equated. Eight rats were trained to lick on fixed-ratio schedules of rewarding lateral hypothalamic stimulation and water delivery, respectively. The effects of d,l-amphetamine (0.1–2.0 mg/kg) were investigated in the lateral hypothalamic stimulation condition and compared with the effects of the drug at 1.0 mg/kg in the water condition. Licking maintained by hypothalamic stimulation was increased after amphetamine administration to approximately 200% of control performance at 1.0 mg/kg. At this dose amphetamine administration resulted in a decrease in water maintained licking. A computer analysis of the temporal patterning of licking in both water and stimulation conditions was carried out. This analysis revealed that amphetamine may attenuate the disruptive effects of hypothalamic stimulation on the lick-interrupt cycle. The differential effects of amphetamine on licking maintained by the two events may reflect either unequal participation of catecholaminergic circuits in the two types of reward, or anti-inhibitory motor effects of amphetamine.

d,l-Amphetamine Hypothalamic stimulation Licking

EFFECTS of drugs on operant or consummatory responding may be influenced by a number of factors including the event that maintains behaviour [2], the topography (i.e., spatial and temporal organization) of the response [26] and the rate at which the response is normally emitted [4]. In attempts to assess the significance of the event that maintains behaviour as a determinant of drug effects, studies have been conducted in which baseline rates of responding have been equated (e.g. [1]). However, differences in the topography of responses have not previously been controlled for, particularly in relation to the response-chain forming the transition from operant to consummatory responding (e.g., the transition from bar-pressing to magazine-entry in experiments with food reinforcement, in comparison with bar-pressing alone in experiments involving non-oral reinforcers). Licking in rats is a well defined behaviour that is reported to be emitted at local rates of between 5 and 8 licks per second [24]. An important feature of this behaviour is that bursts of licking occur evenly, that is, the within-burst patterning of licking is regular. Licking may be maintained not only by fluid delivery, but also by electrical stimulation of the tongue [24], or by non-oral reinforcers such as electrical brain stimulation [10, 21, 23, 25]. As this behaviour is emitted at a relatively constant local rate it may be well suited for comparisons of drug effects on behaviour maintained by different events. Furthermore, the licking response may represent a generally equivalent motor response pattern when maintained either by fluid delivery or by non-oral reinforcers such as rewarding brain stimulation.

Amphetamine is reported to facilitate behaviour maintained by electrical brain stimulation [22] at doses that induce marked anorexia [5]. It is apparent that factors such as response topography, baseline rate and the nature of the reinforcer may significantly influence the behavioural effects of this drug [4, 13, 26]. However, experiments directed to the analysis of drug effects on self-stimulation or food and water

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intake [17] generally do not allow a dissociation of these factors as possible determinants of drug-induced changes in behaviour.

The present experiment was conducted in order to assess the role of the reinforcer as a determinant of the effects of amphetamine. In this study, therefore, the effects of amphetamine were assessed on licking maintained by water delivery or rewarding hypothalamic stimulation.

METHOD

Subjects

Eight adult male hooded rats (Druckray strain) weighing approximately 300 g at the time of surgery were used in this study. The subjects were housed in groups of two or three animals per cage under natural lighting conditions at a constant temperature. The animals had free access to food in the home cages throughout the experiments. During the experiments water was available for a daily 20 minute period after completion of behavioural testing (1700 to 1720 hr).

Surgery and Histology

Each animal was unilaterally implanted with an electrode assembly directed to the medial forebrain bundle at the level of the lateral hypothalamus. The co-ordinates, based on the atlas of Fifková and Maršala [9], were 2.5 mm posterior to bregma, 1.4 mm lateral from the midline, and 8.8 mm ventral to the surface of the dura, with lambda 1 mm below bregma. The electrode assembly consisted of a piece of stainless steel injection tubing (0.5 mm diameter and 15 mm long) serving as a carrier for four enamel insulated nichrome wires (0.1 mm diameter). The nichrome electrodes were fixed to the carrier with epoxy-resin so that they projected 2.0 to 3.0 mm beyond the end of the carrier, their uninsulated tips forming a rhomb in the coronal plane with a distance of 0.7 to 1.0 mm between each tip. The electrode assembly was connected to a miniature transistor socket. This mounting was secured to the skull with steel screws and dental acrylic. Surgery was carried out under Nembutal (40–50 mg/kg) anaesthesia. On completion of the experiments, the deeply anaesthetized animals were sacrificed to verify electrode placements. After perfusion of the heart with 0.9% saline, followed by 10% Formalin, the brains were removed. Frozen sections were taken at 50 μ, mounted and stained using a standard Nissl procedure. All placements were located in and around the medial forebrain bundle in the lateral hypothalamus.

Apparatus

The behavioural test apparatus consisted of a plastic box (30×20×30 cm) equipped with a licking spout accessible through a 12-mm hole located 12 cm above the floor of the box in the middle of one wall. The movement of the rat's tongue across the 3 mm gap between the wall and the spout was monitored with a photoelectric sensor consisting of a miniature light bulb and a phototransistor. A Schmitt trigger was used to convert the photoelectric signal into rectangular pulses, the trailing edges of which (corresponding to tongue retraction) were applied to the input of an 8-bit binary counter. The output of bit 3 (corresponding to the offset of lick 8) triggered a monostable multivibrator, closing a dual relay for 300 msec. One set of contacts initiated intracranial electrical stimulation at a preset current intensity, the other set served to switch on a motor-driven syringe supplying approximately 40 μl of water to the licking spout with every relay closure. The shaped response pulses were also applied to the input of a cumulative recorder and to one analog input of a minicomputer (LINC 8, Digital Equip. Corp.). The output of bit 3 of the binary counter was connected to another analog input of the LINC, serving as a trigger for on-line recording of licking. Electrical stimulation applied to the hypothalamic electrodes was supplied from a constant current source at a fixed sinusoidal frequency of 50 Hz. Throughout the experiments the intensity of brain stimulation was monitored on an oscilloscope (TESLA) as the voltage drop on a 10 KΩ precision resistor, connected in series with the stimulator and the animal.

Procedure

Five to seven days after surgery the animals were placed on a water deprivation schedule, water being available for 20 min per day as described earlier. Each animal was exposed to the behavioural test apparatus for 30 minutes each day, water being available contingent on every 8th lick at the spout (FR 8). After licking was reliably maintained by water delivery (usually after 3 to 5 sessions), each animal's electrode assembly was connected to the stimulator via a flexible counterbalanced cable and trains of hypothalamic stimulation were applied simultaneously with water delivery. The initial current intensity was 20 μA RMS being gradually increased until the animals exhibited licking at the spout in the absence of water delivery. The self-stimulation threshold determined in this way was in the range of 50 to 100 μA RMS for each of the eight animals. With each rat the various electrode pairs (from the 4 poles of the assembly) were tested and the pair yielding the lowest self-stimulation threshold was used throughout the experiment. With current intensity set at approximately 20% above threshold, self-stimulation was maintained over three to five consecutive daily sessions, when overall rates of licking appeared stable. The effects of administration of d,l-amphetamine (0.1, 0.2, 0.5, 1.0, 2.0 mg/kg) were then investigated. d,l-Amphetamine sulphate, dissolved in 0.9% saline, was administered intraperitoneally in a volume of 1 ml/kg ten minutes prior to testing. Each dose was administered once to each animal and a drug day was always preceded by at least three control days on which equivalent injections of saline were given. After completion of the dose-response curve for the self-stimulation sessions, the animals were exposed to the schedule of water delivery in the absence of electrical stimulation. The effects of 1.0 mg/kg of amphetamine were then investigated with licking maintained by water alone. In a final test session the effects of a combination of water delivery and hypothalamic stimulation on the pattern of licking were assessed.

Behavioural Measures

The number of licks emitted during each 30 minute session were established from the cumulative records. A finer analysis of licking was performed with the LINC 8 minicomputer. Termination of lick 4 in the fixed ratio 8 schedule initiated a 2 second interval during which the presence of a lick was recorded in 1024 bins of 2 msec duration. The computer-generated histograms of this analysis were based on the cumulative summation of 256 of these 2 second epochs.

Periodicity of licking was manifested by a high amplitude of the first histogram peak corresponding to lick 5 of the fixed ratio of 8 licks and regular spacing of subsequent

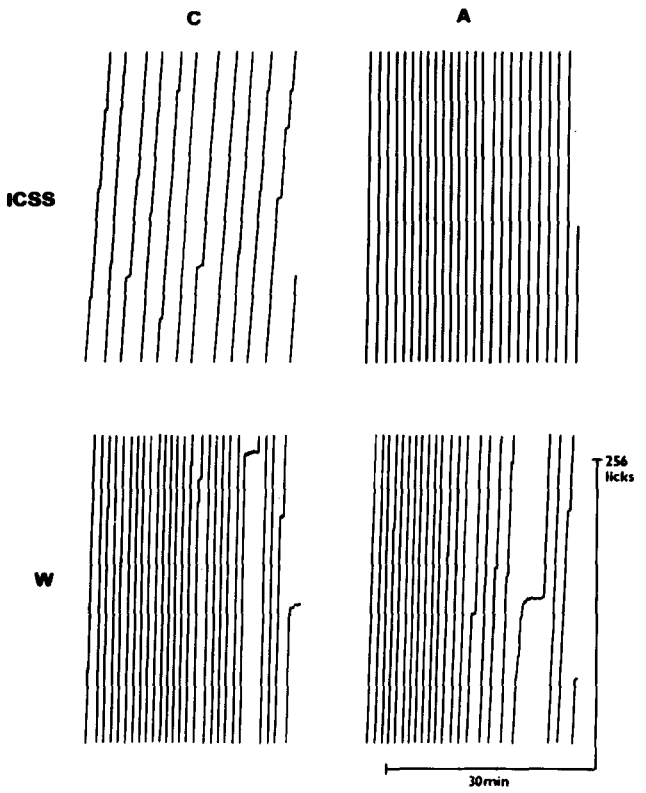

FIG. 1. Cumulative records for one subject illustrating the effects of 1 mg/kg amphetamine (A) on licking maintained by lateral hypothalamic stimulation (ICSS) or by water (W), in relation to control performance (C).

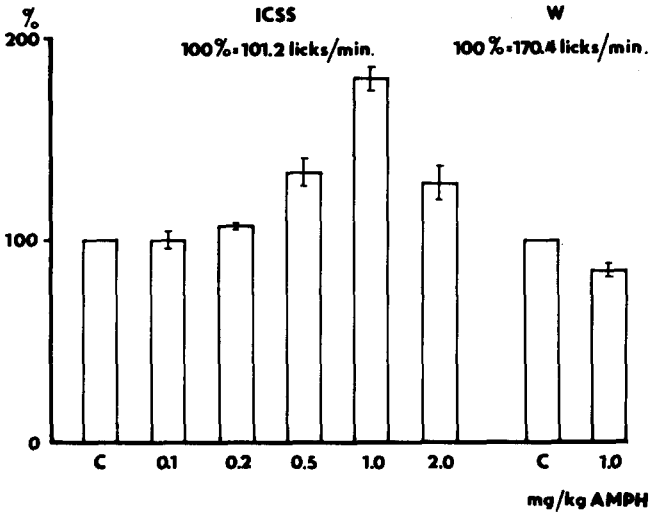

FIG. 2. The effects of amphetamine (abscissa) on licking maintained by lateral hypothalamic stimulation (ICSS) or by water (W). Columns represent overall mean rates ±SEM for the group of 8 rats expressed as a percentage of performance on the day immediately preceding drug treatment (C). Control levels (=100%) in the ICSS and W sessions correspond to different average rates of licking.

peaks, the gradual flattening of which expressed the variability of the inter-lick interval. As the lickometer required a rather long tongue-protrusion for operation, the recorded tongue-spout contact was relatively short (about 40 msec). The combined variability of inter-lick intervals and lick durations is responsible for the incomplete overlap of lick-on signals in the histograms. The amplitude of the histogram remained below 256 (corresponding to 100% of lick-on signals at a particular time in the 2 second epoch) even on lick 5: The probability of occurrence of the nth lick is, therefore, not expressed by the amplitude but rather by the area of the corresponding histogram peak.

RESULTS

Overall Rates of Licking

Licking maintained by hypothalamic stimulation occurred at lower overall rates than that maintained by water delivery. Water-maintained licking was regular and continuous, with occasional brief interruptions towards the end of the 30 min sessions. As all of the water delivered to the drinking spout was consumed, the FR 8 schedule maintained both operant and consummatory licking. The purely operant licking maintained by hypothalamic stimulation was discontinuous, with a 2.0 to 5.0 second postreinforcement pause after each train of electrical hypothalamic stimulation. The differences in overall patterns of licking maintained by the two events are illustrated by the cumulative records for one subject displayed in Fig. 1.

This figure also illustrates the effects of administration of 1.0 mg/kg d,l-amphetamine on licking maintained by water and by hypothalamic stimulation. The cumulative records from the same animal on the right-hand side of this figure show that this dose of d,l-amphetamine facilitated licking maintained by hypothalamic stimulation. Conversely, the overall rate of licking maintained by water delivery was decreased after administration of this dose of amphetamine. However, it is apparent from an examination of these cumulative records that, whereas amphetamine administration resulted in a change in the pattern of stimulation-maintained licking throughout the session, the drug-induced change in licking maintained by water delivery was characterized by the earlier emergence of long pauses in the session relative to the control record.

The effects of administration of amphetamine on licking maintained by hypothalamic stimulation (0.1 to 2.0 mg/kg) and by water delivery (1.0 mg/kg) are displayed for the group of eight animals as a whole in Fig. 2. The average overall rate of licking maintained by water was 101.2 licks per min, the corresponding value for the self-stimulation condition was 170.4 licks per min. A one-way ANOVA with repeated measures revealed that the administration of d,l-amphetamine significantly increased the overall rate of licking maintained by hypothalamic stimulation, $F(5,42)=31.0$, $p<0.01$. Multiple comparison tests using the Newman-Keuls procedure yielded significant effects at the 0.5, 1.0 and 2.0 mg/kg doses; the 1.0 mg/kg dose increased this measure to a greater extent than either the 0.5 or the 2.0 mg/kg doses ($p<0.01$ in each case). Statistical analysis of the effect of administration of the 1.0 mg/kg dose of the drug in licking maintained by water showed that this dose significantly reduced the overall rate of licking in this condition (paired $t(5)=5.1$, $p<0.01$).

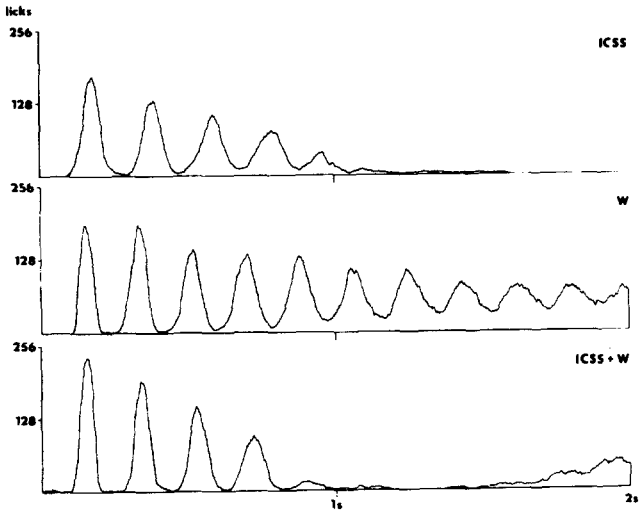

FIG. 3. Patterns of operant licking (FR8) maintained by lateral hypothalamic stimulation and water, alone or in combination. Ordinate: Lick incidence in the 2 sec interval initiated by the offset of lick 4. Abscissa: Time since the offset of lick 4.

Computer Analysis of Licking

The differences in the temporal distribution of licking under baseline and drug conditions described earlier were more pronounced in the computer-plotted histograms. As illustrated by the plots displayed in Fig. 3, water licking gave rise to histograms with a smooth decrease in the amplitude of successive peaks. The middle histogram of this figure illustrates that the regularity of this change in peak amplitude was not interrupted by the transition from operant to consummatory licking with the delivery of water (after lick 8). The histograms of self-stimulation licking were similar to those of water-licking for the pattern of behaviour prior to the onset of stimulation. However, hypothalamic stimulation resulted in an almost complete cessation of operant licking for a period corresponding at least to the end of the 2-second epoch represented in the histogram. This may be seen clearly in the upper histogram in Fig. 3. However, it is evident that this effect may be somewhat obscured by the asynchronous onset of hypothalamic stimulation which is scattered in plots of this type through the descending arc of peak four and possibly over peak five. A comparison of these histograms clearly reveals that licking in approximately the first second after the onset of stimulation did not appear in the intervals corresponding to the pre-stimulation periodicity of licking. Simultaneous delivery of water and hypothalamic stimulation after lick 8 shortened the duration of post-stimulation pauses, as illustrated by the lower histogram of Fig. 3. However, the presence of water did not appear to improve the synchronisation of pre- and post-stimulation licking.

The effects of d,l-amphetamine at the 1.0 mg/kg dose on the temporal distribution of licking maintained by hypothalamic stimulation are represented in a computer generated histogram plot in Fig. 4. This figure also shows the pre-drug plot for the same animal in the saline condition. From this figure it is evident that although administration of the drug resulted in an increased frequency of licking in the period after the onset of stimulation it did not prevent the stimulation-induced desynchronisation of licking. Further analysis of the temporal distribution of self-stimulation licking during the last 80 msec of the peri-stimulation epoch revealed that under control conditions animals (n=5) emitted only 20±9 (SD) licks over 256 stimulations, however, after 1.0 mg/kg of d,l-amphetamine the number of licks in this 80 msec period increased to 56±13.

An examination of the histograms representing the water-maintained and ICSS-maintained lick-distributions showed that the average intervals between the peaks corresponding to the last four pre-reinforcement licks were almost the same under control conditions (210±6 ms for water and 196±4 ms for ICSS). Amphetamine (1 mg/kg) decreased the water-maintained interlick intervals by 15±6 ms, $t(4)=2.6$, $p>0.05$, paired comparison, and increased the ICSS-maintained interlick intervals by 8±6 ms, $t(4)=1.3$, $p>0.05$, paired comparison. Neither difference was statistically significant. It can be concluded, therefore, that the local rates of licking were practically the same before water or ICSS delivery and that they were not significantly influenced by amphetamine.

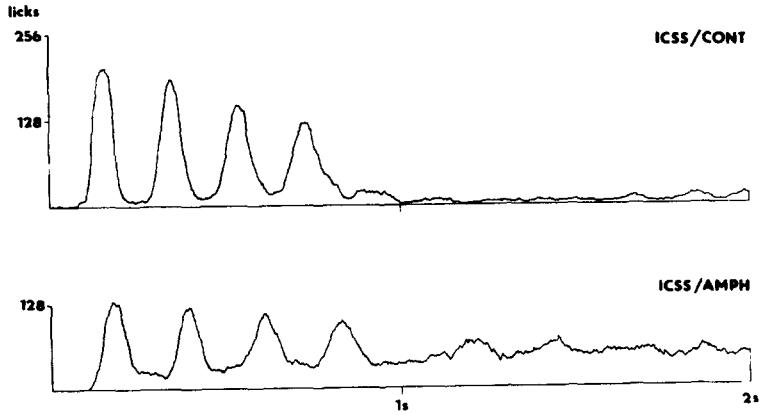

FIG. 4. The effect of amphetamine (1 mg/kg) on the patterns of operant licking maintained by lateral hypothalamic stimulation. Above: control session. Below: amphetamine session—note the shortening of the post-stimulation pause in the drug condition. Ordinate and abscissa as in Fig. 3.

DISCUSSION

The present experiments demonstrated differences in the patterns of licking maintained by water and by electrical hypothalamic stimulation. The most striking difference between these patterns was the emergence of a post-stimulation pause in the self-stimulation condition. Although such pausing is characteristic of fixed-ratio performance with other response requirements such as bar-pressing or key pecking [8], the present post-stimulation interruption of licking is more probably attributable to an interference effect [12].

The facilitatory effects of amphetamine on licking maintained by hypothalamic stimulation are consistent with numerous reports of the effects of this drug on self-stimulation behaviour [19, 23, 27]. It is possible that the amphetamine-induced increase in licking maintained by hypothalamic stimulation may be attributed to a facilitation of brain stimulation reward [19]. Nevertheless, this effect may be related to a drug-induced reduction in the disruptive effects of rewarding hypothalamic stimulation on the lick-interrupt cycle [12]. Independent evidence for the latter interpretation is provided by reports of the facilitation of motor recovery by amphetamine following lesions of motor cortex [6,15]. Although it has been reported that amphetamine may increase the efficiency of licking maintained by water [14], no such effect was observed in the present study.

The overall rate-decreasing effect of the 1.0 mg/kg dose of amphetamine on licking maintained by water is consistent with well documented anorectic and hypodipsic effects of this drug [3, 5, 6, 11, 16, 20]. According to this literature, suppression of food and water intake becomes apparent at 0.5 mg/kg and monotonously increases with amphetamine dosage until it is almost complete at levels exceeding 2 mg/kg. Since no dose of amphetamine increases water consumption during free drinking or low FR responding, the opposite effects of 1 mg/kg amphetamine on water-reinforced and ICSS-reinforced licking cannot be due to a shift of the respective dose-response curves but represents a genuine difference between the two conditions. Further evidence for this interpretation is afforded by the observation that the computer analysis yielded no effect of amphetamine on the local distribution of water-maintained licks.

Previous experiments have demonstrated that baseline rate is a significant determinant of the behavioural effects of amphetamine; in relation to this factor the distinction between overall and local rates is rarely made [18]. In the present study the overall rates of licking maintained by the two events were not equated, and this could possibly be a significant factor in the interpretation of the present results. However, previous studies have reported decreases in high rates of fixed-ratio responding after administration of amphetamine [18]. With both water-licking and self-stimulating-licking, local rates were high in the present experiments. Furthermore, the drug-induced reduction in water-licking was due to an earlier cessation of drinking over the course of the session, without a change in local rates. Thus the present data may not be simply attributed to rate-dependent effects.

The results of the present study demonstrate that, under conditions of equal response requirements, amphetamine has differential effects on behaviour maintained by water and by electrical hypothalamic stimulation. Experiments of this type may provide a useful means for evaluating the involvement of catecholamine activity, or activity in other neurochemical systems, in the maintenance of behaviour with different reinforcers by controlling for gross differences in the topography of the behavioural response set.

REFERENCES

1. Ator, N. A. Differential effects of chlordiazepoxide on comparable rates of responding maintained by food or shock avoidance. *Psychopharmacology* **66:** 227–232, 1979.
2. Barrett, J. E. and J. L. Katz. Drug effects on behaviour maintained by different events. In: *Advances in Behavioral Pharmacology*, vol 3, edited by T. Thompson, P. B. Dews and W. A. McKim. New York: Academic Press, 1981, pp. 119–168.
3. Carlisle, H. J. Differential effects of amphetamine on food and water intake in rats with lateral hypothalamic lesions. *J Comp Physiol Psychol* **58:** 47–54, 1964.
4. Dews, P. B. Studies on behavior. IV. Stimulant actions of metamphetamine. *J Pharmacol Exp Ther* **12:** 137–147, 1958.
5. Epstein, A. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. *J Comp Physiol Psychol* **52:** 37–45, 1959.
6. Falk, J. L. and G. K. Burnidge. Drug antagonism and water intake. *Physiol Behav* **5:** 193–198, 1970.
7. Feeney, D. M., A. Gonzales, H. M. Murray and W. G. Dail. Amphetamine produces an enduring recovery of locomotor function after motor cortex injury in the rat. *Soc Neurosci Abstr* **5:** 802, 1980.
8. Ferster, C. B. and B. F. Skinner. *Schedules of Reinforcement*. New York: Appleton-Century-Crofts, 1957.
9. Fifková, E. and J. Maršala. Stereotaxic atlases for the cat, rabbit and rat. In: *Electrophysiological Methods in Biological Research*, edited by J. Bureš, M. Petráň and J. Zachar. New York: Academic Press, 1967, pp. 653–731.
10. Gibson, W. E., L. D. Reid, M. Sakai and P. B. Porter. Intracranial reinforcement compared with sugar-water reinforcement. *Science* **148:** 1357–1359, 1965.
11. Glick, S. D. and S. Greenstein. Pharmacological inhibition of eating, drinking and prandial drinking. *Behav Biol* **8:** 55–61, 1973.
12. Greenshaw, A. J. and J. Bureš. Rewarding lateral hypothalamic stimulation desynchronises licking in rats. *Neurosci Lett* Suppl **7:** S449, 1981.
13. Kida, M., A. J. Greenshaw, D. J. Sanger and D. E. Blackman. The effects of *d*-amphetamine and chlordiazepoxide on behaviours maintained by a multiple-schedule of food and electrical brain stimulation in rats. *Psychol Record* **31:** 349–356, 1981.
14. Knowler, W. C. and T. E. Ukena. The effects of chlorpromazine, pentobarbital, chlordiazepoxide and d-amphetamine on rates of licking in the rat. *J Pharmacol Exp Ther* **184:** 385–397, 1973.
15. Meyer, P. M., J. A. Horel and D. R. Meyer. Effects of DL-amphetamine upon placing responses in neodecorticate cats. *Physiol Psychol* **56:** 402–404, 1963.
16. Neill, D. B. and S. P. Grossman. Interaction of the effects of reserpine and amphetamine on food and water intake. *J Comp Physiol Psychol* **76:** 327–336, 1971.
17. Rolls, E. T., B. J. Rolls, P. H. Kelly, S. G. Shaw, R. J. Woods and R. Dale. The relative attenuation of self-stimulation, eating and drinking produced by dopamine receptor blockade. *Psychopharmacology* **38:** 219–230, 1974.
18. Sanger, D. J. and D. E. Blackman. Rate—dependent affects of drugs. A review of the literature. *Pharmacol Biochem Behav* **4:** 73–83, 1976.
19. Stein, L. Amphetamine and neural reward mechanisms. In: *Animal Behaviour and Drug Action*, edited by H. Steinberg. Boston: Little, Brown and Co., 1964, pp. 91–113.

20. Stolerman, J. P. and G. D. D'Mello. Amphetamine induced hypodipsia and its implications for conditioned taste aversion in rats. *Pharmacol Biochem Behav* **8**: 333–338, 1978.
21. Von der Porten, K. P. Intracranial stimulation and one-drop licking in the rat. *Physiol Psychol* **5**: 27–33, 1977.
22. Wauquier, A. The influence of psychoactive drugs on brain self-stimulation in rats: a review. In: *Brain Stimulation Reward*, edited by A. Wauquier and E. T. Rolls. New York: American Elsevier Publishing Co., 1976, pp. 123–170.
23. Wauquier, A. and J. E. Niemegeers. A comparison between lick or lever-pressing contingent reward and the effects of neuroleptics thereon. *Arch Int Pharmacodyn Ther* **239**: 230–240, 1979.
24. Weijnen, J. A. W. M. and J. Mendelson. *Drinking Behaviour: Oral Stimulation, Reinforcement and Preference*. New York: Plenum Press, 1977.
25. White, N. Strength-duration analysis of the organization of reinforcement pathways in the medial forebrain bundle of rats. *Brain Res* **110**: 575–591, 1976.
26. Zacharko, R. M. and T. B. Wishart. Facilitation of self-stimulation with high doses of amphetamine in the rat. *Psychopharmacology* **64**: 247–248, 1979.
27. Zarevics, P. and P. Setler. Simultaneous rate-independent and rate-dependent assessment of intracranial self-stimulation: evidence for the direct involvement of dopamine in brain reinforcement mechanisms. *Brain Res* **169**: 499–512, 1979.