Pharmacology Biochemistry & Behavior, Vol. 6, pp. 481–486.

Effects of Schedules of Reinforcement on Brain Catecholamine Metabolism in the Rat[1]

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(Received 3 December 1976)

ALBERT, L. H., M. EMMETT-OGLESBY AND L. S. SEIDEN. *Effects of schedules of reinforcement on brain catecholamine metabolism in the rat.* PHARMAC. BIOCHEM. BEHAV. 6(5) 481–486, 1977. – Rats were trained on a fixed ratio 5 (FR 5) for 0.01 ml water reinforcer, a fixed ratio 20 (FR 20) for 0.01 ml water reinforcer, a fixed ratio 20 (FR 20) for 0.04 ml water reinforcer, a variable time 15 sec (VT 15 sec) for 0.01 ml water reinforcer, a variable time 30 sec (VT 30 sec) for 0.01 ml water, variable time 60 sec (VT 60 sec) for 0.01 ml water, or a variable time 60 sec (VT 60 sec) for 0.10 ml water. In all these schedules, the rate of NE metabolism varied with the number of water presentations but not with the number of responses or the volume of water. The rate of metabolism of DA was increased over control values in rats on the VT schedules but no significant differences could be found in DA metabolism among the rats performing on the various VT schedules or receiving various volumes.

Schedules of reinforcement α-Methyl-p-tyrosine Brain catecholamine metabolism
Tyrosine-hydroxylase inhibitor Nonadrenergic neuronal activity

THERE IS evidence implicating a relationship between norepinephrine (NE) and operant behavior [2, 8, 16] and a relationship between the schedule of reinforcement and activity of central noradrenergic neurons. Schoenfeld and Selden [15] have found that the same doses of α-methyl-p-tyrosine (AMPT), a potent tyrosine-hydroxylase inhibitor [11] resulted in lower brain NE and DA levels in rats responding on a VI 30 sec schedule of reinforcement than in nonresponding control rats. This was interpreted as indicating an increase in noradrenergic neuronal activity. These results were confirmed by Lewy and Seiden [12] using radioactive tracer labelling of the NE in brain. Rats responding on a VI 30 sec for water reinforcers were found to have a lower specific activity of tritiated NE than control rats. This was interpreted as being secondary to an increase of noradrenergic neuronal activity in the performing rats.

In both these papers, water was used as the reinforcer. Slangen and Miller [18], Courey [4] and Setler [17] have all shown that intracranial injection of NE leads to drinking behavior. The question arises, therefore, whether the changes in norepinephrine and its metabolism seen by Schoenfeld and Seiden [15] and Lewy and Seiden [12] are secondary to the presentation of water or to lever pressing behavior.

In the present experiments, norepinephrine turnover in brainstem-diencephalon was compared in rats receiving the same amount of a reinforcer for different numbers of lever press responses and in rats receiving different amounts of reinforcer for the same amount of lever press responding. A correlation was found between NE turnover and number of reinforcements but not between NE turnover and quantity of reinforcer or number of responses. In another experiment, deprived rats received water noncontingently and CA synthesis was inhibited with AMPT. It was found that the depletion of NE in brainstem was correlated to the number of presentations, but not to the quantity of water presented. Further, DA, although depleted more than in nonreinforced controls, did not correlate to either the number of reinforcers or the quantity of water presented.

METHOD

The rate of NE metabolism was estimated either by the injection of tracer doses of ^{3}H-NE (Intraventricularly) or the injection of α-methyl-p-tyrosine (IP). Male Sprague-Dawley rats (Holtzman) involved in radioactive tracer studies had indwelling cannula implanted in the right ventricle by the method of Hayden *et al.* [9]. Coordinates for placement of the cannula are given by Lewy and Seiden [12]. Rats were cannulated at 120 days of age. Rats were injected with 1.0μCi of ^{3}H-NE (New England Nuclear) in 10.0μl Merlis solution [7] via the cannula at the beginning of the experimental session. At the end of this session, rats were decapitated and brains dissected for cerebral cortices and brainstem-diencephalon by the method of Iversen and Glowinski [10]. The cortices were individually weighed and

[1] This research was supported by U.S. Public Health Service National Institute of Mental Health Grant MH–11191–11.
[2] Supported by Training Grant USPHS 2 TO5 GM 01939–07 (MSTP).
[3] Supported by Research Career Development Award 5 KO2 MH 10562–08.

homogenized in 82% (v/v) cold ethanol and total radioactivity was determined for each sample. A ratio of counts per minute of right to left cortex was calculated. A ratio of less than 4.0 was considered indicative of an intraventricular injection while a ratio of 4.0 or greater was considered to indicate an injection into the cerebral cortex rather than the ventricle.

The brainstem-diencephalons were individually weighed and their content of ^{3}H-NE and NE determined by the method of Pujol [14] with the modification of using Triton X100 rather than Bray's solution as the scintillation cocktail.

In those experiments using AMPT, 60 day old male Sprague-Dawley rats were injected with 125 mg/kg dl-AMPT or vehicle intraperitoneally one hr prior to the experimental sessions. Experimental sessions lasted two hr. At the end of the session, the rats were decapitated and pons-medulla and caudate were dissected. The caudate nuclei were removed by coronal section at the level of the optic chiasm and at the level of the rostral border of the olfactory bulb. The caudate nuclei were then dissected away from the cortex corpus callosum and striatum surrounding them. The pons-medulla was obtained by peeling away the cerebellum, reflecting the telencephalon forward and then sectioning the brainstem-diencephalon at the level of the mammilary bodies. Dopamine (DA) concentrations were determined by the method of Carlsson and Waldeck [3]. NE concentrations were determined by the method of Bertler *et al.* [1].

RESULTS

Experiment 1

Sixty day old Sprague-Dawley male rats (Holtzman) were trained on FR 5 or FR 20 schedules using either 0.01 ml or 0.04 ml of water as reinforcer. The turnover of NE in the pons-medulla was determined using radioactive tracer methods and it was found that the metabolism of NE was influenced by either the schedule of reinforcement, the number of reinforcers, or both, but not by the total volume of water presented as reinforcement.

Rats were randomly assigned to one of four groups, a control and 3 experimental groups. Rats were housed in pairs in 25 cm × 36 cm × 19 cm cages with ad lib food (Rockland). Rats were water deprived for 48 hr prior to beginning the experiment. Experimental rats were trained to lever press on a fixed ratio (FR 5) for 0.01 ml water reinforcer, fixed ratio (FR 20) for 0.01 ml water reinforcer, or FR 20 for 0.04 ml water reinforcer in operant conditioning chambers (Lehigh Valley Model 1316) programmed with solid state logic modules (Massey Dickinson Co). The protocol for conditioning is given in Table 1. Sessions lasted 1/2 hr. During training sessions, rats in the control group were placed in individual holding cages for the duration of the session. At the end of the session all rats were returned to their home cages. Fifteen min later rats were given ad lib water in individual watering cages. Length of access to ad lib water was determined by maintaining average daily body weight to 300 g ± 5 g (80% normal body weight). In the last week of the experiment, it was necessary on empirical grounds to give sessions every other day and allow ad lib water for 6 min on alternate days to maintain body weights. Rats were considered trained when their daily response output for 3 successive sessions was ± 10% the average of the three sessions. Rats were cannulated on the 62nd day of the protocol. No deficit in responding appeared post cannulation. When rats were fully trained they were injected with 1.0 μ Ci ^{3}H-NE intraventricularly immediately before the experimental session. After the session, rats were decapitated and the pons-medulla-diencephalon assayed for ^{3}H-NE and NE as described in the METHOD section.

The specific activity of the ^{3}H-NE in the brainstem-diencephalon in the FR 5 group was significantly lower ($p = 0.05$) than control, FR 20–0.01 ml and the FR 20–0.04 ml groups (Table 2). As can be seen from Table 2, neither the ^{3}H-NE content nor the NE content of these

TABLE 1

TRAINING PROTOCOL FOR MEASURING NE METABOLISM IN THE BRAINS OF ANIMALS PERFORMING ON FIXED RATIO SCHEDULES

Group	Day 1	2	3	4	5	6	7-60	61	62	63	64	65	66	67	68	69	
Control	—	—	—	—	—	—	—	Ad lib water No test session	CANNULATION	—	AD	—	AD	—	AD	INJECTION OF ^{3}H-NE	—
							0										
FR 5 0.01 ml	FR1	FR1	FR5	FR5	FR5	FR5	FR5			FR5	LIB	FR5	LIB	FR5	LIB		FR5
FR 20 0.01 ml	FR1	FR1	FR5	FR10	FR15	FR20	FR20			FR20	WAT ER	FR20	WAT ER	FR20	WAT ER		FR20

TABLE 2

NE METABOLISM IN THE BRAINS OF RATS THAT HAD PERFORMED UNDER FR SCHEDULES OF REINFORCEMENT

Group	Specific Activity nCi/μg NE ± SEM	^{3}H-NE nCi ± SEM	NE μg/gm tissue ± SEM	Brain wt. gm ± SEM	N
Control	198.1 ± 17.9	40.4 ± 3.8	.364 ± 0.26	.621 ± .038	18
FR 5	155.2 ± 11.5*	35.4 ± 4.3	.404 ± .043	.611 ± .033	17
FR 20 - .01 ml	190.6 ± 19.4	36.6 ± 4.9	.327 ± .062	.710 ± 0.65	10
FR 20 - 0.04 ml	214.6 ± 27.6	37.7 ± 5.6	.348 ± .023	.533 ± .008	9

*Significantly different from all other groups, ($p = 0.05$) (Welch's t-test).

four groups was significantly different. This is because these total levels depend on the recovery of the assay. Variability in the recovery is reflected as variability in the total content ^{3}H-NE and NE. This variability masks significant differences. The specific activity, however, being a ratio, is independent of assay recovery, and thus reflects the significant differences in rates of metabolism of NE among the four groups.

In comparing the schedule related variables (Figs. 1–3) to the biochemical variables (Table 2), it is apparent that only the total number of reinforcements and the schedule of reinforcement itself parallel the differences found in the specific activity of ^{3}H-NE among the four groups.

Experiment 2

These results were verified using a different technique in the followup experiment. Sixty day old male Sprague-Dawley rats (Holtzman) exposed to various variable-time (VT) schedules showed reductions in NE levels in pons-medulla which correlated with the square root of the number of presentations of a water reinforcer (Fig. 5). Dopamine levels in the caudate on the other hand, were non-specifically lowered on the various VT schedules (Fig. 6).

Rats were exposed to daily 2 hr sessions for 5 days on one of the following variable time (VT) schedules: VT 15 sec, VT 30 sec or VT 60 sec. Each schedule presented 0.01 ml of water after the interval had elapsed. One hr before the session on Days 4 and 5, rats were injected with 2.0 ml of vehicle IP. One hr before the experimental session on the 6th day, rats in the experimental group were injected with 125 mg/kg AMPT. At the end of the 2-hr session (3 hr after injection) the rats were killed by decapitation, dissected and assayed as described above.

Rats receiving water on any of a VI 15, VI 30 or VI 60 sec schedule of reinforcement had lower levels of NE in the pons-medulla than AMPT treated controls not receiving water although the difference was not significant (Fig. 4). However, the pons-medulla NE was negatively correlated with the square root of the frequency of water presentation ($r = .74$; 39 df; $p<0.01$, Fig. 5). Caudate dopamine, on the other hand, was equally lowered by all three schedules of reinforcement (Fig. 6).

Experiment 3

On the three VT schedules of this experiment, rats were presented with unequal numbers and quantity of reinforcement. Rats on theVT 15 sec schedule were presented with an average of 4.8 ml of water during the session. Rats on the VT 30 sec schedule were presented with an average of 2.4 ml of water and rats on the VT 60 sec schedule were presented with an average of 1.2 ml of water during the session. To rule out differences in catecholamine metabolism due to differences in quantity of reinforcement, the following experiment was performed. Rats were trained as

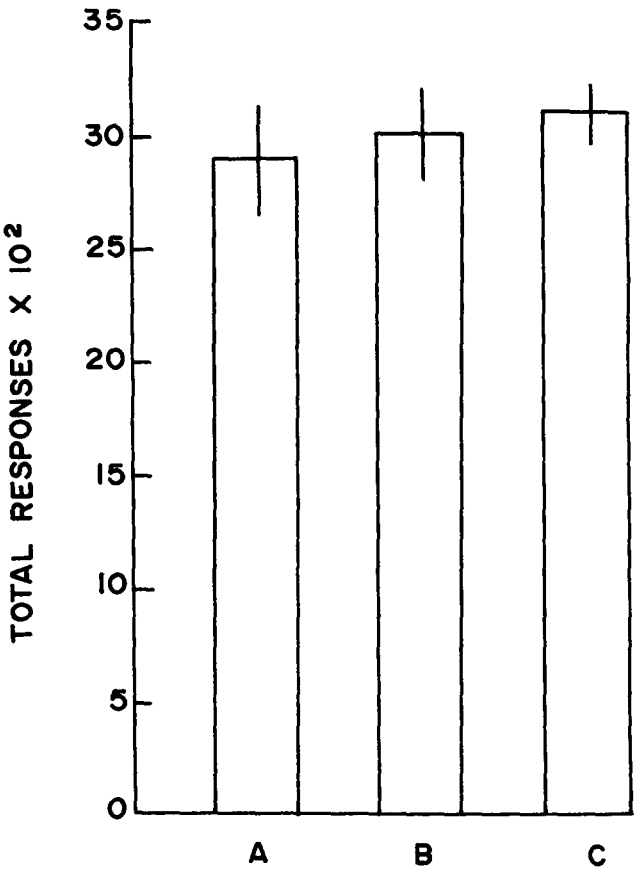

FIG. 1. The total number of responses during a one-half hr session. Values are group means ± SEM. Panel A is the total number of responses made by animals responding on a FR 5 schedule. Panel B is the total number of responses made in the experimental session by rats responding on an FR 20 schedule for 0.04 ml of water. Panel C represents the total number of responses ± SEM given by animals responding on an FR 20 schedule for 0.01 ml of water.

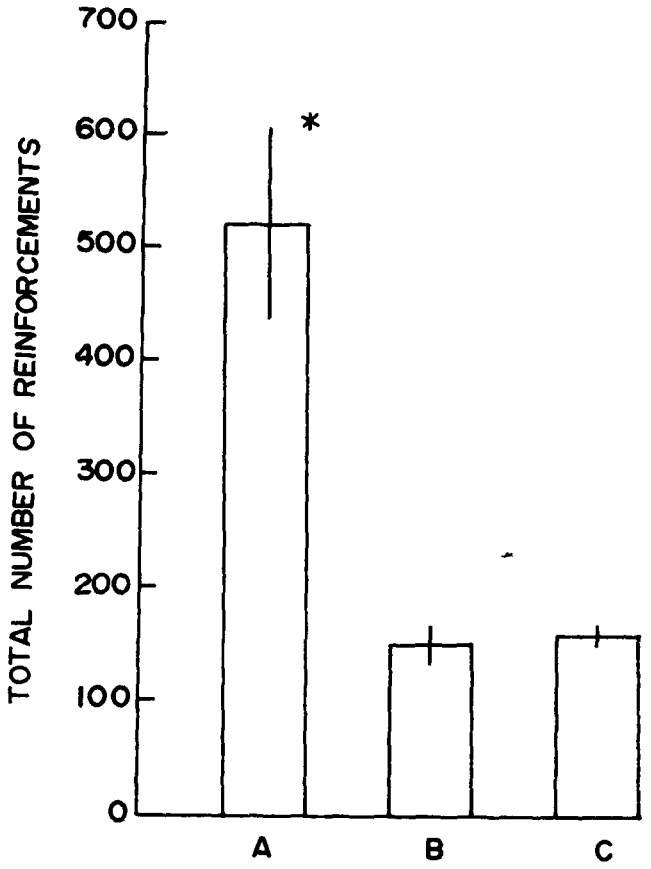

FIG. 2. The total number of reinforcements presented during the one-half hour experimental session in Experiment 1. Values are expressed as group means ± SEM. Panel A represents the total number of reinforcements presented to rats responding on an FR 5 schedule. Panel B represents the total number of reinforcements presented to rats responding on an FR 20 schedule for 0.04 ml water. Panel C represents the total number of reinforcements presented to rats responding on an FR 20 schedule for 0.01 ml water. *The value given by Panel A is significantly higher ($p<0.05$) than the other two groups' values (Welch's t-test).

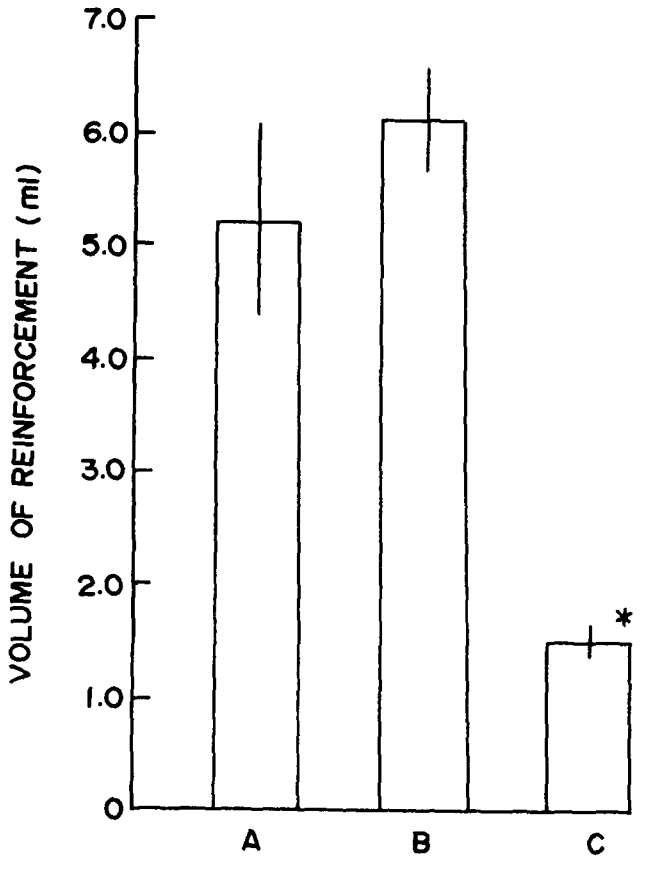

FIG. 3. Total volume of water reinforcement during one-half hr experimental session of Experiment 1. Values are group means ± SEM. Panel A represents the total volume of water presented to rats responding on an FR 5 schedule for 0.01 ml water per reinforcement. Panel B presents the total volume of water presented to rats responding on an FR 20 schedule for 0.04 ml water per reinforcement. Panel C represents the total volume of water presented to rats performing on an FR 20 schedule for 0.01 ml water per reinforcement. Panel C is significantly ($p<0.05$) lower than the other two groups (Welch's t-test).

above on a VT 60 sec schedule except that one group received 0.01 ml water per reinforcement, another group 0.04 ml water, and another 0.10 ml water per reinforcement. Since the schedule was the same for all three groups, the number of reinforcements would be equal. Because of the differences in dipper size, however, the total quantity of water presented would be unequal, and therefore, any inequality in catecholamine metabolism in these three groups could be attributed to differences in total volume of water presented. Conversely, no differences among the three experimental groups of this experiment would rule out any effect of total volume of reinforcement on CA metabolism.

Both pons-medulla NE and caudate DA were lower in groups receiving water than in AMPT-treated controls. There was, however, no correlation between dipper size and either pons-medulla NE or caudate DA (Figs. 7,8).

DISCUSSION

The changes in NE metabolism seen in the present

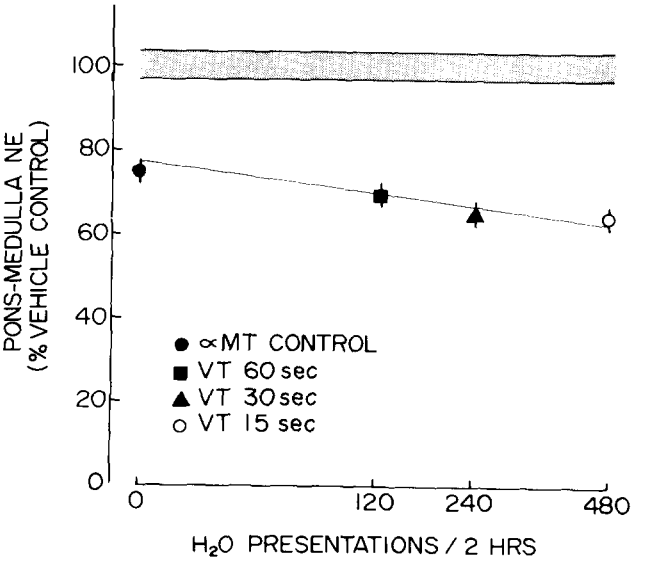

FIG. 4. Pons-medulla NE as a function of water presentation in rats receiving 0.01 ml water on a VT 15 sec, VT 30 sec, or VT 60 sec schedule.

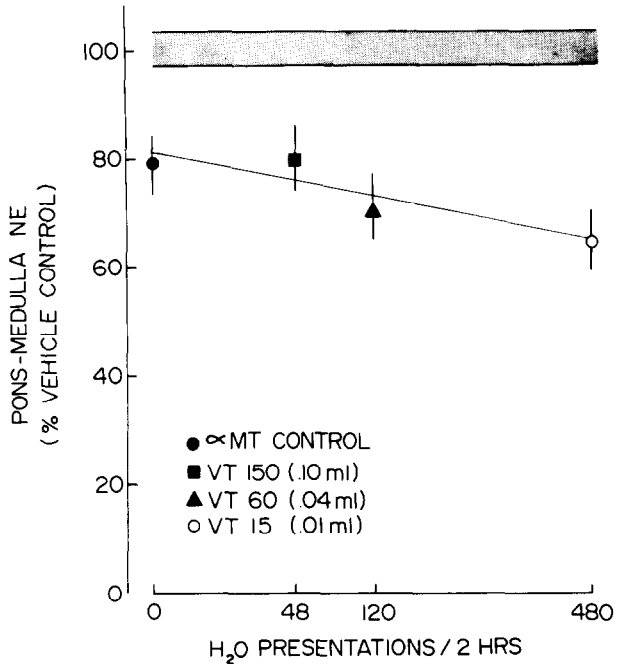

FIG. 5. Relation of pons-medulla NE to square root of number of reinforcers in rats receiving 0.01 ml water on VT 15 sec, VT 30 sec, or VT 60 sec schedule.

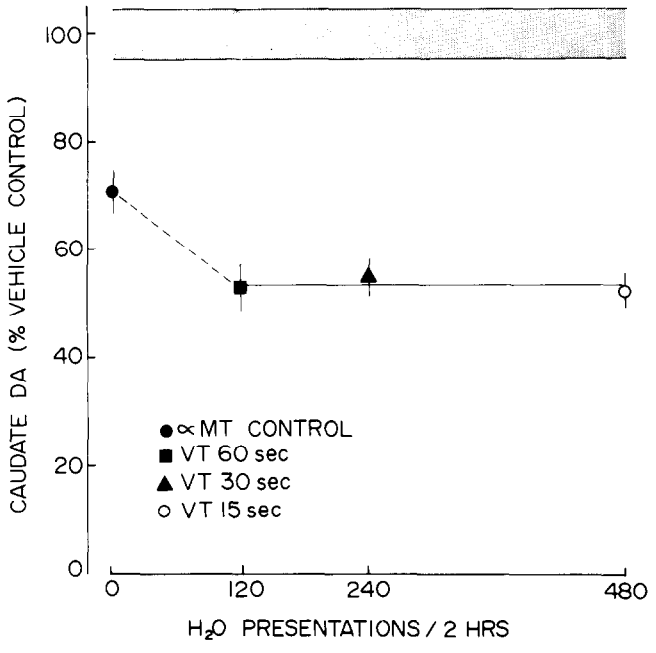

FIG. 6. Caudate DA as a function of water presentation for rats receiving 0.01 ml water on VT 15 sec, VT 30 sec, or VT 60 sec schedule.

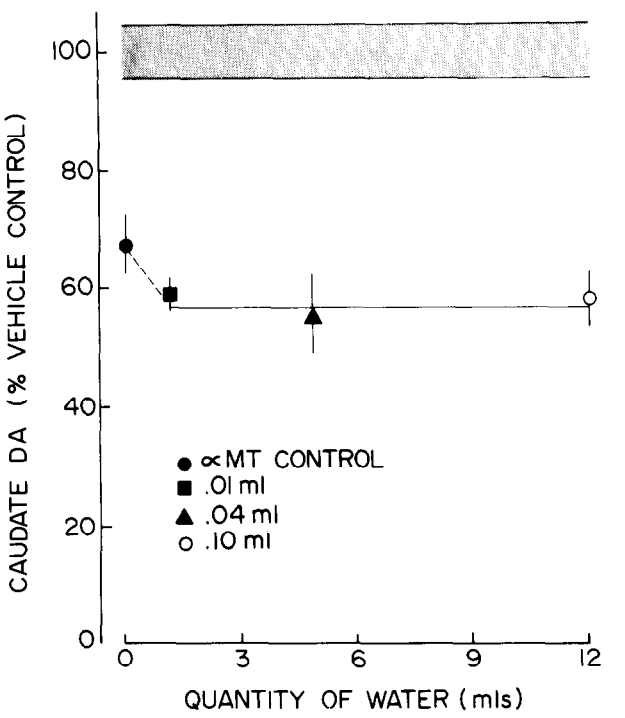

FIG. 7. Caudate DA as a function of volume reinforcer after AMPT pretreatment in rats receiving 0.01 ml water on a VT 60 sec schedule.

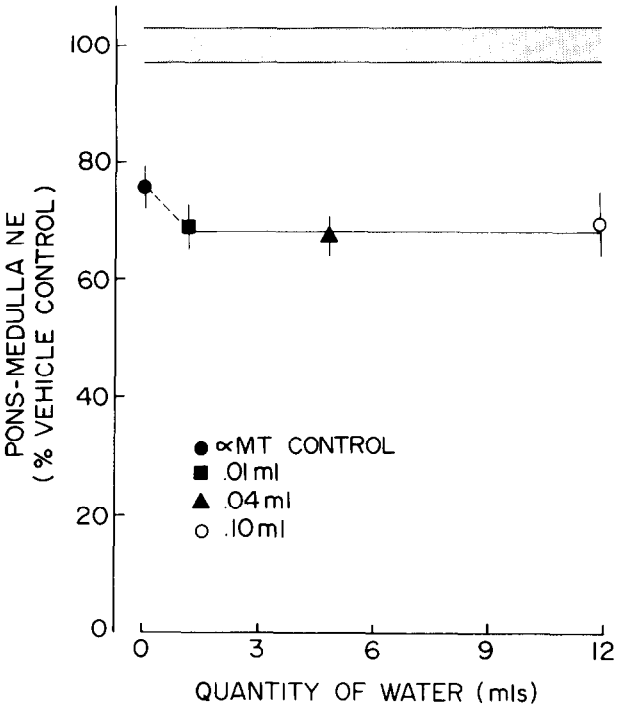

FIG. 8. Pons-medulla NE after AMPT pretreatment in rats receiving 0.01 ml, 0.04 ml, or 0.10 ml water on a VT 60 sec schedule.

experiments may be due to the schedule of reinforcement. This would agree with Dew's findings [5,6] that the effects of drugs vary according to the schedule of reinforcement. Since in the FR studies the number of reinforcers was in part determined by the schedule of reinforcement, and in the VT studies, the number of presentations was determined by the schedule, it is impossible to say whether it was the schedule of reinforcement or the number of reinforcers, or both, which had an effect on the rate of NE

metabolism. If indeed it was the number of reinforcers that affected the NE metabolism, it is possible that either the presentation or the consumption of the reinforcer was primarily responsible for altering the rate of NE metabolism.

Emmett-Oglesby *et al.* (in preparation) attempted to answer this question by measuring the rate of NE metabolism in rats receiving water not contingent on their behavior. They found that NE metabolism is affected by stimuli previously paired with reinforcement rather than either presentation of a reinforcer per se or consumption of reinforcer. These biochemical results compare well with anatomical and electrophysiological data indicating that the reticular activating system (RAS) is largely noradrenergic [19] and that pairing of a neutral stimulus with a reinforcing event will lead to activity of the RAS on presentation of the initially neutral stimulus [13]. The hypothesis that the rate of NE metabolism is influenced by conditioning rather than positive reinforcement per se provides another possible explanation for the data of Wise and Stein [20]. The changes they see in noradrenergic activity in intracranial self stimulation studies may be due not to self stimulation per se but to the previous pairing of reinforcing stimulation with initially neutral environmental stimuli and events.

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