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Uptake of ^{3}H-Uridine into Brain and Incorporation into Brain RNA of Rats Exposed to Various Training Tasks – A Biochemical Analysis[1]

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SMITH, J. E., G. T. HEISTAD AND T. THOMPSON. *Uptake of ^{3}H-uridine into brain and incorporation into brain RNA of rats exposed to various training tasks – a biochemical analysis.* PHARMAC. BIOCHEM. BEHAV. 3(3) 447–454, 1975. – Operant schedules were used to isolate component parts of a training task and rates of incorporation of ^{3}H-uridine into the brain and brain RNA were determined. Rats that developed a discrimination in responding to a visual stimulus absorbed more radioactivity into the brain and incorporated a higher percentage of this radioactivity into total and cytoplasmic RNA than littermates exposed to the visual stimulus only. Of the component parts of the training task, the discrimination accounted for the greatest increase in absorption of radioactivity and incorporation of it into RNA. The schedule change had the second largest effect and the stimulus change the least.

RNA changes during training Neurochemical correlates of training Rat brain RNA changes Brain RNA RNA

IN the last decade, a great deal of research has focussed on the molecular events of information processing and storage by the central nervous system. One major area of research has been the demonstration of neurochemical changes in proteins [17,24], RNA [37] and DNA [34] during training.

Neurochemical changes have been demonstrated with both positive (presentation of a reinforcing stimulus) and negative (removal or avoidance of an aversive stimulus) training procedures. With a shock avoidance training procedure, RNA changes in whole brain [4, 28, 38], brain areas [22, 31, 32, 39], and specific RNA species [1, 2, 10, 11] and protein changes in whole brain [16] and in brain areas [6] have been demonstrated. Several training tasks have been used to study neurochemical changes with positive procedures: wire climbing maintained by food reinforcement [13], change of handedness in rats retrieving food from a narrow tube [21], Y-maze running maintained by water reinforcement [8,23], key pecking by pigeons [7], and imprinting chicks to a flashing light [5]. RNA changes in whole brain [13], brain areas [8, 19, 23], and specific RNA species [19] and protein changes in whole brain [7], brain areas [5,20] and specific protein species [21] have been demonstrated in animals trained in these tasks. In many of these studies, the total difference observed between comparison groups has been attributed to the hypothesized central process responsible for the observed changes in response probabilities (learning), when there have been stimulus and environmental differences between the comparison groups.

Neurochemical changes in the form of increased protein and RNA synthesis have been demonstrated in animals receiving visual [37], electrical [27], olfactory [33], and motor [3,18] stimulation. These stimuli are concurrent with most training tasks. The purpose of this study was to use operant training procedures to isolate and measure the effects of some of the component parts of a training task on the rate of uptake and distribution of ^{3}H-uridine-5 into the brains and brain RNA of rats exposed to various portions of a training task. RNA changes were investigated to maximize the probability of detecting biochemical changes, since changes in RNA are one of the first responses observed when the genome is stimulated. Therefore, if changes in the brain that involve enzyme induction, structural changes, or nearly any known physiological or biochemical response occur, then induction and a resulting increase in RNA synthesis is likely. Such changes have not been dem-

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onstrated to occur as a direct result of the specific central process responsible for the observed changes in response probabilities.

METHOD

Behavioral

Animals. Thirty-six male Fischer F-344 strain 90–120 day old rats (Hilltop Labs, Chatsworth, CA) were used in 9 groups of 4 littermates. The experiments were begun with 9 animals in each of 4 treatment conditions. Because of loss of some fractions during the experimental manipulations, there are less than 9 per group in some of the comparisons (Tables 1 and 2). The animals were maintained at 80% of their free-feeding weight with free access to water in group cages (4 littermates to each cage) in a temperature and humidity controlled environment with the room illuminated continually.

Procedure. In each group of littermates, the 4 rats were trained to lever press on a fixed ratio 20 (FR 20) schedule of food reinforcement (45 mg Noyes pellets) until stable baselines (±5 responses per min) were obtained. A fixed-ratio 20 schedule of reinforcement is one in which a response is reinforced upon completion of 20 responses counted from the preceding reinforcement [14]. The FR 20 baseline training procedure was used to control for experience in the training environment (the number of reinforcements obtained, number of responses emitted, and number of training sessions administered were held constant within each experimental group [litter]). The response rates of the animals were also similar. Littermates from a highly inbred strain were used to minimize genetic differences that might effect the background level in neurochemical pathways being studied.

After stable baselines were obtained (approximately 35 sessions), the four littermates were lightly anesthetized with ether and injected with 50 μc of ^{3}H-uridine-5 (specific activity 28 C/mM) in 50 μl of saline into each cerebral hemisphere approximately 4 mm lateral to the superior sagital suture, 2 mm anterior to the posterior lateral suture, and 3 mm below the skin with a 27 g needle using the method of Campagnoi [9]. A total of 3.57 nanomoles of uridine was administered which is less than 0.6 percent of the free UTP pool in the brain [26]. One hour after administration of the ^{3}H-uridine, the animals were exposed with random assignment to one of the following training conditions for 60 min:

(a) A multiple 2 minute FR 20 2 minute extinction condition (MULT). The multiple schedule consisted of two alternating schedules of reinforcement: FR 20 and extinction (EXT–reinforcement is withheld) with a different stimulus present during each. In this condition the light that had been present during FR 20 training (session light) alternated between being lit for 2 min during which the FR 20 component was in effect and being extinguished for 2 min during which the extinction component was in effect and food was withheld. The stimulus change was a cue for the portion of the schedule that was present. As a result the animal in this condition developed a discrimination and responded at a high rate when the light was on and the FR 20 component was in effect and responded very little when the light was out and the extinction component was in effect.

(b) A mixed FR 20 EXT with a random interpolated stimulus change (MIX). In the MIX condition, reinforcement was programmed by the same two schedules (FR 20 and EXT) alternating at random with no stimuli correlated with the onset of either. In this condition the session light was randomly lit and not lit 50 percent of the time during each schedule component. This animal received the same quantity of schedule change (30 min FR 20 and 30 min EXT) and stimulus change (30 min of light and 30 min of no light) as the MULT animal, but the stimulus change was not correlated with the schedule change. The animal exposed to this condition had no cue to which protion of the schedule was present and, therefore, did not develop a discrimination and responded at a high rate during both components (FR 20 and EXT).

(c) FR 20 stimulus change (FR 20 Stimulus). In this condition the session light was lit 2 min and extinguished 2 min in the same alternating sequence as in the MULT condition, however there was no schedule change. The stimulus had no consequence and thus was novel.

(d) FR 20. This animal was exposed to no change from the original training procedure and served as comparison for the effects of the baseline training condition.

These four training conditions were used to separate and measure the neurochemical effects of the component parts of the total training task (MULT minus FR 20). In each litter the effect of the stimulus change was estimated by comparing the FR 20 stimulus animal with the FR 20 animal. The effect of the contingency change (schedule change plus the discrimination in responding) was estimated by comparing the MULT animal with the FR 20 stimulus animal since both were exposed to the same stimulus change. The effect of the discrimination was estimated by comparing the MULT with the MIX animal since both were exposed to the same amount of physical stimulus and schedule change.

The differences in the pattern of responding to the stimulus and training conditions were quantified by computing response rate ratios (RRR) for the animals. Fifteen RRR's were computed for 4 min intervals of the 60 min training session as follows: (1) MULT RRR equals $\frac{\text{responses during 2 min light}}{\text{responses during 2 min no-light}}$ which in this condition equals $\frac{\text{responses during 2 min FR 20}}{\text{responses during 2 min EXT.}}$ (2) MIX RRR equals $\frac{\text{responses during 2 min light}}{\text{responses during 2 min no-light}}$ for the stimulus. (3) MIX RRR equals $\frac{\text{responses during 2 min FR 20}}{\text{responses during 2 min EXT}}$ for the contingency. (4) FR 20 Stimulus RRR equals $\frac{\text{responses during 2 min light}}{\text{responses during 2 min no-light.}}$ (5) FR 20 RRR equals $\frac{\text{responses during 2 min}}{\text{responses during previous 2 min.}}$

Immediately after the 60 min training condition, the animals were sacrificed by immersion in Freon 12 cooled in liquid nitrogen (-155°C). Stainless steel tubes (12 x 2½ in.) were used both for transporting devices during training, and as freezing tubes that could be easily lowered into a Dewar flask of Freon 12. In this manner stress effects of the sacrifice procedure were minimized. The brains were removed without being allowed to warm in a vise partially submerged in liquid nitrogen with chisels that were cooled in liquid nitrogen. The cerebellum and olfactory bulbs were

discarded and the brains stored in liquid nitrogen (-197°C) until extraction and analysis. A modification of a previously reported procedure [29,30] was used for the isolation of cell fractions and the extraction of cytoplasmic and nuclear RNA.

Biochemical

Isolation of cellular fractions. The brains were pulverized in a pulverizer cooled in liquid nitrogen (-197°C), and homogenized with a Dounce homogenizer on ice in a hypotonic buffer, RSB-PVS-DEP (0.01 M Tris-HCl, 0.01 M NaCl, 0.01 M EDTA, 0.0015 M $MgCl_2 \cdot 6H_20$, 0.01% polyvinyl sulfate (PVS), 0.05% diethyl procarbonate (DEP), pH 7.4). (All manipulations prior to the phenol stage were carried out at 4°C). The homogenate was placed in a 35 ml disposable plastic syringe and passed through two Swinney adapters (13 mm dia.) in tandem, each containing five 100 mesh stainless steel screens and then through three Swinney adapters in tandem, each containing eleven 170 mesh stainless steel screens to obtain a high degree of cell breakage. At this point a 0.1 ml aliquot was taken for determination of total radioactivity in the homogenate. The nuclei were separated from the cytoplasmic fraction by centrifugation at 1600 x g for 5 min. The supernatant solution (cytoplasmic fraction) was decanted into a tube containing 2 volumes of 95 percent ethanol. The nuclei were then washed by resuspension in 4 ml of RSB-PVS-DEP and redeposited by centrifugation and the supernatant combined with the cytoplasmic fraction. This washing of the nuclei was repeated twice more, each time combining the supernatant with the cytoplasmic fraction. The nuclear pellet was resuspended in 4 ml of RSB-PVS-DEP, 0.6 ml of the following mixture was added (one part of a 10 percent w/v solution of the ionic detergent sodium deoxycholate in water and two parts of 10 percent v/v solution of the nonionic detergent Tween 40, polyoxyethylene sorbital monopalmitate, in water) and the mixture shaken for 10 sec. Immedaitely, 10 ml of RSB-PVS-DEP was added, the nuclei deposited by centrifugation, and the supernate added to the cytoplasmic fraction.

Digestion of the nuclei. The nuclei were resuspended in 2 ml of a high salt buffer, HSB (0.5 M NaCl, 0.05 M $MgCl_2 \cdot 6H_20$, 0.1 M Tris-HCl, pH 7.4), 200 μg of electrophoretically purified DNase was added and the mixture stirred vigorously at 37°C for 1 min. Immedaitely the mixture was brought to 0.05 percent DEP and 0.01 percent PVS concentration, 0.4 ml of a 0.5M EDTA–pH 7.4 buffer and 0.1 ml of a 25 percent SDS solution were added and the mixture mixed for 2 min. Immediately, 3 ml of a 88 percent freshly redistilled phenol solution was added and mixed for 1 minute.

Treatment of cytoplasmic fraction. The cytoplasmic fraction was centrifuged at 5000 x g for 10 min. The supernate was decanted and the pellet resuspended in 3 ml of resuspension buffer (0.01 M Tris, 0.01 M NaCl, 0.001 M EDTA, 0.01 percent PVS, 0.05 percent DEP, pH 7.4), 0.1 ml of 25 percent SDS and 0.6 ml of 0.5 M EDTA pH 7.4 was added and the mixture stirred at 30°C for 1 min. Immediately, 3 ml of 88 percent phenol was added and the solution mixed for 1 min.

Extraction of the RNA. The nuclear and cytoplasmic fractions were heated to 65°C for 2 min with continual stirring and then mixed for 1 min with a vortex mixer; 3 ml of chloroform-1 percent isoamyl alcohol were added and the mixture again heated to 65°C for 2 min with continual stirring. The solution was mixed 1 min with a vortex mixer, the phases separated by centrifugation at 2000 rpm for 2 min at room temperature in a clinical centrifuge, and the organic layer (bottom) removed and discarded. Three more extractions were performed with chloroform–1 percent isoamyl alcohol alone at room temperature. The final purified aqueous phase (upper) was removed and 0.5 ml of 5X RSB buffer (2.5 M NaCl, 0.25 M $MgCl_2 \cdot 6H_20$, 0.5 M Tris-HCl, pH 7.4) was added along with two volumes of 95 percent ethanol. The RNA was allowed to precipitate overnight at -20°C. The RNA was collected by centrifugation at 27,000 x g for 10 min at 4°C. The supernate was decanted and the RNA dissolved in 0.2 ml of sample buffer (80 percent 0.12 M Tris-HCl, 0.06 M sodium acetate, 0.006 M EDTA, pH 7.4; and 20 percent glycerol).

Quantification of RNA and Determination of Radioactivity. The RNA concentration was determined with a modified micro-orcinol procedure [25]. Aliquots of the homogenate and RNA solutions were counted for radioactivity in a Beckman LS-200 liquid scintillation counter using a TLA (Beckman) 70 percent toluene–30 percent absolute methanol scintillation cocktail and the efficiency of counting determined from a quench curve corrected by automatic external standardization (A. E. S.).

RESULTS

Behavioral

The cumulative records for one group of littermates on the FR 20 training schedule the day before sacrifice and the day of the 60 min treatment procedure are shown in Fig. 1. The patterns of responding to the stimulus change is different in the MULT condition from the MIX and FR 20 stimulus conditions. This is evident in Fig. 2 which is a plot of the response rate ratios. As the session progresses, the stimulus develops control of lever pressing behavior in the animal exposed to the MULT schedule as is shown by the large RRRs. The stimulus develops no control over lever pressing behavior in the animals in the MIX and FR 20 stimulus conditions which is demonstrated by the RRR's of 1.0 for these animals.

A paired t-analysis of the RRRs for the 9 groups of littermates shows that the RRRs for the MULT animals differ significantly from the MIX and the FR 20 stimulus animals in the last 8 minutes of the 60 minute contingency exposure (MULT vs. MIX: paired $t = 3.16$, df = 2, $p = 0.05$; MULT vs. FR 20 Stimulus: paired $t = 3.79$, df = 2, $p = 0.03$). Log transformations were used to normalize the data and one tailed t-tests were performed since apriori predictions of direction were possible. The pattern of responding of the MULT group is different than the pattern of responding of the MIX and FR 20 stimulus groups; the stimulus developed control over the MULT aminals lever pressing behavior.

Biochemical

DPM per mg of brain tissue in the homogenate. The level of radioactivity in the homogenate of the trained animals is shown in Fig. 3A and Table 1. The amount of radioactivity in the homogenate is related to the complexity of the training task. The MULT animals were exposed to a stimulus change, a schedule change, and developed a discrimination in responding, and thus showed the highest level of uptake

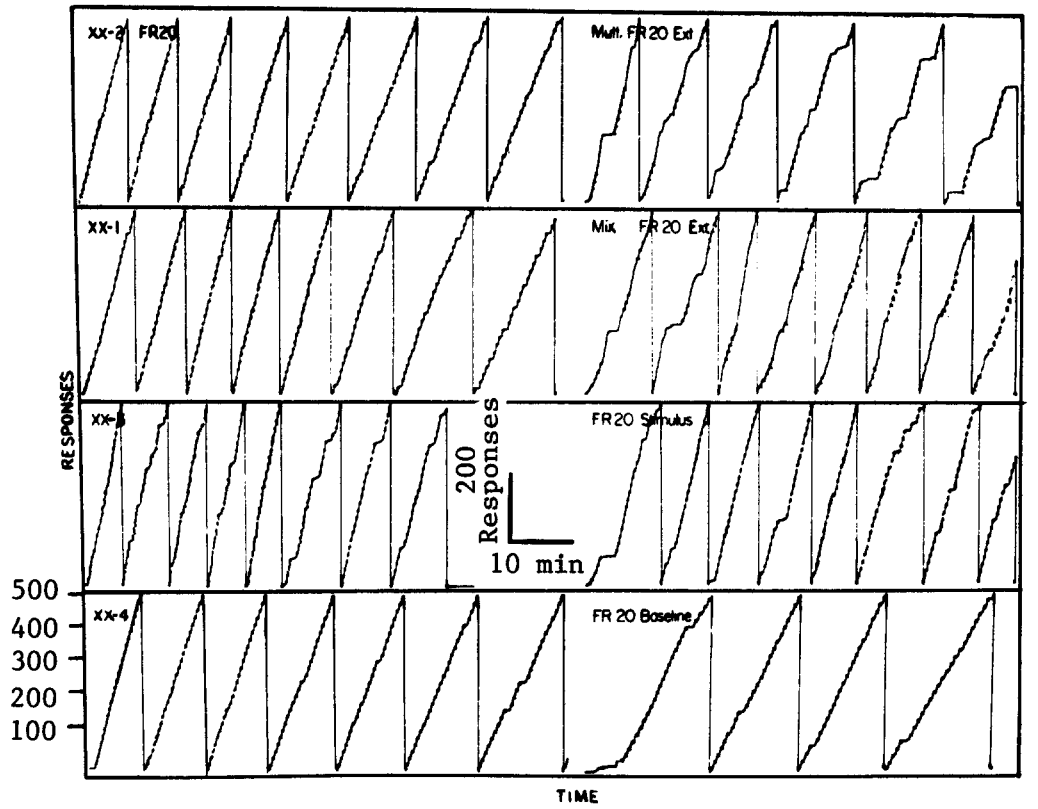

FIG. 1. Cumulative records of lever pressing behavior the day before (left) and the day of exposure (right) to the 60 min training session for one group of littermates.

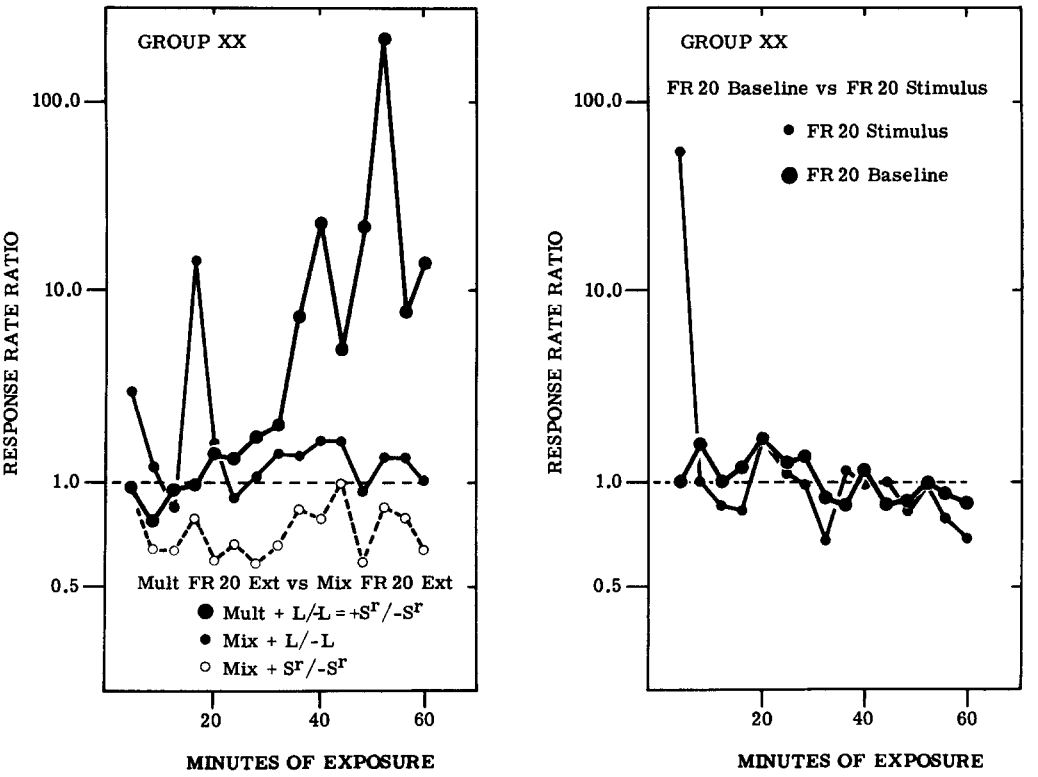

FIG. 2. Response rate ratios for the 60 minute training session for the group of littermates from Fig. 1. (L^+ – stimulus light on; L^- = stimulus light off; + S^r = with food = FR 20, and $-S^r$ = with no food = EXT).

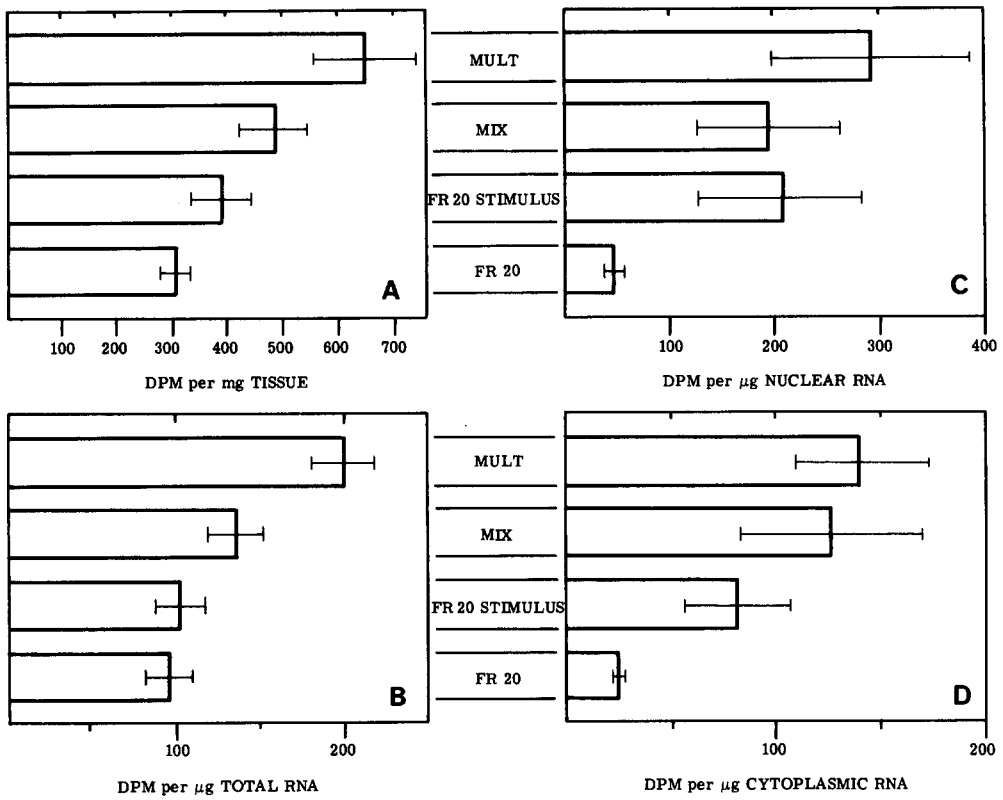

FIG. 3. DPM in brain tissue and brain RNA of trained littermates (bars represent standard error of the mean). (A) DPM per mg brain tissue in the homogenate (n = 8). (B) DPM per μg of total RNA per gram of tissue (n = 9). (C) DPM per μg of nuclear RNA (n = 6). (D) DPM per μg of cytoplasmic RNA (n = 6).

TABLE 1

RADIOACTIVITY IN HOMOGENATE, TOTAL, NUCLEAR, AND CYTOPLASMIC RNA OF TRAINED LITTERMATES

	DPM per mg Tissue in Homogenate	DPM per μg Total RNA	DPM per μg Nuclear RNA	DPM per μg Cytoplasmic RNA
MULT	647 ± 188	191 ± 70	288 ± 196	138 ± 66
MIX	481 ± 119	137 ± 54	192 ± 127	128 ± 83
FR 20 Stimulus	387 ± 115	105 ± 49	203 ± 147	81 ± 49
FR 20	306 ± 48	92 ± 45	46 ± 14	23 ± 4
Sample Size	N = 8	N = 9	N = 6	N = 6

Values represent means ± SEM.

of radioactivity into the brain. The MIX group was exposed to only the stimulus and schedule change and did not develop a discrimination in responding, and thus showed the second highest level of uptake. The FR 20 stimulus group received the stimulus change only and showed the third highest uptake. The FR 20 group was exposed to no change from baseline training and showed the lowest uptake. Analysis of the effects of the component parts of the training task on the level of radioactivity in the homogenate showed that exposure to the contingency change produced a significant change in the uptake of ^{3}H-uridine into the brain (p = 0.05) (Table 2).

The total net mean increase in DPM per mg of tissue in the homogenate as a result of the total training task (MULT

TABLE 2

PAIRED t AND p VALUES FOR THE EFFECTS OF THE COMPONENT PARTS OF THE TRAINING TASK ON THE RADIOACTIVITY ABSORBED INTO THE BRAIN AND INCORPORATED INTO TOTAL, NUCLEAR, AND CYTOPLASMIC RNA

	DPM per mg Tissue in Homogenate	DPM per μg Total RNA	Percent Total Radioactivity Incorporated into RNA	DPM per μg Nuclear RNA	DPM per μg Cytoplasmic RNA
Stimulus	$t = 0.55$	$t = 0.018$	$t = 0.72$	$t = 1.14$	$t = 1.25$
(FR 20 Stimulus vs FR 20)	$p = 0.30$	$p = 0.43$	$p = 0.24$	$p = 0.15$	$p = 0.14$
Contingency	$t = 1.92$	$t = 2.08$	$t = 2.66$	$t = 1.70$	$t = 2.10$
(MULT vs FR 20 Stimulus)	$p = 0.05$*	$p = 0.04$*	$p = 0.01$*	$p = 0.08$	$p = 0.05$*
Discrimination	$t = 1.05$	$t = 1.47$	$t = 2.28$	$t = 0.33$	$t = 0.33$
(MULT vs MIX)	$p = 0.17$	$p = 0.09$	$p = 0.03$*	$p = 0.12$	$p = 0.38$
Sample Size	N = 8	N = 9	N = 8	N = 6	N = 6

One-tailed t tests were performed since previous data [1, 2, 5, 6, 8, 10, 11, 22, 23, 36, 38, 39] allowed an apriori prediction of the direction of change.

*p values statistically significant

minus FR 20) was separated into the amount that each component part (discrimination, schedule change, and stimulus change) contributed as follows: (1) stimulus change equals FR 20 stimulus minus FR 20. (2) discrimination equals MULT minus MIX. (3) Schedule Change equals MULT minus FR 20 Stimulus minus Discrimination.

A percentage of the total increase (MULT minus FR 20) was computed for each component by dividing by the total increase. By computing the contribution of the component parts to the total training task as outlined above, it was found that the discrimination accounted for 49 percent (166 DPM/mg), the schedule change 28 percent (94 DPM/mg), and the stimulus change 23 percent (81 DPM/mg) of the total net increase (341 DPM/mg) in DPM per mg of tissue in the homogenate.

DPM per μg of total RNA. The specific activities of total RNA for the trained animals is shown in Fig. 3B. The MULT and the MIX groups have more highly labelled RNA than the other two groups (Table 1). The MULT and the MIX groups are the only groups that have been exposed to a schedule change (EXT) from baseline training (FR 20).

Analysis of the effects of the component parts of the training task on the specific activity of total RNA showed that the animals exposed to the MULT incorporate more label into brain RNA than animals exposed to the stimulus only ($p = 0.04$) (Table 2).

Of the total net mean increase in DPM per μg of RNA (99 DPM/μg) the discrimination accounts for 55 percent (55 DPM/μg), the schedule change 33 percent (32 DPM/μg) and the stimulus change 12 percent (12 DPM/μg).

Percent of total radioactivity incorporated into RNA. The MULT group incorporated 2 percent of the available radioactivity into RNA while the other groups incorporated less (MIX 0.9 percent, FR 20 Stimulus 1.0 percent, FR 20 1.4 percent). The largest effects are the contingency change ($p = 0.01$) and the discrimination ($p = 0.03$). The observed changes in specific activity of RNA from the brains of the trained animals cannot simply be attributed to differences in the availability of radioactive precursor. The behavioral manipulations appear to effect the rate of incorporation of ^{3}H-uridine into RNA.

DPM per μg of nuclear RNA. A large body of data indicates that ribosomal and messenger RNA are synthesized in the nucleus and transported to the cytoplasm [12]. A species of RNA called heterogeneous nuclear RNA (Hn RNA) (because of its heterogeneous sedimenting characteristics), has been characterized [30]. Hn RNA is synthesized and found in the nucleus and is though to have some regulator function. All of these RNA species (transfer, ribosomal, messenger, and heterogenous nuclear) have different functions that may vary with changes in physiological states. Changes in the rate of incorporation of radioactivity into nuclear and cytoplasmic RNA may indicate changes in these different RNAs.

The MULT group has more radioactivity in the nuclear RNA fraction than the other groups (Fig. 3C and Table 1). The MIX and FR 20 Stimulus groups have very similar levels while the FR 20 group has the least. Analysis of the effects of the component parts of the training task on the specific activity of nuclear RNA showed that there is no significant change in the rate of incorporation of radioactivity as a result of the training tasks (Table 2).

DPM per μg of Cytoplasmic RNA. The MULT and the MIX groups incorporate more radioactivity into cytoplasmic RNA than the FR 20 Stimulus or the FR 20 groups (see Fig. 3D and Table 1). Analysis of the effects of the component parts of the training tasks on the specific activity of cytoplasmic RNA showed that animals exposed to a

contingency change with a correlated stimulus (MULT) incorporate more radioactivity into cytoplasmic RNA than animals exposed to the stimulus change alone (FR 20 Stimulus) ($p = 0.05$) (Table 2).

DISCUSSION

A training task has several components, some of which are (a) a stimulus component, (b) a motor component, and (c) a central component that relates to the probability of that behavior occurring again. A rat that learns to jump to a ledge to avoid shock emits a motor response to a stimulus in the environment. The motor response and the stimulus components of the training task are linked together somehow so that the probability of that response occurring again to that stimulus increases. In a training situation there are physical stimuli in the environment (visual, auditory, tactile, olfactory, and visceral) that impinge upon the behaving organism. These stimuli alone may cause neurochemical changes [35]. In a training task, a motor response is usually required of the behaving organism which may alone cause neurochemical changes [18]. It has been postulated and widely accepted that the learning process involves central phenomena other than the stimulus and motor components of a training task [15]. In training tasks that have been used to study neurochemical correlates of behavior, the differences between trained groups and comparison groups have been attributed to the specific effect of learning when actually it is the sum of all the non-specific effects (stimulus and motor components) and the specific effects (learning). Increases in the rate of RNA synthesis have never been demonstrated for these specific factors separate from the non-specific factors.

This study used operant techniques to separate and measure the magnitude of effect some of these non-specific factors in a training task have on the rate of incorporation of ^{3}H-uridine into brain RNA of rats exposed to different amounts of the training task. The training procedure allowed the measurement of the component parts of the training task. Each animal within each experimental group (litter) entered the 60 minute training session with equivalent genetic, environmental, and training backgrounds so that observed changes could be attributed to the 60 minute training session. The animals exposed to the FR 20 baseline schedule received no change from baseline training and therefore the incorporated radioactivity should represent the motor and baseline training effects. The FR 20 group consistently ranks fourth in most measures of the rate of incorporation of ^{3}H-uridine into brain RNA. The animals in the FR 20 Stimulus condition were exposed to only the stimulus change from the baseline training procedure. The difference in the incorporation of radioactivity between the FR 20 and the FR 20 Stimulus groups should represent the effects of the stimulus change alone. The animals in the MIX condition were exposed to the same amount of total stimulus change (30 minutes light and 30 minutes no light) and total contingency change (30 minutes FR 20 and 30 minutes Extinction) as the animals exposed to the MULT schedule. The only difference between these two groups was the stimulus, which in the MULT condition was correlated with the schedule change (FR 20-Extinction) but was not in the MIX condition. The MIX animals had no cue as to which portion of the schedule was in effect and therefore did not develop a discrimination in responding like the MULT animals. To the MULT animals the stimulus change was a cue as to which portion of the schedule was in effect. As a result, the animals in this condition responded at a high rate during FR 20 and did not respond (or responded at a low rate) during the EXT component. The MULT group consistently ranks first in all measures of the rate of incorporation of ^{3}H-uridine into brain RNA.

The design of this experiment has allowed the measurement of the contribution of some of the component parts of the training task to the observed increase in the incorporation of radioactivity into the brain and brain RNA. The results indicate that the component parts of a training task have neurochemical consequences and that observed differences between groups represent the sum total of stimulation, motor, and learning differences. The distribution of ^{3}H-uridine into nuclear and cytoplasmic RNA species and into brain areas using the techniques of polyacrylamide gel electrophoresis and high resolution autoradiography have been studied in animals trained with the same methods set forth above.

REFERENCES

1. Adair, L., J. Wilson and E. Glassman. Brain function and macromolecules. IV. Uridine incorporation into polysomes of mouse brain during different behavioral experiences. *Proc. Natn Acad. Sci. U.S.A.* **61**: 917–922, 1968.
2. Adair, L., J. Wilson, J. Zemp and E. Glassman. Brain function and macromolecules. III. Uridine incorporation into polysomes of mouse brain during short-term avoidance conditioning. *Proc. Natn Acad. Sci. U.S.A.* **61**: 606–613, 1968.
3. Altman, J. Differences in the utilization of ^{3}H-leucine by single neurons in normal and exercised rats: an atuoradiographic investigation with microdensiometry. *Nature* **199**: 777–780, 1963.
4. Altschuler, H., M. Kleben, M. Gold, M. Lawton and M. Miller. Neurochemical changes in the brain of albino rats as related to aversive training. *J. Biol. Psychol.* **9**: 33–38, 1969.
5. Bateson, P., G. Horn and S. Rose. Effects of early experience on regional incorporation of precursors into RNA and protein in the chick brain. *Brain Res.* **39**: 449–465, 1972.
6. Beach, G., M. Emmens, D. Kimble, and M. Lickey. Autoradiographic demonstration of biochemical changes in the limbic system during avoidance training. *Proc. Natn Acad. Sci. U.S.A.* **62**: 692–696, 1969.
7. Bogoch, S. *The Biochemistry of Memory*, New York: Oxford University Press, 1968.
8. Bowman, R. and D. Strobel. Brain RNA metabolism in rat during learning. J. comp. physiol. Psychol. **67**: 448–456, 1969.
9. Campagnoni, A., G. Dutton, H. Mahler and W. Moore. Fractionation of the RNA components of rat brain polysomes. *J. Neurochem.* **18**: 601–611, 1971.
10. Coleman, M., B. Pfingst, J. Wilson, and E. Glassman. Brain function and macromolecules. VIII. Uridine incorporation into polysomes of hypophysectomized rats and ovarectomized mice during avoidance conditioning. *Brain Res.* **26**: 349–360, 1971.
11. Coleman, M., J. Wilson and E. Glassman. Brain function and macromolecules. VII. Uridine incorporation into polysomes of mouse brain during extinction. *Nature* **229**: 54–55, 1971.
12. Darnell, J. RNA from animal cells. *Bact. Rev.* **32**: 262–290, 1968.
13. Dellweg, H., R. Gerner, and A. Walker. Quantitative and qualitative changes in RNA of rat brain dependent on age and training experiments. *J. Neurochem.* **15**: 1109–1119, 1968.
14. Ferster, C. and B. Skinner. Schedules of Reinforcement. New York: Appleton-Century-Crofts, 1957.

15. Geller, A. and M. Jarvik. The role of consolidation in memory. In: *Biochemistry of Brain and Behavior,* edited by R. Bowman New York: Plenum Press, 1970, pp. 245.
16. Gold, M., M. Miller, H. Altschuler, M. Kleban and M. Lawton. The relationship of acid-soluble nitrogen in rat brain to avoidance behavior. *Psychon. Sci.* **20:** 13–14, 1970.
17. Haljamae, H. and P. Lange. Calcium content and conformational changes of S-100 protein in the hippocampus during training. *Brain Res.* **38:** 131–142, 1972.
18. Hyden, H. Biochemical and functional interplay between neuron and glia. In: *Recent Advances in Biological Psychiatry,* edited by J. Wortis. New York: Plenum Press, 1964, pp. 31.
19. Hyden, H. Biochemical and molecular aspects of learning and memory. *Proc. Am. Phil. Soc.* **3:** 326–342, 1967.
20. Hyden, H. and P. Lange. S-100 brain protein: Correlation with behavior. *Proc. Natn Acad. Sci. U.S.A.* **67:** 1959–1966, 1970.
21. Hyden, H. and P. Lange. Protein synthesis in hippocampal nerve cells during reversal of handedness in rats. *Brain Res.* **45:** 314–317, 1972.
22. Kahan, B., M. Krigman, J. Wilson and E. Glassman. Brain function and macromolecules. VI. Autoradiographic analysis of the effect of a brief training experience on the incorporation of uridine into mouse brain. *Proc. Natn Acad. Sci. U.S.A.* **65:** 300–303, 1970.
23. Kottler, P., R. Bowman and W. Haasch. RNA metabolism in the rat brain during learning following intravenous and intraventricular injections of ^{3}H-cytidine. *Physiol. Behav.* **8:** 291–297, 1972.
24. Levitan, I., G. Ramirez and W. Mushynski. Amino acid incorporation in the brains of rats trained to use the non-preferred paw in retrieving food. *Brain Res.* **47:** 147–156, 1972.
25. Lin, R. and O. Schjeide. Micro estimation of RNA by the cupric ion catalyzed orcinol reaction. *Analyt. Biochem.* **27:** 473–483, 1969.
26. Mandel, P. and M. Jacob. Regulation of transcription in nerve cells. In: *Protein Metabolism of the Nervous System,* edited by A. Lajtha, New York: Plenum Press, 1970, pp. 129.
27. Marichich, E. and I. Isquierdo. The dependence of hippocampal RNA levels on the frequency of afferent stimulation. *Naturwissenschaften* **7:** 254, 1970.
28. Nasello, A. and I. Isquierdo. Effect of learning and of drugs on the RNA concentration of brain structures of the rat. *Expl. Neurol.* **23:** 521–528, 1969.
29. Penman, S. RNA metabolism in the He Le cell nucleus. *J. molec. Biol.* **17:** 117–130, 1966.
30. Penman, S., C. Vesco and M. Penman. Localization and kinetics of formation of nuclear heterodesperse RNA, cytoplasmic heterodesperse RNA and polyribosome associated messenger RNA in He Le cells. *J. molec. Biol.* **34:** 49–69, 1968.
31. Pohle, W. and H. Mathies. The incorporation of ^{3}H-uridine monophosphate into the rat brain during the training period. A microautoradiographic study. *Brain Res.* **29:** 123–127, 1971.
32. Quevedo, J., P. Bosque, M. Andres and M. Garcia. Cytoplasmic RNA in the rat hippocampus after a learning experience, an autoradiographic study. *Acta anat.* **79:** 360–366, 1971.
33. Rapport, D. and A. Daginalol. Changes in nRNA of brain induced by olfaction in catfish. *J. Neurochem.* **15:** 991–1000, 1968.
34. Reinis, S. Autoradiographic study of ^{3}H-thymidine incorporation into brain DNA during learning. *Physiol. Chem. Phys.* **4:** 391–398, 1972.
35. Rose, S. Changes in visual cortex on first exposure of rats to light. Effects on incorporation of ^{3}H-lysine into protein. *Nature* **215:** 253–255, 1967.
36. Shashoua, V. RNA metabolism in goldfish brain during acquisition of new behavioral patterns. *Proc. Natn Acad. Sci. U.S.A.* **65:** 1, 160–167, 1970.
37. Singh, V. and G. Talwar. Identification of protein fraction in the occipital cortex of the monkey rapidly labelled during the exposure of animal to rhythmically flickering light. *J. Neurochem.* **16:** 951–959, 1969.
38. Zemp, J., J. Wilson, K. Schlesinger, W. Boggan and E. Glassman. Brain function and macromolecules. I. Incorporation of uridine into RNA of mouse brain during short-term training experiences. *Proc. Natn Acad. Sci. U.S.A.* **55:** 1423–1431, 1966.
39. Zemp, J., J. Wilson and E. Glassman. Brain function and macromolecules. II. Site of increased labelling of RNA in brains of mice during a short-term training experience. *Proc. Natn Acad. Sci. U.S.A.* **58:** 1120–1125, 1967.