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Effects of a Novel Experience on Rat Brain Chromatin[1,2]

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TEDESCHI, B. AND L. UPHOUSE. *Effects of a novel experience on rat brain chromatin.* PHARMAC. BIOCHEM. BEHAV. 11(3) 253–258, 1979.—In this study adult Fischer, inbred rats experienced (1) training to avoid footshock (2) unavoidable footshock or (3) no training or footshock. Each animal was sacrificed at one of several time points (1–60 hours) following experience. Brain chromatin was extracted and used as template for RNA synthesis in vitro. Both groups which received the novel experience demonstrated greater template activity than the unshocked, untrained groups. This effect was brief. The two groups which received the experience did not differ from each other. These results suggest that a brief, novel experience can temporarily alter the transcriptional activity of brain chromatin.

Footshock Brain chromatin RNA synthesis in vitro Macromolecular changes in CNS

IT IS now becoming clear that macromolecular responses in the mammalian central nervous system (CNS) can be influenced by experience. Various kinds of experiential events, which presumably alter the functional activity of the CNS, have been found to correlate with the relative level or turnover of RNA and/or protein in the brain [8, 11, 25]. Recent studies suggest that some of these macromolecular changes may result from altered transcription of the CNS genome. Using DNA-RNA hybridization techniques, both Uphouse and Bonner [24] and Grouse *et al.* [12] demonstrated that whole brain RNA from rats reared in an enriched environment hybridized to more single-copy DNA than did RNA from rats reared in a standard colony environment. When chromatin was examined for its capacity to support in vitro RNA synthesis, it was found that differential rearing altered this capacity in both whole brain [26] and parts of brain [23]. Possible transcriptional responses of the CNS to other environmental challenges, e.g., drug administration, have also been reported. Hodgson *et al.* [13] found that morphine treatment and the development of tolerance to morphine were correlated with a decrease in the template activity of brain chromatin.

Although each of these studies provide evidence that the CNS chromatin is responsive to experiential events, the study of transcriptional changes has been restricted to relatively long-term environmental challenges. It is difficult to compare these findings to the larger literature of macromolecular changes following acute experiential events. Shock avoidance has been found to elicit changes in brain nuclear proteins [18,19]. Since nuclear chromosomal proteins and phosphoproteins have been implicated as regulators of gene expression [9], experiential modifications of these cellular components may be indicative of changes in brain transcription.

In the following experiment, we have examined the effect of brief experiences on the ability of rat brain chromatin to support in vitro RNA synthesis.

METHOD

Animals

Seventy-five, male, Fischer inbred rats, purchased from Charles Rivers Laboratories (North Wilmington, Massachusetts) at 60 days of age, were used in the experiment. Immediately upon arrival in the laboratory, the animals were housed 4 to 6 per cage in stainless steel cages (62×33×18 cm) with Purina Laboratory Chow and water available ad lib. The colony room, maintained at 25.5°C, was kept on a 12–12 light-dark cycle with lights off from 12 p.m. to 12 a.m. All animals were used within three weeks of their initial arrival but were given at least one week to adjust to the housing conditions before experimenter intervention.

Materials

(5-^{3}H) Uridine 5′-triphosphate (22.7 Ci/mM) was purchased from New England Nuclear. RNase free sucrose was purchased from Schwartz-Mann and nitrocellulose membrane filters (0.45 μm) were purchased from Schleicher and Schuell. All other chemicals were obtained from Sigma Chemical Company.

Behavioral Procedures

All behavioral procedures took place between 1 p.m. and 5 p.m. during the colony room dark cycle. At the start of each procedural day, three rats were assigned to either trained, shocked or quiet conditions and were individually

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housed in clear plastic cages (41×21×19.5 cm) for transport to the experimental room.

Trained and shocked rats received experience in a training apparatus similar to that described by Machlus *et al.* [19]. The apparatus, a straight runway (38.7 cm long, 15 cm wide, 54.2 cm high), constructed of black Plexiglas, contained a grid floor made of 3/32 in. brass rods, the centers of which were 8.5 mm apart. The grid floor was connected to a shock generator and scrambler from which shock could be administered. At one end of the apparatus, the start box (15 cm long, 11 cm wide, 54.2 cm high) was partitioned from the remainder of the runway by a black, Plexiglas trap door. At the other end of the runway, a safety platform (29.5 cm long, 13.7 cm wide, 38 cm high) made of white Plexiglas, was elevated 8 cm above the grid floor.

In the training condition, an animal was removed from the transport cage, lowered onto the grid floor of the runway with the trap door raised, and allowed 5 min to explore the apparatus. Training immediately followed this exploration period. On the first training trial, the rat was lowered by the tail into the start box with the trap door closed. The door was immediately opened and 5 sec later a 1.0 mA footshock was delivered. The rat could avoid the shock by traversing the runway and stepping onto the safety platform within the 5 sec period. If the rat did not avoid the shock, footshock was continued until the rat stepped onto the safety platform or until 15 sec of shock had elapsed. If a rat did not escape the shock within the 15 sec period, then the animal was lifted by the tail and placed onto the safety platform. Each trial lasted 30 sec from the opening of the trap door. If, during this time, a rat stepped back onto the runway grid from the safety platform, it received footshock until it remounted the platform, but in no case was a rat allowed to experience more than 15 sec of consecutive shock. At the end of each trial, the rat was picked up by the tail and placed into the start box for the next trial. Total time of training was 10 min for each animal, but most rats acquired the response within 3 trials.

In the shock condition, an animal received the same handling and shock treatment as a comparison trained animal, but the safety platform was turned around so that shocked animals were unable to either escape or avoid the shock.

In the quiet condition, an animal was placed into the carrying cage and transported to the experimental room. However, the animal received no experience in the training apparatus.

After both the shocked and trained animals received experience in the apparatus, all three animals were returned to the colony room where they remained individually housed until the time of sacrifice. The three animals were later sacrificed in random order within 15 min of each other and remained matched throughout the remaining procedures. Each matched triplet was sacrificed by decapitation at either 1, 24, 36, 48, or 60 hr following termination of experience in the runway. Brains were removed, quick-frozen in liquid nitrogen, and stored at −85°C until chromatin extraction.

Biochemical Procedures

Preparation and analysis of chromatin. Chromatin was extracted from brain tissue according to the method of Bonner *et al.* [2] as modified for brain by Uphouse and Moore [26]. The purified chromatin pellet was dissolved in 10 mM tris-HCl, pH 8.0 and dialyzed overnight against the same buffer. Chromatin was prepared in matched triplets so that trained, shocked, and quiet groups were simultaneously prepared. All later comparisons of chromatin were made among the matched triplets.

DNA and protein in chromatin were determined, respectively, by the method of Burton [4] with calf thymus DNA as standard and the method of Lowry *et al.* [17] with bovine serum albumin (BSA) as standard.

Assay for in vitro RNA synthesis by chromatin. Assay of RNA synthesis in vitro was similar to that described by Uphouse and Moore [26]. After dialysis, chromatin absorbance in 10 mM Tris-HCl, pH 8.0, was measured at 230, 260, and 280 nm with a Varian Techtron Model 635 spectrophotometer and quartz cuvettes of 1 cm pathlength. Each chromatin sample was diluted with Tris buffer to a final concentration of 2.0 ± 0.5 absorbance units at 260 nm per ml (A_{260}/ml). Chromatin concentrations were determined in 9 volumes of 1 N NaOH. In vitro RNA synthesis took place in the following reaction mixture with a final volume of 0.26 ml: 44 mM Tris-HCl, pH 8.0; 10 mM $MgCl_2$; 140 mM KCl; 0.8 mM ATP, CTP, and GTP each; 3.6 μM (^{3}H) UTP; 11% glycerol; 2.5 units of *Escherichia coli* RNA polymerase (R-0501, Type II); and varying amounts (50–150 μl) of chromatin. Samples were incubated for 20 min at 37°C. The reaction was stopped by reduction in temperature to 4°C and precipitation with 100 μl of 50% trichloroacetic acid (TCA). Precipitation of the RNA was facilitated by the addition of 50 μl BSA. The precipitate was collected onto membrane filters, washed with 10% TCA and 95% ethanol, air dried, and counted in 10 ml of Aquasol (New England Nuclear) in a Beckman LS-150 liquid scintillation counter.

Enzyme assays. The contribution of endogenous RNA polymerase to the in vitro RNA synthesis was determined by incubating chromatin in the absence of *E. coli* RNA polymerase.

The possibility of differential RNase activity was determined in the following manner: Chromatin samples were incubated as in the standard assay procedure except the reaction was not stopped at the end of 20 min. At this point, an excess of unlabeled UTP (10 mM) was added to the samples and incubation was continued for an additional 20 min. The acid-precipitable cpm at the end of this 40-min incubation were then compared to the cpm obtained without the unlabeled UTP chase.

The possible inhibition of *E. coli* RNA polymerase by chromatin was measured by incubating liver DNA with brain chromatin. DNA was extracted from nonexperimental rat liver as described by Uphouse and Moore [26]. Acid-precipitable cpm obtained from the combined chromatin and DNA samples were then compared with the cpm obtained from the incubation of liver DNA samples alone.

Statistical analyses. Although varying amounts (50–150 μl) of chromatin, containing 3.2 to 9.6 μg of DNA, were used to assure linearity of each assay for in vitro RNA synthesis, only the 150 μl aliquots were used for statistical comparisons. For each chromatin preparation, 150 μl chromatin aliquots were assayed at least in triplicate and the mean cpm/150 μl chromatin obtained. These values were then compared across the three treatment groups (trained, shocked, quiet).

A two-factor repeated measures ANOVA was performed on the data to compare the three treatment groups at the five post-experience time points. Since the absolute cpm varied from day to day, and the matched triplet comparisons were made within each day, days were parceled out as a repeated factor. Post hoc comparisons between specific treatment group means were made with the Newman-Keuls'

test [16]. Comparisons within groups across time were hampered by the day-to-day variability in cpm. The way in which treatment groups may have changed relative to one another across time post-experience was examined by the use of ratios of the raw data. Within each triplet comparison, trained/quiet (T/Q) and shocked/quiet (S/Q) cpm ratios were calculated. A trend analysis was then performed on these ratios [16].

RESULTS

Physical Characteristics of Brain Chromatin

The A_{230}/A_{260} ratio (mean=1.12 ± 0.09, N=25), A_{280}/A_{260} ratio (mean=0.58 ± 0.03, N=25) and protein/DNA ratio (mean=3.4 ± 0.67, N=10) are similar to that previously reported for adult brain chromatin [3,26]. Table 1 shows how the treatment groups compared on these measurements at 48 hr postexperience. No differences between any of the treatment groups were found on any of these measures.

Reaction Kinetics and Enzyme Assays

Figure 1 shows that the incorporation of ^{3}H-UTP into acid-insoluble product was linear for the 20 min incubation period. Figure 2 shows that the acid-insoluble incorporation of label was linear over the concentration range used in the experiment.

The incorporation of ^{3}H-UTP into acid-insoluble product was dependent upon the presence of exogenous RNA polymerase. Cpm did not exceed background levels when chromatin was incubated in the absence of *E. coli* RNA polymerase.

In Vitro RNA Synthesis by Brain Chromatin

Figure 3 shows the change in the acid-insoluble cpm of the trained and shocked groups, relative to the quiet group, across the time periods examined. An ANOVA performed on the T/Q and S/Q ratios revealed a significant time effect, $F(4,40)=3.37$, $p<0.05$. A trend analysis showed some tendency for the trained and shocked groups to change, relative to the quiet group, both linearly, $F(1,8)=3.91$, $p<0.1$ and quartically, $F(1,8)=4.00$, $p<0.1$.

Analysis of variance on the raw cpm data revealed a significant experiential condition by time after experience interaction, $F(8,40)=4.92$, $p<0.05$. Analysis of this interaction showed that the difference in cpm between the three treatment groups was only significant at 48 hr postexperience, $F(2,8)=4.92$, $p<0.05$. A post-hoc Newman-Keuls test $\alpha=0.05$) on the treatment group means at this time point showed that the brain chromatin from both trained and shocked animals supported more in vitro RNA synthesis than the brain chromatin from quiet animals. The trained and shocked groups did not differ from one another ($p>0.05$). The effect of footshock experience on brain chromatin 48 hr after experience was a consistent finding across matched triplets. As shown in Table 2, in four out of the five comparisons, the trained group was higher than the quiet group. Similarly, the shocked animal was greater than the quiet in five out of the five matched comparisons.

Thus, the template activity of chromatin from both the trained and shocked animals appears to increase with time, relative to the quiet animals, until 48 hr following the experience. By 60 hr, the template activity is the same for all three groups.

TABLE 1

PHYSICAL CHARACTERISTICS OF BRAIN CHROMATIN AT 48 HR TIME POINT

Physical Measurement	Treatment Group* T	S	Q
$A_{230/260}$	1.12 ± 0.05	1.10 ± 0.05	1.09 ± 0.04
$A_{280/260}$	0.57 ± 0.02	0.58 ± 0.02	0.58 ± 0.03
Protein/DNA	3.39 ± 0.35	3.74 ± 0.70	3.47 ± 0.60

*The mean values were based on 5 preparation samples from each treatment group for both $A_{230/260}$ and $A_{280/260}$ ratios and 4 preparation samples from each treatment group for protein/DNA ratios.

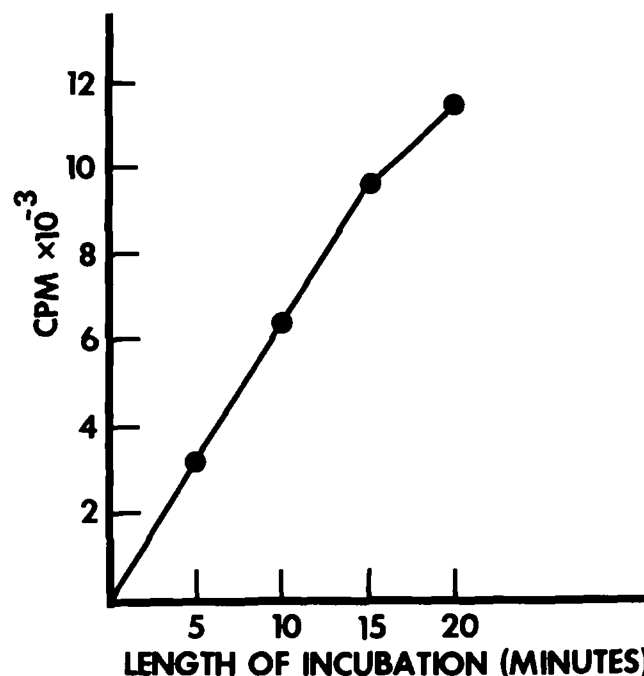

FIG. 1. Incorporation of (^{3}H) UTP into acid precipitable product by brain chromatin. Chromatin, 9.6 μg DNA, was incubated with 2.5 units of *E. coli* RNA polymerase as described in Method. Each point is the mean of two incubations.

It does not appear that the present results are due to differential RNase activity in the chromatin samples. The cpm recovered following the 20 min unlabeled UTP chase did not substantially differ between groups (Trained=93% recovery, Shocked=91% recovery, Quiet=91% recovery). While RNase activity may have been present in the chromatin, it does not appear to have contributed to the observed differences in template activity.

Similarly the results do not appear to be the consequence of differential inhibition of *E. coli* RNA polymerase by chromatin. Were the results due to differential inhibition of polymerase, there should have been a selective reduction in acid-precipitable cpm when liver DNA was incubated in the presence of quiet group chromatin. Table 3 shows that the cpm recovered in the presence of quiet group chromatin was not lower than that recovered in the presence of either trained or shocked group chromatin.

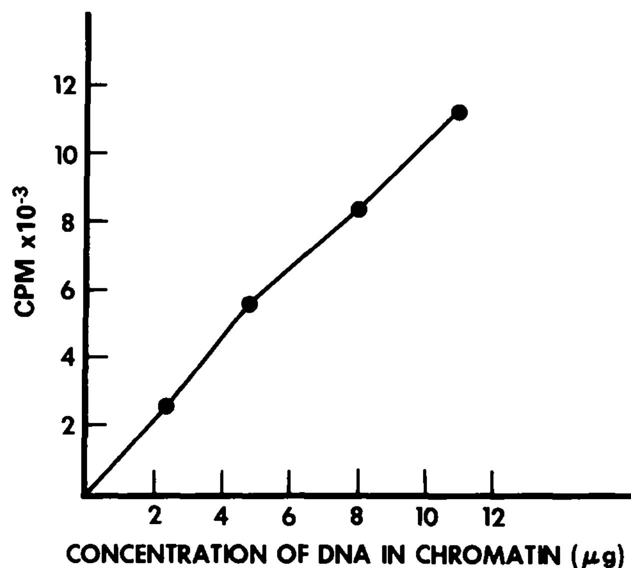

FIG. 2. Incorporation of (^{3}H) UTP into RNA with increasing concentrations of brain chromatin. Chromatin was incubated with 2.5 units of *E. coli* RNA polymerase as described in Method. Each point is the mean of two incubations.

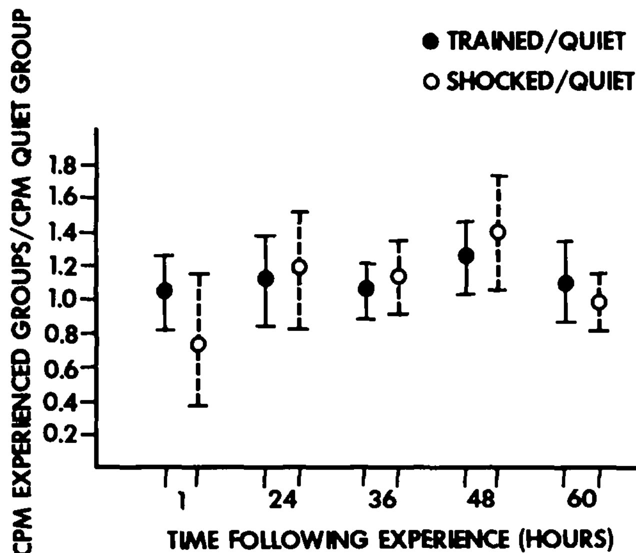

FIG. 3. Change in cpm of trained and shock group, relative to quiet group, over the postexperience time points. In each matched comparison, ratios of the cpm of trained/quiet and shocked/quiet were calculated. Each bar is based on the mean of five ratios.

It was possible that the increase in in vitro RNA synthesis seen 48 hr after experience was due to changes across time in the template activity of the chromatin from the quiet group. This possibility was tested by comparing, within the same day, the acid-insoluble cpm from chromatin of quiet animals that were sacrificed immediately (0 hr), 1 hr, or 48 hr following their transfer from the colony cages to the plastic carrying cages. Ratios of 1 hr/0 hr=0.97 and 48 hr/0 hr=1.03 were obtained. Thus the changes in template activity appear to be due to changes in the chromatin from the experienced groups and not to changes in the quiet group chromatin.

TABLE 2

ALL MATCHED GROUP COMPARISONS OF TEMPLATE ACTIVITY AT 48 HR POSTEXPERIENCE TIME POINT

Group	Mean CPM	Ratio to Quiet*
	Matched Comparison 1	
T	11620	1.12
S	19267	1.86
Q	10355	—
	Matched Comparison 2	
T	19100	1.51
S	21492	1.70
Q	12603	—
	Matched Comparison 3	
T	18671	0.95
S	22400	1.14
Q	19577	—
	Matched Comparison 4	
T	25543	1.38
S	21934	1.18
Q	18434	—
	Matched Comparison 5	
T	38300	1.31
S	33198	1.14
Q	29090	—

*Mean CPM of Trained or Shocked group/mean CPM of Quiet group.

TABLE 3

ASSAY FOR DIFFERENTIAL INHIBITION OF *E. COLI* POLYMERASE ACTIVITY BY CHROMATIN SAMPLES*

Template Source	CPM	% of DNA CPM
DNA	55,493	—
T + DNA	61,527	110
S + DNA	74,165	133
Q + DNA	78,345	141

*150 μl rat liver DNA incubated in the presence or absence of 150 μl chromatin as described in Methods. Treatment group chromatin samples are from the 48 hr postexperience time point. CPM are the average of duplicate samples.

DISCUSSION

Three major findings are presented in this paper. First, an acute experiential event can alter the capacity for rat brain chromatin to support RNA synthesis in vitro. In the present instance, exposure to a novel experience was associated with an increase in brain chromatin RNA synthesis in vitro. Second, the effect of experience on chromatin template activity varies as a function of the time following experience. There appears to be a general trend toward greater template

activity in the chromatin from the experienced groups from 1 to 48 hr postexperience but the difference in template activity between the chromatin of the experienced groups and quiet control is only significant at 48 hr. The effect appears to be transient, however, since no differences in template activity are seen at 60 hr postexperience. Third, repeated handling and/or footshock experience is sufficient for the appearance of the macromolecular response. This result appears to conflict with those studies which show training-specific changes in various macromolecular events [5, 14, 15, 18]. However, it is in agreement with the suggestion that the general activational aspects of experience, inherent in stress and arousal, may modulate gene expression. The delay in the brain chromatin template response to footshock is consistent with the delay in the chromatin template responses of some non-neural target tissues (e.g., liver, uterus, chicken oviduct) to hormonal stimulation [21,22].

The fact that different macromolecular events have been measured may explain the apparently discrepant findings between the present study and studies purporting to show training specific changes. Very soon after an acute experience (e.g., stress, imprinting, training, etc.) changes can be seen in nuclear macromolecular activity in the CNS [7, 14, 15, 20], but the changes in chromatin template activity are not detectable until 48 hr after the novel experience. Immediately after an acute experience, altered macromolecular metabolism may reflect modulation of those RNA transcripts which have recently been synthesized or are in the process of being synthesized. Thus the early macromolecular changes following experience may represent manifestations of ongoing macromolecular activity. The effects of experience on transcription may not occur until later or may be dependent upon these earlier macromolecular events. The brain genome may be modulated by certain molecular events which are triggered by experience and the general time course may be similar to hormonal modulation of non-neural target tissue [22]. Thus the early changes which have been found in certain brain nuclear proteins following avoidance training to footshock [18,19] may be related to the later template responses of brain chromatin.

Alternatively, the discrepant time course may result from the use of *E. coli* RNA polymerase. Chromatin differences present at earlier time points may not be distinguished by the exogenous polymerase. Although the use of *E. coli* RNA polymerase limits our ability to extrapolate directly to the in vivo mammalian system, in vitro chromatin template activity has been found to generally reflect the in vivo synthesis of RNA. For example, RNA products transcribed in vitro have been found to be similar (though not identical) to the in vivo transcriptional products [1,10]. Also the metabolic activity of a tissue in vivo has been found to correlate with the in vitro template activity of the tissue chromatin [6]. The present results therefore do suggest a change in CNS chromatin as a consequence of acute environmental stimuli and these changes may be related to analogous in vivo transcriptional events.

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