Chemical variation in brain loci during shock avoidance

JOHN GAITO, JAMES MOTTIN, and JAMES H. DAVISON,
York University, Toronto, and Waterloo University, Waterloo,
Ont., Canada

RNA/DNA, protein/DNA, and protein/RNA ratios, supplemented by radioisotope indices, indicated that portions of the ventral cortex and dorsal cortex in the rat may contribute uniquely to shock avoidance behavior.

Chitre & Talwar (1963) reported that RNA/DNA and protein/DNA ratios change during behavioral events. In preliminary experiments in this laboratory with auditory stimulation or forced motor activity for various periods, these ratios (and protein/RNA) showed an orderly change in the cortical site appropriate to the event, but not in other sites. There were increases at 10 and 20 min whereas 30 min results tended to be the same as at 0 min. The present experiments used these ratios, supplemented by radioisotope incorporation, to determine brain sites contributing to shock avoidance conditioning.

METHOD

The task was one way active avoidance conditioning with the shock level set at 2.5 mA. Eight or more littermates (Wistar strain) were used for all conditions in each of four experiments; their ages varied from 60 to 210 days over the four experiments but within each experiment the ages were similar. All but three litters were males.

In Experiment 1 there were three groups: Shock Avoidance, E (given 15 trials in 15 min); Shock, S (received the same duration of shock, and at the same points in the 15 min session, as had the littermate in the E Group); and Controls, C (in the shock chamber for 15 min without any treatment). All rats were allowed a 15 min adaptation period in the shock chamber. Because the S rats might have learned aspects other than to avoid shock, this group was replaced in the second experiment by animals (A) that were anesthetized during the time in the apparatus. However, the anesthetic introduced contaminating changes in the neurochemistry; thus, in the other experiments only two groups of rats were used, E and C.

At the end of the 30 min period in the apparatus each rat was sacrificed by immersion in liquid nitrogen for 10 sec. The brain was rapidly removed and sectioned into 10 parts (Fig. 1): anterior ventral cortex (AV), medial ventral cortex (MV), posterior ventral cortex (PV), anterior dorsal cortex (AD), medial dorsal cortex (MD), posterior dorsal cortex (PD), cerebral hemispheres (CH), cerebellum (CB), upper brain stem (UBS), and lower brain stem (LBS) (sectioned just below the inferior colliculi). RNA, DNA, and proteins were extracted by a modified Schmidt-Thannhauser procedure and the amounts of each determined by spectrophotometric analysis. In Experiment 3 each rat was injected intracranially with 5 μ c of orotic acid -6-C¹⁴ (precursor of RNA; specific activity - 34.7 mc/mM) and in Experiment 4 with 5 μ c of valine-C¹⁴ (precursor of protein; specific activity - 117 mc/mM to

Fig. 1. Rat brain: (a) Ventral view, (b) Dorsal view.

determine incorporation rates into RNA and protein, respectively.

The dependent variables in Experiments 1 and 2 were: RNA, DNA, and protein (all per gram of tissue, wet weight), RNA/DNA, Prot/DNA, and Prot/RNA; in Experiment 3, relative specific activity of the RNA fraction (R.S.A. RNA), i.e., specific activity of the RNA fraction/specific activity of the cell pool fraction; in Experiment 4, relative specific activity of the protein fraction (R.S.A. Prot), i.e., specific activity of the protein fraction/specific activity of the cell pool fraction, and the six dependent variables of Experiments 1 and 2. Littermates by Conditions analyses of variance were conducted with the rejection region set at a p of .10 so as to decrease the p of Type II errors. Because Type I errors increase in this case, only tissues which showed significant results in two or more chemical measures were considered real ones.

RESULTS AND DISCUSSION

In the four experiments, the E rats had six or more avoidance responses in the 15 trials. The neurochemical results for each experiment are presented in Table 1; only tissues which indicated more than one significant result are shown; others are considered to be Type I errors.²

The results of these experiments suggest the involvement of MV and MD. The ventral cortex may provide important contributions in the shock avoidance behavior; the three ventral cortices (AV, MV, and PV) consistently showed greater specific activities in the RNA, protein, and cell pool fractions and greater relative specific activity of the protein and RNA fractions than did other brain tissue.

MD consistently provided significant differences between E and C. It is possible that differences in MD in the shock avoidance task reflected the pain and somesthetic stimulation resulting from the electrical shock. The medial dorsal cortex appears to be a somesthetic area (Zubek, 1951). However, MD may provide unique contributions for the learning process.

The CB tissue indicated differences also. Such results probably reflect the running involved in avoiding and/or escaping the shock source.

There was a tendency for $E < C$ in the specific activity of RNA, protein, and cell pool fractions and in ratios involving RNA and/or protein for most tissues. Such results appear to be inconsistent with ideas presented by individuals who believe that RNA performs a unique role in learning behavior (see Gaito, 1966). These individuals maintain that an increase in RNA and/or protein is required for learning to occur. In these experiments the learning animals were indicating clearly that a learning process was underway. But learning events were occurring during decreased RNA and protein synthesis,

Table 1
Significant Results in Various Tissues

		Tissues		
		MV	MD	CB
Exp. 1	Prot/DNA ($E = S < C$)		RNA/DNA ($E < S = C$)	
Exp. 2	DNA ($E > A = C$)		DNA ($E = A > C$)	RNA ($E < A = C$)
	Prot ($E = A < C$)		Prot ($E < A < C$)	RNA/DNA ($E = A < C$)
	RNA/DNA ($E < A = C$)		Prot/DNA ($E < A < C$)	
	Prot/DNA ($E < A = C$)		Prot/RNA ($E < A < C$)	
Exp. 3			R.S.A. RNA ($E < C$)	R.S.A. RNA ($E > C$)
Exp. 4				R.S.A. Prot ($E < C$)

suggesting that an increase in neither RNA nor protein synthesis is necessary for the acquisition of a learned response. Other research with RNA and protein synthesis inhibitors (actinomycin-D and puromycin) has indicated that learning can proceed even with greatly reduced levels of RNA and/or protein being synthesized (see Gaito, 1966). Yet it is possible that only small amounts of unique RNA and/or protein species are required to sustain learning events.

In these learning experiments, the consistent significance of MV and MD could indicate changes due to learning, an interpretation which agrees with results by other investigators (see Gaito, 1966). However, the differences noted in these sites may be due to the stressing agent involved (electric shock). Studies with other learning tasks and lesion studies are now underway to determine whether the differences reflect neurochemical events unique to learning or to other processes such as stress.

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NOTES

1. This work was supported by Office of Naval Research Contract Nonr-4935(00) and grants from the National Research Council of Canada.
2. Complete data may be obtained by writing the first author.