Chemical variation in brain loci during water maze performance[1]

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Four experiments showed consistent differences in neurochemical measures between learning and nonlearning animals in the medial ventral cortex.

In previous experiments (Gaito, Mottin, & Davison, in press, a, b), it was found that chemical measures in the medial ventral cortex (MV) of the rat consistently differentiated between shock avoiding and nonbehaving animals. The significant differences, however, might have been caused by the stress involved in the task. In one experiment a shocked group was introduced but this group may have shown learning other than that involved in avoiding shock. In a second experiment an anesthetized group was used to eliminate this aspect; the anesthetic produced chemical changes which contaminated the results. Thus, it was decided to resort to two types of experiments to examine further the role of MV: (a) studies with other learning tasks which do not utilize shock, e.g., water-maze performance, and (b) studies in which lesions are produced in MV. This article is concerned with four replicated experiments in a water-maze task.

METHOD

In each of four experiments, eight to 10 littermates (Wistar strain) were used; the ages varied from 83 to 155 days. All but nine litters were males.

The experiments were conducted in a six unit wooden T-maze, 18 in. deep, placed in a large plastic wading pool; the pool was filled with 12 in. of water. Fifteen min before training commenced, a learning rat (E) was placed on a small wooden ledge just out of the water in the last unit of the maze. When training commenced, E was given five trials in the water to familiarize him with the maze pattern. Ten test trials followed during which time to reach the wooden ledge and number of blind alley entries were recorded. Reaching the ledge within 30 sec was considered a successful run. The 15 trials were completed in approximately 15 min. The C rats received no training and remained on the ledge for 30 min.

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 as in the previous experiments: 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 were determined by spectrophotometric analysis. In Experiments 3 and 4 each rat also was injected intracranially with 5 μc of L-valine-C^{14} (specific activity–124 mc/mM) to determine incorporation rates into protein.

The dependent variables were: Experiments 1 and 2–RNA, DNA, and protein (all per gram of tissue, wet weight), RNA/DNA, Prot/DNA, and Prot/RNA; Experiments 3 and 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 and/or experiments were considered real ones.

RESULTS AND DISCUSSION

In the four experiments, all but three E rats had six or more successful runs in the 10 trials; these three had four or five responses. The neurochemical results are presented in Table 1; only tissues which had more than one significant result are shown; others are considered to be Type I errors.[2]

The results of the shock avoidance experiments suggested the involvement of MV. In the present experiments MV appears to be implicated in water-maze learning also. In the shock avoidance experiments, differences between C and E animals occurred in several cases in PV as well. In the water-maze experiments only two significant results occurred for PV. Likewise, AV provided significant differences in several of the present experiments.

It is possible that the ventral cortex provides important contributions in both the shock-avoidance and water-maze tasks with the major focus being in MV but tissue in AV and PV showing some contribution. 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

Table 1
Significant Results in Various Tissues

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
AV		Prot (E > C)	RNA (E > C)	RNA (E > C)
MV	DNA (E < C)		Prot (E > C) Prot/RNA (E > C) R.S.A. Prot (E < C)	RNA/DNA (E < C)
PV		RNA/DNA (E < C)	R.S.A. Prot (E > C)	
MD				R.S.A. Prot (E > C)
AD		RNA/DNA (E > C)	Prot/DNA (E < C) Prot/RNA (E < C)	Prot/RNA (E > C)
CB			R.S.A. Prot (E > C)	R.S.A. Prot (E < C)

(Continued on page 48)

levers to obtain food when continued use of the initially preferred lever resulted in shock to another rat. This occurred even though, for most Os, the nonpreferred lever required twice as much force to activate.

Correlations between Os that changed preference and possible intervening variables such as (a) number of depressions of each lever, (b) total number of trials, and (c) lever first learned, were all quite low or nonexistent by visual inspection of the data, and were not considered to be significant to the occurrence of preference change.

DISCUSSION

The phenomenon of discrimination might adequately explain the absence of altruistic behavior in Experimental Group 2, without recourse to any speculation regarding motivation. In Group 1 continued pressing of the preferred lever brought about an abrupt stimulus change (i.e., squealing and struggling of the V) in addition to food delivery. In Group 2, however, continued pressing of the preferred lever did not bring about any stimulus change in addition to food delivery, since V was in an ongoing distress state. Thus, in Group 2, there were no discriminable stimuli to be associated with Os' behavior.

The differential results are found in Experimental Group 1. In this group Os that had been shocked prior to the experiment showed altruistic behavior. Two existing hypotheses to account for altruistic behavior are: (a) that it is innate or "instinctive" (Masserman et al, 1964; Rice & Gainer, 1962); (b) that it serves merely to reduce the intensity of physical noxious stimuli (Lavery & Foley, 1963).

Neither of these hypotheses adequately explains the results of this experiment. According to each, nonshocked Os in Subgroup 1A should have shown behavior changes similar to those of shocked Os in Subgroup 1B. However, the hypothesis of Lavery and Foley becomes tenable if another concept is considered – the concept of sensitization as espoused by Church (1959). As he sees it, "a group of animals that have been shocked may be more responsive to all stimuli, including the pain responses of others [1959, p. 133]. Thus, if Church's idea is valid, the "altruistic" Os in this experiment, having experienced shock themselves, could have been sensitized to the extent that they would perceive the abrupt increase in level of stimulation (i.e., squealing and squirming of V) as noxious, and thus would work to reduce it. This explanation seems more likely than one based on innate factors, since (a) shock-naive Os were not altruistic, and (b) it has been shown that both stimulus increase and/or decrease can serve a reinforcing function (Hunt & Quay, 1961; Roberts, Marx, & Collier, 1958).

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NOTE

1. Now at Norwich Hospital, Norwich, Connecticut. The assistance and encouragement of Dr. William James, Department of Psychology, University of Georgia, are sincerely appreciated.

(Continued from page 46)

fractions than do other brain tissue in both shock-avoidance and water-maze experiments.

The AD and CB tissues consistently indicated differences between E and C in the shock-avoidance experiments as well as in the water-maze task. Such results probably reflect the running involved in avoiding and/or escaping the shock source in the former task and the swimming in the latter.

In some of the shock-avoidance experiments MD consistently provided significant differences between E and C, but this was not the case in the other experiments. It is probable 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).

There was a strong tendency for $E < C$ in the specific activity of RNA, protein, and cell pool fractions and in ratios involving RNA and/or protein in some shock avoidance and water maze experiments. These results appear to be inconsistent with the expectations of individuals who believe that RNA performs a unique role in learning behavior (Gaito, 1966). One would expect that an increase in RNA and/or protein would be required for learning to occur. In these experiments the learning animals were indicating clearly that a learning process was under way during decreased RNA and protein synthesis, 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) indicated that learning can proceed even with greatly reduced levels of RNA and/or protein being synthesized (see Gaito, 1966). However, only small amounts of unique RNA and/or protein species may be required to sustain learning events.

The consistent significance of MV (and possibly PV and AV) could indicate changes due to learning; such interpretation is consistent with results by other investigators (Gaito, 1966). However, the differences noted in these sites could be due to the stressing agents involved in each case. Lesion studies are now under way to determine whether the differences reflect neurochemical events unique to learning or to other processes such as stress.

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NOTES

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2. Complete data may be obtained by writing the first author.