is randomly varied. Nonrandom target presentation, with the animal displaying a behavior that suggests an ability to predict target position, reveals a different mode of eye-head coordination. Under these conditions the head moves before the target is presented. A saccade follows the head movement by up to 150 msec; therefore, the sequence of eye movements during head turning is characterized first by a compensatory movement, then a saccade, and then a second compensatory eye movement. Thus, there are clearly different strategies of eye-head turning available to the animal.

Finally, we believe that knowledge about the coactivation of the oculomotor and neck motor system could be helpful to the eye-movement physiologist since it would be very easy to misinterpret single unit data if the tight coupling between these two systems were not taken into account. Although artificially restraining the head may eliminate the overt manifestation of movement, it will not prevent the delivery of neural activity to the neck muscles. On the other hand, the possibility of bringing under control different strategies of eye-head coordination may be a useful tool for differentiating between the discharge of central neurons related to head movements and those concerned with movements of the eye.

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References and Notes

1. R. S. Woodworth and H. Schlosberg, *Experimental Psychology* (Holt, New York, 1954), pp. 518-523.
2. G. R. Wendt, in *Handbook of Experimental Psychology*, S. S. Stevens, Ed. (Wiley, New York, 1951), p. 1191.
3. H. W. Bond and P. Ho, *Electroencephalogr. Clin. Neurophysiol.* **28**, 206 (1970).
4. E. S. Szebenyi, *Atlas of Macaca mulatta* (Fairleigh Dickinson Univ. Press, Cranbury, N.J., 1970).
5. A. E. Bartz, *Science* **152**, 1644 (1966).
6. R. Dodge, *J. Exp. Psychol.* **6**, 247 (1921).
7. E. Bizzi, R. E. Kalil, P. Morasso, V. Tagliasco, in preparation.
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Electroshock Effects on Brain Protein Synthesis: Relation to Brain Seizures and Retrograde Amnesia

Abstract. *The effects of electroshock on brain seizure activity and brain protein synthesis were studied in male mice. A significant but short-lasting inhibition of brain protein synthesis and an increase in the amount of free leucine were produced by electroshock at intensities above the brain seizure threshold. Electroshock at intensities below the brain seizure threshold did not affect brain protein synthesis.*

It is well known that electrical stimulation of the brain can impair memory for experiences that occur shortly prior to the brain stimulation (*1*). Much is known about the conditions under which correlate with the effects of ES type of memory impairment, which is referred to as retrograde amnesia (RA). For example, the degree of RA produced by ES varies directly with the current intensity and inversely with the time between the experience and the ES treatment (*2, 3*). However, although these effects have been studied for over two decades, little is known about the bases of the effects.

It is generally assumed that ES causes RA by interfering with the neurobiological processes involved in memory storage. But, what kind of interference is essential for the occurrence of RA? In recent research we have found that the passage of current through the brain is not a sufficient condition for producing RA. In order for ES current to produce RA, the current must be administered at intensities at or above the threshold for producing brain seizures (*3, 4*). For example, current levels which produce brain seizures and RA in normal mice do not affect memory in mice in which the brain seizures are prevented by light anesthetization with diethyl ether just prior to the ES treatment (*4*). In general, brain seizures are a highly reliable correlate of RA in animals given ES after training.

Seizures provide an electrophysiological sign of neurobiological disturbance. If it is assumed that memory storage involves neurochemical processes, it should be possible to find effects of ES on brain neurochemistry which correlate with the effects of ES on brain seizures and memory. The findings of many studies suggest that memory storage requires protein synthesis. Drugs such as cycloheximide, which impair protein synthesis, are reported to produce RA (*5*). In the experiment reported here we examined the effect of ES on brain protein synthesis. We wished to know whether or not ES inhibits protein synthesis and, if so, whether the degree of inhibition varies with ES current. The findings indicate that ES at intensities below the threshold for brain seizures and for RA does not significantly affect protein synthesis. However, at intensities above that necessary for producing seizures and RA, ES has a short-lasting but significant inhibitory effect on brain protein synthesis.

Male Swiss-Webster mice (*6*), housed individually in small cage pans, were used. They were approximately 80 days old when killed for the determinations of protein synthesis. The mice were first implanted with bilateral cortical electrodes (*7*) and used in studies of ES (administered through transcorneal electrodes) effects on brain seizures and RA (*3, 4*). After approximately 3 weeks the mice were used to study the effect of ES on brain protein synthesis. The experimental conditions used for ES administration were based on the findings of previous behavioral and electrophysiological studies.

To examine the effect of ES on brain protein synthesis at various times after treatment, we administered a 4-minute pulse of L-[1-^{14}C]leucine at varying intervals after the ES treatment. The use of a short labeling pulse enabled us to measure the time-dependent effects of ES on the rate of protein synthesis. Animals were injected in a tail vein with 4 μc of L-[1-^{14}C]leucine (30 mc/mmole, New England Nuclear). All animals were killed 4 minutes after the injection of the isotope to measure protein synthesis. For the measurement designated "immediate" (Table 1), animals were injected 2 minutes prior to ES and killed 2 minutes after. For all other groups, times indicated represent the times between ES and the beginning of the 4-minute incorporation period.

The ES consisted of 60-hz constant current delivered for 200 msec through transcorneal electrodes. Two ES current levels were used: 12 ma and 30 ma. From the earlier studies (*3, 4*) we determined that, with the stimulus parameters used, 12 ma produced both

brain seizures and RA in normal mice but was below the threshold for seizures and RA in animals lightly anesthetized with ether. The 30-ma ES was well above the seizure and RA threshold in both untreated and ether-treated mice. In the experiment reported here some of the groups were lightly anesthetized with ether prior to the administration of ES. Ether was administered by placing the mouse in a 500-ml jar containing ten gauze-covered cotton balls wetted with 2.0 ml of diethyl ether at room temperature (22°C). Each mouse was retained in the jar until it lost its righting reflex; typically this occurred within 25 seconds. Electrocorticograms were recorded for approximately 45 seconds after the ES. The electrocorticograms obtained were comparable to those obtained in the earlier research. The 12-ma ES produced brain seizures in the nonetherized mice. However, in mice anesthetized with ether, brain seizures occurred only with the 30-ma ES.

After the mice were decapitated, the brains were rapidly removed and the whole brain was used for the determination of protein synthesis. The radioactivity of brain fractions soluble and insoluble in trichloroacetic acid (TCA) were determined according to procedures previously described (*8*). When certain conditions concerning the time course of protein labeling are satisfied (*9*), the rate of protein synthesis is directly related to the quantity a_p/a_l, where a_p and a_l are, respectively, the specific activities of protein and precursor. Under many conditions the absolute concentration of either protein or precursor (in micromoles of free leucine per gram of brain) would not be expected to change. In this case, the ratio of the total radioactivity (in counts per minute) in the protein (q_p) and in the precursor (q_l) is also proportional to the rate of protein synthesis. This ratio, q_p/q_l, is readily measured when L-[1-^{14}C]leucine is used as the precursor because the TCA-soluble radioactivity accurately represents the free leucine radioactivity (*8*). Metabolites soluble in TCA do not accumulate because the radioactivity is lost as ^{14}C-labeled CO_2. This procedure enabled us to measure the rate of protein synthesis on the basis of data from a single animal. Further, the rate was readily measured after a short pulse (4 minutes) of radioactive label.

The rate of protein synthesis, as indexed by the quantity q_p/q_l (Table 1, column 2), decreased significantly immediately after ES. The degree of inhibition varied with the ES current. In comparison with the controls (*10*), the ratio q_p/q_l in mice given 12-ma ES without ether anesthesia was reduced by 40 percent ($P < .01$, by a two-tailed t-test). In mice given 30-ma ES after ether treatment the q_p/q_l ratio was reduced by 26 percent immediately after ES ($P < .01$) but by only 17 percent 10 minutes after ES ($P < .01$). Thirty minutes after ES this ratio was significantly greater than that of controls (15 percent, $P < .05$) but declined to control values within 2 hours with the exception of the 12-ma, 120-minute group (*11*). No effect on q_p/q_l values was seen in mice given 12-ma ES after ether anesthesia.

As discussed above, the use of the q_p/q_l ratio to measure the rate of protein synthesis is based on the assumption that the total quantity of free leucine is not influenced by the experimental conditions. Although the amounts of free leucine could not be measured accurately in individual animals, the validity of this assumption was examined by pooling the TCA fractions within the various groups. We were surprised to find (Table 1, column 4) that ES influenced the amount of free leucine. Increases in the leucine concentration of from 10 to 27 percent of the control were found in both no-ether and ether groups injected immediately after ES and in ether-treated groups injected 10 minutes after ES. Leucine concentrations returned to those of the controls within the accuracy of the analysis (± 3 to 5 percent) (*12*) within 10 minutes for the group given 12-ma ES and within 30 minutes for the 30-ma, ether-treated group. The quantities of free leucine were correlated with the q_p/q_l values. Increases in the amount of free leucine, such as those found in the groups given 30-ma ES or ES without ether, will result in a decrease in precursor specific activity and a concomitant decrease in protein labeling. In order to obtain a more accurate estimate of the rate of protein synthesis, it was necessary to measure specific activities and to calculate a_p/a_l values. With these corrected values (Table 1, column 5) significant inhibition ($P < .01$) was found only immediately after ES in groups given ES without ether (24 percent decrease) or 30-ma ES after ether treatment (19 percent decrease). The small difference in percent inhibition between these two groups was not statistically significant. No effect was found with 12-ma ES after ether treatment or with 30-ma ES at longer intervals after ES.

Thus, when the effect of ES on the rate of protein synthesis is measured by the ratio a_p/a_l, which takes into account the precursor specific activity, we find a short but significant decrease immediately after ES in groups given ES without ether or 30-ma ES after

Table 1. The time-dependent effect of ES on the amounts of free leucine and on the rate of protein synthesis. Column 2 shows the rate of protein synthesis as a percentage of the control, on the assumption of a constant content of precursor (q_p/q_l, mean ± standard deviation). The leucine concentration is shown in column 4 (as a percent of the control) and can be seen to change as a result of ES. For this reason, the value q_p/q_l shown in column 2 is not a valid measure of the rate of protein synthesis. Column 5 shows the corrected value for the rate of protein synthesis when the change in leucine concentration is taken into account (a_p/a_l). The amount of free leucine was determined by amino acid analysis of the TCA-soluble fraction (*8*). The control value represents a concentration of 0.063 μmole/g; n is the number of animals in each group.

Time after ES	Percentage of control				
	q_p/q_l		Leucine concentration	a_p/a_l	
	Mean	± S.D.		Mean	± S.D.
Controls, $n = 37$ (*10*)	100	± 13.2	100	100	± 13.2
Immediate					
No ether, 12 ma, $n = 16$	60	± 15.3*	127	76	± 19.5*
Ether, 12 ma, $n = 16$	92	± 13.5	106	98	± 14.4
Ether, 30 ma, $n = 6$	74	± 14.4*	110	81	± 15.8*
10 minutes					
Ether, 12 ma, $n = 7$	105	± 14.6	98	103	± 14.3
Ether, 30 ma, $n = 9$	83	± 11.6*	127	105	± 14.7
30 minutes					
Ether, 12 ma, $n = 7$	108	± 18.1			
Ether, 30 ma, $n = 5$	115	± 13.0†	95	110	± 17.2
60 minutes					
Ether, 12 ma, $n = 6$	103	± 17.9			
Ether, 30 ma, $n = 7$	114	± 10.5†			
120 minutes					
Ether, 12 ma, $n = 10$	116	± 15.5*			
Ether, 30 ma, $n = 6$	104	± 9.4			

* Significantly different from control value, $P < .01$. † Probability $P < .05$.

ether. Because the free leucine concentration increases, the ratio q_p/q_l, which does not take into account the decrease of precursor specific activity, is not indicative of the rate of protein synthesis. Use of this ratio alone gives overestimates of the magnitude and duration of the ES inhibition of protein synthesis. The largest changes in q_p/q_l were observed for groups in which q_p/q_l was measured immediately after ES (no ether, 12-ma and ether, 30 ma). After correction for free leucine the differences became smaller but remained significant. The significant decrease in q_p/q_l found at 10 minutes after ES in mice given 30-ma ES after ether treatment is due to dilution of the specific activity of the precursor and does not reflect a real change in the rate of protein synthesis. The significance of the decrease in q_p/q_l in the 30-ma group at 30 minutes is difficult to interpret.

These findings indicate that ES administered at an intensity sufficient to produce brain seizures (and RA as indicated by previous findings) has two effects: it produces a significant but short-lasting inhibition of brain protein synthesis and it increases the amount of free leucine in the brain. These results are consistent with other evidence suggesting that ES may produce a short-lasting inhibition of protein synthesis in the brain (*13*). In addition, the increases in free leucine might indicate an increase in protein catabolism at early times.

The most important finding in this study is that the inhibition of protein synthesis is not produced by the passage of current per se. Inhibition was produced only at ES levels above that necessary for eliciting brain seizures. Thus, it appears that the ES effects which produce brain seizures also result in an inhibition of brain protein synthesis. In view of our earlier finding that memory losses (RA) are produced by ES only if the current is at or above the brain seizure threshold intensity, it appears that an inhibition of brain protein synthesis is also found with ES levels which produce RA. It should be noted, however, that the degree of inhibition of protein synthesis produced by ES in this study (a maximum of 24 percent) is considerably less and of shorter duration than that produced by antibiotic drugs such as cycloheximide (*14*). In general, the degree of RA produced by antibiotic drugs is less than that produced by ES. Thus, it does not seem likely that the amnesia produced by ES is due to inhibitiion of protein synthesis per se. Rather, it seems more likely that brain seizures and inhibition of protein synthesis are signs that the coordinated function of the cells of the brain is altered to such a degree that the processes involved in memory storage are disrupted.

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References and Notes

1. J. L. McGaugh and H. P. Alpern, *Science* **152**, 665 (1966).
2. R. Kopp, Z. Bohdanecky, M. E. Jarvik, *ibid.* **153**, 1547 (1966).
3. S. Zornetzer and J. McGaugh, *Physiol. Behav.*, in press.
4. J. McGaugh and S. Zornetzer, *Commun. Behav. Biol.* **5**, 243 (1970).
5. S. H. Barondes, in *Molecular Approaches to Learning and Memory*, W. L. Byrne, Ed. (Academic Press, New York, 1970), p. 27.
6. The mice were obtained from Simonsen Laboratory, Gilroy, California.
7. S. Zornetzer, *Physiol. Behav.* **5**, 739 (1970).
8. G. Banker and C. Cotman, *Arch. Biochem. Biophys.* **142**, 565 (1971).
9. The transfer of label between two homogeneous compartments can be described by means of the following equation [C. W. Sheppard and A. S. Householder, *J. Appl. Phys.* **22**, 510 (1951): J. M. Reiner, *Arch. Biochem. Biophys.* **46**, 53 (1953)]:

$$K_{1p} = \frac{Q_p \, da_p/dt}{a_1 - a_p}$$

where, for the case under consideration, K_{1p} is the rate of protein synthesis, a_p and a_1 are the specific activities of the protein and precursor, respectively, and Q_p is the total quantity of protein present. We have previously shown (*8*) that under similar conditions the increase in a_p is linear with time during the first 8 minutes after the administration of precursor and that a_p is negligible as compared to a_1. In this case

$$da_p/dt = ca_p$$

and

$$K_{1p} = \frac{cQ_p a_p}{a_1}$$

where c is a constant. In a pilot study it was verified that Q_p remains constant after ES so that

$$K_{1p} = c' a_p/a_1$$

where $c' = cQ_p$. The use of these quantities as a measurement of the rate of protein synthesis is based on the assumption that precursor and product compartments are homogeneous. When a variety of products are made at different rates, the measured rate becomes less accurate with increasing incorporation time [C. W. Sheppard and A. S. Householder, *J. Appl. Phys.* **22**, 510 (1951)]. Although this seems to be the case for brain proteins [A. Lajtha, *J. Neurochem.* **3**, 358 (1959)], measurement of the rate over a short, constant incorporation time should allow meaningful comparison of control and ES-treated animals.
10. Control animals were etherized and treated in the same manner as animals in the other groups but did not receive ES. The incorporation of amino acid into protein was measured immediately after and 2 hours after ether treatment. The two control groups were identical. Further, S. Roberts and B. S. Morelos [*J. Neurochem.* **12**, 373 (1965)] have reported that ether does not affect brain protein synthesis. In view of this finding, a more detailed analysis of the effect of ether was not undertaken.
11. At 120 minutes after ES (12 ma with ether) a small increase was seen which was statistically significant ($P < .01$). This effect appears to represent a delayed stimulation of the rate of protein synthesis, although alterations of the precursor pool cannot be ruled out.
12. S. Blackburn, *Amino Acid Determination: Methods and Techniques* (Dekker, New York, 1968), p. 103.
13. J. MacInnes, E. McConkey, K. Schlesinger, *J. Neurochem.* **17**, 457 (1970); B. Agranoff, in *Molecular Approaches to Learning and Memory*, W. L. Byrne, Ed. (Academic Press, New York, 1970), p. 35.
14. The percentage of inhibition may not represent maximum inhibition since a 4-minute pulse was used and in the "immediate" group the ES was given 2 minutes after the injection of the isotope.
15. We thank C. Green, K. Domoto, and B. Longacre for technical assistance. Supported by research grants MH 12526, training grant MH 11095-04, and predoctoral fellowships MH 36372-03 and MH 50166-01 from the National Institute of Mental Health; research grant NS 08597 and biomedical sciences support grant RR-07008-05 from the National Institutes of Health; and research grant NSF-GB-14491 from the National Science Foundation.
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Ethanol Preference in the Rat as a Function of Photoperiod

Abstract. *Ethanol-drinking was induced in laboratory rats that were maintained in total darkness. The established preference for ethanol was not reversed under conditions of constant illumination although a decrease in ethanol intake occurred. Administration of melatonin to rats maintained under "normal" photoperiods (9 hours of darkness during a 24-hour day) also induced ethanol-drinking.*

The search for an experimental model of alcoholism has generated considerable research dealing with those variables that are critical for establishing ethanol preference or self-selection of ethanol by laboratory animals. Richter and Campbell (*1*) accomplished this through a gradual day-to-day increase of the percentage concentrations of ethanol offered their rats. Other methods for inducing ethanol preference in laboratory rats include caloric restriction (*2*), intraventricular administration of minute quantities of ethanol (*3*), and exposure of animals to ethanol as their sole drinking fluid over a period of time (*4*). Induction of ethanol preference also has been attempted by subjecting animals to stressful or anxiety-provoking situations (*5*). The present investigation evolved from an unsuccess-