This arrangement of amino acid residues within this histone raises the possibility that the carboxyl-terminal region might be designed to interact strongly with DNA, whereas the amino-terminal half might be designed for specific interaction with some other agent, for example, one which is involved in processes of repression and derepression.

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Amino Acid Incorporation into Rat Brain Proteins during Spreading Cortical Depression

Abstrast. *Unilateral spreading cortical depression was elicited by applying potassium chloride solutions to the dura of conscious, freely moving rats. Incorporation of ^{3}H-leucine into soluble cortical proteins was decreased in the depressed hemisphere relative to the control side, while soluble brainstem proteins from both sides had the same specific activity. Various subfractions of soluble cortical proteins were affected to equal degrees.*

Spreading cortical depression (SCD) (*1*) consists of repeated waves of depressed electrocortical activity that can be induced by topical application of KCl to a small area of the cerebral cortex. The depression spreads to the rest of the cortex of the hemisphere on the side to which KCl has been applied, but not to the other hemisphere or to subcortical areas. Ruščák (*2*) has shown that SCD in anesthetized rats is accompanied by decreased incorporation of ^{35}S-methionine into total brain proteins. We now report experiments which extend his findings to conscious, freely moving animals, and which suggest that none of the soluble proteins is preferentially affected.

Male Sprague-Dawley rats (250 to 350 g) were used; we implanted bilateral epidural cannulas on either side of the sagittal suture just anterior to the lambdoid suture (*3*). The cannulas were filled with sterile, 0.9 percent saline. Between 24 and 48 hours after surgery, unilateral SCD was induced in conscious rats by rinsing one cannula with 10 percent KCl, and the existence of SCD was verified by observing the loss of the placing response in the contralateral hind limb (*4*). Leucine-4,5-^{3}H (5 c/mmole, Tracerlab, Waltham, Mass.) was then injected intraperitoneally in a dose of 3.5 μc per gram of body weight.

After the period designated for the incorporation of the tracer, the animals were killed by cardiac perfusion with cold saline under sodium pentobarbital anesthesia, in order to ensure the removal of blood proteins from the brain. The brain was exposed and sectioned transversely through the posterior colliculus and the longitudinal fissure, and the cerebrum (minus olfactory lobes) was quickly removed. The left and right halves were separated, rinsed in cold saline, and immediately frozen in a mixture of dry ice and acetone. Each hemisphere was divided roughly into "cortex" and "brainstem" (*5*).

Extracts of the cortex and brainstem of each hemisphere were prepared in sodium-potassium phosphate buffer (*p*H 7.4, ionic strength 0.1) by homogenizing in a Ten Broeck tissue grinder at 0° to 4°C, with 10 ml of buffer per gram of tissue (wet weight). The homogenates were centrifuged for 1 hour at 50,000*g* in the No. 40 rotor of a Spinco model L centrifuge. The pellets were discarded, and the clear supernatants containing the soluble proteins were analyzed. The proteins soluble at *p*H 7.4 in the extraction buffer were separated into two classes, designated as *p*H 5–insoluble and *p*H 5–soluble proteins. The specific activity of each of these two fractions was determined by first bringing a portion of the extract to *p*H 5. About half of the protein precipitated. After centrifugation, the soluble fraction was removed, and trichloroacetic acid (TCA) was added to precipitate the remaining protein. A portion of each brain extract at *p*H 7.4 was also added directly to half the volume of 30 percent TCA in order to precipitate all the protein. The washed *p*H 5 precipitate and the two TCA precipitates were dissolved in 97 percent formic acid. The formic acid solution (0.2 ml) was added to 20 ml of scintillation fluid (*6*). Radioactivity was determined by counting in a liquid scintillation counter equipped with an external standard and corrected for quenching (*6*). A portion of each TCA supernatant was also counted by adding it directly to the scintillation fluid. The protein concentration of each extract and each *p*H 5–soluble supernatant was determined (*7*), with bovine serum albumin as a standard. Specific activity (count min^{-1} mg^{-1}) was calculated from the radioactivity (count min^{-1} ml^{-1} in protein) and the protein concentration (mg ml^{-1}) of the extract.

We first determined the kinetics of

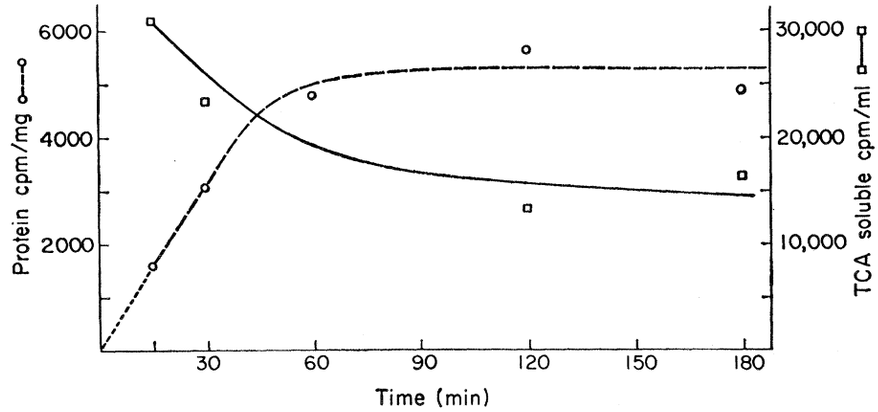

Fig. 1. Change with time in specific activity of total soluble proteins (○—○) and radioactivity of the acid soluble fraction (□—□) of rat cortex, after intraperitoneal injection of ^{3}H-leucine (3.5 μc per gram body weight). Each point represents the average of determinations on two or more animals.

Table 1. Effect of spreading depression on incorporation of ^{3}H-leucine into cortical proteins.

Group	Total protein in extract*	pH 5–insoluble protein*	pH 5–soluble protein*	TCA soluble fraction†
(A) NaCl on right and left cortex; $N = 6$; 2-hr incorporation	$1.01 \pm .06$ $P < .6$	$1.02 \pm .07$ $P < .4$	$1.01 \pm .06$ $P < .6$	$0.97 \pm .04$ $P < .3$
(B) KCl on right cortex, NaCl on left; $N = 7$; 2-hr incorporation	$0.86 \pm .05$ $P < .01$	$0.87 \pm .05$ $P < .01$	$0.84 \pm .08$ $P < .01$	$1.03 \pm .04$ $P < .1$
(C) KCl on right and left cortex; $N = 1$; 2-hr incorporation	0.99	1.00	0.95	1.05
(D) KCl on right cortex, NaCl on left; $N = 2$; 30-min incorporation	0.87	0.82	0.92	1.04

* Results are expressed as the ratio of specific activity of the right cortex to that of the left cortex. Ratios were calculated for each animal and are reported for the N animals in the group as mean ± standard deviation. † Results are expressed as the ratio of the number of counts per minute per milliliter of extract from right cortex to that from left. Ratios were calculated for each animal and are reported as mean ± standard deviation; P values were obtained by use of the t-test for significant differences from the value of 1 of radioactivity and specific activity ratios.

^{3}H-leucine incorporation into protein in unoperated rats (Fig. 1). The specific activity of protein increased linearly for about 30 minutes and then leveled off. Radioactivity of the TCA soluble fraction attained a maximum value within 15 minutes and declined thereafter. When brainstem proteins were compared with cortical proteins from the same animal, no differences were found in the shape of the curve or in the magnitude of specific activity. Also, no differences were detected between pH 5–soluble and pH 5–insoluble proteins.

A 2-hour incorporation period was initially chosen for studies on the effects of SCD, in order to obtain maximum specific activity (Table 1). We have calculated the specific activity ratio of the right hemisphere (experimental) to the left hemisphere (control) for each animal. The use of this ratio eliminates individual variations in absolute specific activity and shows the percentage of difference in specific activity between the two hemispheres.

In group A animals (Table 1), both cannulas were rinsed with saline; in group B animals, the left cannula was rinsed with saline and the right with 10 percent KCl. Both groups were otherwise treated identically, and both were occasionally tested for the placing response in each of the hind limbs. When saline was applied to both sides (A, Table 1), the cortical proteins from both hemispheres had equal specific activities. In animals with unilateral spreading depression (B, Table 1), the specific activity of cortical proteins on the depressed side was only 86 percent of that on the control side. Specific activity ratios for these animals were significantly different ($P = .01$) from the corresponding ratios for group A animals. The differences observed between the pH 5–soluble and pH 5–insoluble proteins within each group were found to be not significant.

Although the radioactivity in the TCA soluble fraction was the same in both hemispheres 2 hours after the injection of ^{3}H-leucine, the possibility existed that the rate of uptake of isotope was slower in the depressed hemisphere. This might not be evident after 2 hours, at which time the kinetic curve of the radioactivity in the TCA soluble fraction is in a declining phase (Fig. 1), but it could be reflected in specific activity of protein. Therefore, several animals with SCD in the right hemisphere were killed 5 and 10 minutes after the injection of ^{3}H-leucine, and the radioactivity of the TCA soluble fractions of cortical extracts was determined. At both times the mean of the right to left ratios of the TCA soluble fractions of the extracts was one or more (1.03 after 5 minutes, mean of three animals; 1.10 after 10 minutes, mean of two animals). This suggests that the depressed hemisphere takes up leucine at a rate equal to or slightly greater than that of the control hemisphere, and that the decreased protein specific activity in the depressed hemisphere is not due to differences in the rate of ^{3}H-leucine uptake. A small increase during SCD in the rate of leucine uptake could be explained by the concomitant vasodilation (*1*).

The absolute value of the specific activity of proteins from the control hemisphere of unilaterally depressed animals (B, Table 1) (5640 ± 1120 count min^{-1} mg^{-1}) was the same as the value for group A (5640 ± 970 count min^{-1} mg^{-1}), showing that application of KCl to the right hemisphere did not affect the left hemisphere. Moreover, in one animal under bilateral SCD (C, Table 1), there was no difference in specific activity between the right and left cortex, suggesting that neither hemisphere is preferentially susceptible to the effects of SCD on protein metabolism.

Two animals under unilateral spreading depression were killed after only 30 minutes of incorporation (D, Table 1). The magnitude of the effect was the same as after 2 hours (B, Table 1).

Soluble proteins of the brainstem do not show a significant decrease in the specific activity ratio during SCD (Table 2). This is consistent with physiological evidence (*4*) suggesting that SCD does not spread to subcortical areas. Our results cannot distinguish between a lack of effect on the brainstem and a bilateral effect, for there are some secondary physiological changes in the subcortical regions which are not necessarily unilateral (*4*).

The equal magnitude of the effect of spreading depression on pH 5–soluble

Table 2. Effect of spreading cortical depression on incorporation of ^{3}H-leucine into brainstem proteins. Results are expressed as in Table 1.

Group	Total protein in extract*	pH 5–insoluble protein*	pH 5–soluble protein*	TCA soluble fraction†
(A) NaCl on right and left cortex; $N = 4$; 2-hr incorporation	$0.96 \pm .06$ $P < .2$	$0.90 \pm .07$ $P < .05$	$0.97 \pm .06$ $P < .3$	$1.02 \pm .06$ $P < .5$
(B) KCl on right cortex; NaCl on left cortex; $N = 4$; 2-hr incorporation	$0.95 \pm .03$ $P < .1$	$0.96 \pm .11$ $P < .5$	$1.00 \pm .03$	$0.95 \pm .05$ $P < .1$
(C) KCl on right and left cortex; $N = 1$; 2-hr incorporation	0.91	0.93	0.93	0.95

and *p*H 5–insoluble cortical proteins (Table 1) suggests that no one protein subfraction was preferentially affected. This is also indicated by our finding that the specific activity of insoluble cortical proteins (that is, those contained in the 50,000*g* sediment) was decreased during SCD to the same extent as the soluble proteins. In order to confirm this suggestion, the *p*H 5–soluble proteins were further fractionated (*8*) by disc electrophoresis on polyacrylamide gels (*9*). The unstained gels, 12.5 mm in diameter and 10 cm long, were cut into 40 cross-sectional slices, and the radioactivity of each slice was measured (*10*). Duplicate gels were stained, and specific activity was estimated by comparing the radioactivity in each slice with the dye intensity of the corresponding area of a stained gel. Although the analysis was limited by the assumption that the intensity was directly proportional to protein concentration and by the low radioactivity in each slice, there was no indication of preferential incorporation into any of the protein bands.

The primary event in spreading depression is believed to be the loss of intracellular potassium and the rise of the extracellular potassium (*11*). Other biochemical alterations have also been reported. Concentrations of glucose, glycogen, and creatine phosphate fall, while the amounts of lactate, citrate, inorganic phosphate, and total ninhydrin-positive substances increase (*12*). These changes are enhanced by simultaneous induction of ischemia and indicate a generalized alteration in the oxidative metabolism of cortical cells, possibly through a stimulation of the sodium-potassium pump. Similar biochemical events have been reported in brain slices during electrical stimulation (*13*) and in media of high potassium concentration (*14*). These observations suggest that the effect of spreading cortical depression on incorporation of labeled leucine into protein is a nonspecific, secondary consequence of the diversion of energy sources or amino acids (or both) into different metabolic pathways.

Our results confirm those of Ruščák (*2*) and extend them to conscious, freely moving animals. The findings suggest the design of experiments to detect changes in the synthesis of particular proteins as a result of more specific alterations in neural activity such as learning and memory. Several groups have reported experiments showing that unilateral spreading cortical depression appears to restrict learning to the normal hemisphere (*4*, *15*). This experimental system might therefore be used to detect possible biochemical alterations in a "trained" hemisphere relative to the depressed hemisphere.

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6. The scintillation fluid consisted of 50 g of naphthalene, 500 ml of dioxane, 70 ml of Liquifluor (Pilot Chemicals, Inc., Watertown, Mass.) brought to 1 liter with toluene. The liquid scintillation counter (model 574) is from Packard Instrument Co., Downers Grove, Ill.
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Adrenergic Blood Pressure Responses in the Shark

Abstract. *Both nurse and lemon sharks recovered slowly from the pressor effect of epinephrine and from the depressor effect of isoproterenol. The recovery time increased with the dose of epinephrine. Grading of dosage and the use of dibenzyline revealed that these species exhibit alpha and beta adrenergic vascular responses in a manner qualitatively similar but quantitatively dissimilar to that for mammals.*

The pressor response of the shark to epinephrine has been described as prolonged, with accompanying desensitization to subsequent doses (*1*). Little else is known about the nature of the pressor response in sharks. We have studied such responses in small (1 to 6 kg) nurse sharks *Ginglymostoma cirratum* and lemon sharks *Negaprion brevirotris* (*2*). *d*-Tubocurarine (3.0 mg/kg) was injected into the caudal vein. The animal was then restrained in a supine position and anesthetized by perfusion of tricaine methanesulfonate (1:10,000 in seawater) across the gills. The gills were continuously perfused with fresh seawater throughout the experimental period. A deep incision was made through the ventral surface of the tail which was then reflected dorsally exposing the caudal artery and vein, and polyethylene cannulas were inserted in these vessels. Arterial blood pressure was recorded with a Statham P23 DC transducer and a Grass model 79-2 polygraph. Drugs were diluted in an elasmobranch solution (Nicholl) (*3*) and administered by way of the venous cannula.

No differences in response attributable to sex or species were observed among 19 lemon sharks and 17 nurse sharks. Control arterial systolic pressures ranged from 20 to 36 mm-Hg, diastolic pressures from 16 to 30 mm-Hg, and pulse pressures from 3 to 7 mm-Hg. Control heart rates ranged from 16 to 44 beats per minute.

When initial doses of epinephrine chloride (100 to 1000 μg/kg) were administered, mean arterial pressure increased by 10 to 20 mm-Hg. This pressor response decreased only slightly during the subsequent 3-hour period. During the peak pressor response, the administration of additional epinephrine or norepinephrine did not elicit further pressor responses. When the initial doses of epinephrine were below 10 μg/kg, blood pressure returned to that of the control within 60 minutes after administration. Doses of epinephrine below 0.5 μg/kg failed to elicit a pressor response.

In mammals low doses of epinephrine will often elicit a depressor response. Doses of epinephrine as low as 0.0001 μg/kg were administered to