other brain regions similar to the male pattern, but we restricted consideration to the nuclei mentioned. For mapping, autoradiograms were analyzed at intervals of approximately 300 μm.

17. These measurements were made under the microscope with the aid of an ocular micrometer. Autoradiograms were analyzed at intervals of about 40 μm.
18. The lateral archistriatum shows no sign of containing labeled cells. In three male and three female brains, we counted the number of grains found within a micrometer grid of fixed size (about the size of one cell) when this grid was placed over each of 100 archistriatal cells in each animal. The frequency distributions of grain counts for each animal were not significantly different from the Poisson distribution (G test, $P > .05$) [R. Sokal and F. Rohlf, *Biometry* (Freeman, San Francisco, 1969)].
19. The mean estimated cell sizes for males and females, respectively, were: HVc, 44.2 and 36.5 μm^2; MAN, 65.8 and 45.2 μm^2; ICo, 35.8 and 37.1 μm^2; nXIIts, 322 and 290 μm^2; and PVM, 36.1 and 37.1 μm^2.
20. In these analyses, a large ocular micrometer grid was superimposed over the field of interest, and all cells whose centers lay within the grid were analyzed. The counts include both neurons and nonneural cells stained with thionine. The grid was moved and the process repeated until 150 cells or more were counted in each animal in each brain region. (In nXIIts, 90 cells were counted.) In nXIIts we counted only cells larger than 160 μm^2, which is low enough to include virtually all motoneurons in both sexes and yet exclude counts of other cell types in this nucleus. In HVc, MAN, and nXIIts, the fields of cells sampled were chosen randomly from within the borders of these nuclei to avoid histological and autoradiographic artifacts. The portion of PVM sampled was centered approximately 250 μm ventral to the anterior commissure and 190 μm from the midline in all animals. Since ICo is a heterogeneous population of cells (in that cells in different regions have different sizes, shapes, and amounts of labeling), we restricted sampling in all brains to one portion of ICo just medial to nucleus mesencephalicus lateralis, pars dorsalis (MLd), and separated from both MLd and the reticular formation by fiber laminae. Accordingly, we do not generalize our results to all cell populations within ICo.
21. We have examined the data to determine if some differences among animals in preparation of the autoradiograms might have artifactually produced the observed sex difference. For example, the males were injected with 6.5, 16.5, and 27.7 ng of testosterone per gram of body weight, and the females with 9.8, 16.4, and 34.2 ng/g. We have examined the variables of dose of hormone, dose of radioactivity, length of exposure of the autoradiograms, background density in the autoradiograms, and combinations of these variables (for example, dose of hormone multiplied by exposure period). In each case, there is extensive overlap between the sexes in the values of these variables, yet there is no overlap in the percentages of labeled cells in male and female HVc or male and female MAN. This, together with the observation that three of the brain regions show no difference in the percentage of labeled cells, indicates that the observed difference is indeed a difference between the sexes, not an artifact of autoradiographic procedures.

    One factor might reduce the disparity in percentages of labeled cells in male and female HVc and MAN. The number of cells which appear labeled in an autoradiogram are an underestimate of the actual number of labeled cells. In the 6-μm tissue section, labeled neurons relatively far from the emulsion will not appear labeled, since the beta particles emitted by isotope within these cells will not penetrate the intervening tissue. The number of small labeled cells may be underestimated to a greater extent than the number of large ones [appendix in (*4*)]. Since female HVc and MAN cells are smaller than male cells on the average, this factor might overestimate the sex difference in the percentage of labeled cells. This error is quite small, however, and cannot account for the sex difference in percentages of labeled cells: (i) Males have a higher percentage of labeled cells in all size classes in HVc and in six of eight of our arbitrary size classes of MAN cells. (ii) One may calculate the approximate correction factor for the percentages of labeled cells of each size class [appendix in (*4*); A. P. Arnold, *J. Comp. Neurol.*, in press]. The error reduces the difference in the percentage of labeled cells in male and female MAN by less than 2 percent, and actually increases the difference for HVc.
22. Since the boundaries of MAN in the female are poorly defined in Nissl-stained sections, measurement of the volume of this nucleus in females is difficult.
23. A. P. Arnold, in preparation.
24. The small, necrotic ovarian remnants are also unlikely to have secreted a steroid to competitively block the accumulation of testosterone or its metabolites in HVc and MAN but not in other brain regions.
25. The intrinsic difference between male and female cells in HVc and MAN could be a difference in steroid metabolism, so that, for example, a specific metabolite of testosterone, normally produced in male but not female HVc cells, is the active metabolite accumulated by cells in this region. For the purposes of this discussion, an intrinsic sex difference in steroid metabolism has implications similar to an intrinsic difference in the accumulation mechanism per se.
26. We assume that the observed differences in hormone accumulation indicate differences in hormone action on these neurons. This assumption still requires experimental proof.
27. Such hormone-dependent growth of neurons has been demonstrated in cultures of neonatal brain cells in mice [C. D. Toran-Allerand, *Brain Res.* **106**, 407 (1976)].
28. This statement assumes that these neurons in both sexes are exposed to similar hormones during development; embryonic chick testes and ovaries both synthesize androgens and estrogens [J. E. Woods and E. S. Podczaski, *Gen. Comp. Endocrinol.* **24**, 413 (1974)].
29. Sexual difference in steroid accumulation must also have casual antecedents, such as genetic or hormonal differences during development.
30. We thank C. Arnold for preparing some of the histology and D. Mills for help with analysis and typing. This study was supported by NSF grant BNS 77-05973 to A.P.A.

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Synaptic Proteins After Electroconvulsive Stimulation

Abstract. *Quantitative immunoelectrophoresis of rat brain synaptic proteins following a series of electroconvulsive stimulations demonstrated changes suggestive of an increase in the number of synaptic vesicles, in a preparedness for glycolytic demands, and a delayed development of a certain area in the brain. The increased synaptic remodeling may be important for the action of electroconvulsive therapy.*

Electroconvulsive therapy (ECT) is the most efficacious treatment of psychotic depression (*1*). Its mode of action, however, is far from clear, although it is evident that seizure activity generated from upper brainstem centers is a prerequisite for therapeutic effect (*2*). Because both the sustained beneficial effects and the transient, unwanted influence on memory functions appear only after a number of induced seizures, a hypothesis of the mode of action of ECT should be based mainly on effects demonstrable after a period of time following the last seizure. The importance of short-lasting effects, however, such as increased blood-brain barrier permeability (*3*, *4*), should not be overlooked. Studies of cerebrospinal fluid metabolites in humans undergoing ECT have so far not lent support to the monoamine theory of affective disorder (*5*), whereas animals given monoaminergic nerve transmitter agonists showed behavioral changes indicative of increased functional (postsynaptic) activity of monoamines (*6*, *7*). These findings are in accordance with the demonstration of a specific increase of monoamine oxidase and tyrosine hydroxylase in the nerve endings in rat brain, as shown by studies involving sustained administration of electroconvulsions (*8*, *9*).

The present study was designed to demonstrate a direct effect of repeated electroconvulsions on the brain synapses, using the specific rat brain synaptic proteins synaptin, D1, D2, D3, and 14-3-2 as markers. Subcellular fractions of both synaptic vesicles and synaptosomal plasma membranes are enriched with synaptin (*10*, *11*). It is located on the outside of the vesicles and on the inside of the plasma membranes (*12*). The function of synaptin is assumed to be related to the exocytotic process of synaptic vesicles during nerve impulse transmission (*13*). Synaptosomal membrane fractions are enriched with D1, D2, and D3, but these proteins are absent from both synaptic vesicles and cultured astroglial cells (*10*, *11*, *14*). Both D1 and D2 are located on the outside, whereas D3 is on the inside of the synaptosomal membrane (*15*). In an ontogenetic study in mice the postnatal concentrations of synaptin, D1, and D3 rose to adult levels, while the concentration of D2 just after the brain growth spurt gradually decreased to a steady adult level of 50 percent of the level on day 12 (*16*); it may be speculated that D2 is involved in intercellular recognition during synaptogenesis (*15*). The 14-3-2 isolated from brain has been localized to the neuronal cytoplasm (*17*–*19*); the two electrophoretically different forms, 14-3-2 ac and 14-3-2 cc, are probably identical with enolase isoenzymes (*20*).

The study was carried out with chloroform-anesthetized Wistar rats, 35 days old at the start. Electroconvulsive stimulation (ECS) was given via electrodes in the earholes, using a stimulus of 50 mA for 0.9 second. The following situations were studied: (i) ECS three times weekly for 4 weeks, (ii) ECS three times weekly for 8 weeks, and (iii) ECS three times weekly for 4 weeks followed by 4 weeks of sham treatment. All experimental animals were compared to their sham-treated littermates. The animals were

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anesthetized with chloroform and were decapitated 24 hours after the last treatment (ECS or sham). The brains, divided into an occipital cortex sample and the remaining forebrain (*21*), were weighed and homogenized separately in a buffer [73 m*M* tris buffer, 24 m*M* barbital, 2 m*M* NaN_3, and aprotinin protease inhibitor (100 KI unit/ml), *p*H 8.6], of which exactly 1 ml was used for the occipital cortex and exactly 10 ml for the forebrain. The homogenates were stored at −80°C before analysis.

On the day of analysis samples of the thawed homogenates were mixed with two volumes of 4 percent Triton X-100 and incubated for 60 minutes at 0°C. The solubilized proteins were not separated from the residuals. The suspensions were analyzed by crossed immunoelectrophoresis (*10, 14, 22*) with polyspecific antiserum to rat brain synaptic membranes (SPM 0176) in the case of D1, D2, D3, and 14-3-2, and with antiserum to rat brain synaptic vesicles (anti-VES 1173) in the case of synaptin. The areas of the immunoprecipitates were measured by planimetry (*22*) and quantitated by use of a standard dilution series for each protein. Specimens from ECS and control animals were analyzed together on the immunoelectrophoretic plates. All determinations were made in triplicate on different occasions and the median values of the percentage deviations from the sham-treated animals were calculated for each litter [100 ×(ECS − sham)/sham]. The statistical analysis was by Student's paired *t*-test. As all samples of either occipital cortex or residual forebrain were homogenized to the same volume, the calculated percentage deviations indicate changes in the amounts of the parameters analyzed. The values for the whole forebrain shown in Fig. 1b were calculated from the values for the occipital cortex (contribution about 10 percent) and those for the residual forebrain (about 90 percent), using the tissue weight as an adjusting factor.

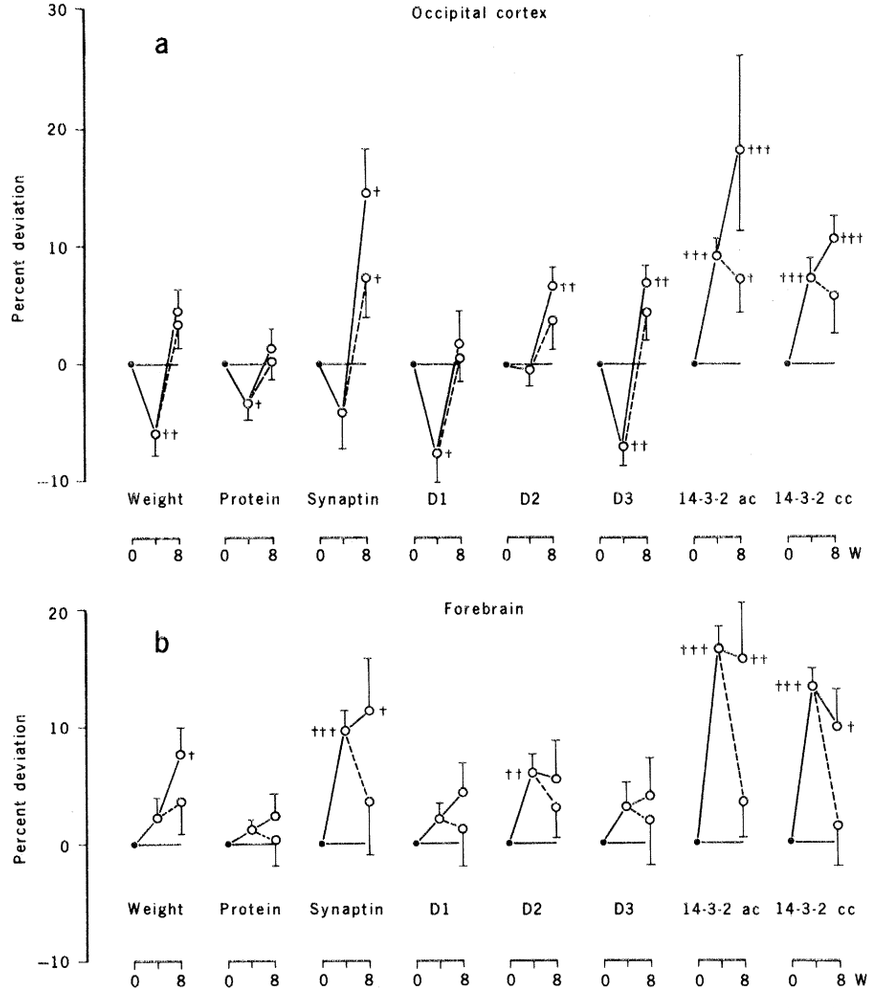

Fig. 1. Rats were given ECS for 4 weeks [N = 10, solid line from (●) to 4-week (○) point], for 8 weeks [N = 10, solid line from 4-week (○) point to 8-week (○) point], or for 4 weeks followed by 4 weeks of sham treatment (N = 10, dashed line). The results indicate percentage deviations between the littermate controls of the same age and the ECS rats. Means and standard errors are given. Significance levels: (+) $P < .05$, (++) $P < .01$, and (+++) $P < .001$.

As shown in Fig. 1a, the occipital cortex of rats given ECS for 4 weeks showed a decrease in both weight and tissue protein. The amounts of D1 and D3 clearly decreased, synaptin and D2 were not significantly changed, and both forms of 14-3-2 increased. In the same situation the values for forebrain (Fig. 1b) were somewhat different: weight and tissue protein were unchanged, as were D1 and D3. Synaptin and D2 showed increased values. In the occipital cortex the 14-3-2 forms were largely increased. The results for the occipital cortex are quite similar to those obtained with rats housed in an impoverished environment for 4 weeks (*21*), apart from the increased amount of the 14-3-2 forms, which in the latter situation did not increase. The ECS-induced increase of the 14-3-2 forms related to enolase activity may be understood in light of the heavy glycolytic demand on the neurons during seizures.

In forebrain this increase of 14-3-2 was even more pronounced; the increased amount of synaptin may be related to the reported increase of synthesis and turnover of brain monoamines stored in the synaptic vesicles (*6, 13*). Since neither D1 nor D3, both represented in the synaptosomal membrane, increased, we must assume that the amount of synaptic membranes remained unchanged. The clear increase of D2 may be related to its assumed function as a recognition protein active during synaptogenesis. In the experimental situation of repeated seizures the increase could well be brought about by an increased turnover of synapses, eventually stimulating new synaptic patterns in the brain.

When ECS was continued for another 4 weeks, to a total of 8 weeks, synaptin, D2, D3, 14-3-2 ac, and 14-3-2 cc were significantly increased in the occipital cortex compared to the values for sham-treated controls. Animals given ECS for 4 weeks followed by 4 weeks of sham treatment displayed changes in the same direction, but the difference from purely sham-treated controls was significant only for synaptin. Thus, the values in occipital cortex after ECS for 8 weeks were similar to those in forebrain after ECS for 4 weeks. The similarity between the effects of an impoverished environment and of ECS on the occipital cortex indicates that ECS causes delayed development of the occipital cortex, as has been assumed for an impoverished environment (*21*).

The forebrains of rats treated for 8 weeks differed from those treated for 4

weeks only with respect to brain weight. As seen in Fig. 1b, 4 weeks of ECS followed by 4 weeks of sham treatment led to normalization. The significant increase of synaptin, D2, and the 14-3-2 isoantigens was therefore a reversible phenomenon.

In conclusion, we found an increase of synaptin, D2, and 14-3-2 in the rat brain. This increase was seen in forebrain after ECS three times weekly for 4 weeks, and in occipital cortex after ECS three times weekly for 8 weeks. The occipital cortex showed a decrease of those proteins after 4 weeks of ECS, which may indicate a reversible delay of development of this area of the brain. The reversible increase of 14-3-2 may indicate increased neuronal preparedness for glycolytic demands. The reversible increase of forebrain synaptin may indicate an increase of synaptic vesicles, and the reversible increase in forebrain D2 may indicate increased synaptic remodeling. These findings are in accordance with the concept that ECS leads to a sustained change in postsynaptic nerve transmission (*6, 7*). Furthermore, the increased amount of D2 measured in the cerebrospinal fluid of patients recovering from psychotic (endogenous) depression, regardless of the type of therapy (*23*), indicates that the present model for the study of ECS may prove valuable in the search for the etiology of affective illness.

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Differential Behavioral and Biochemical Effects of Right and Left Hemispheric Cerebral Infarction in the Rat

Abstract. *Following ligation of the right middle cerebral artery, rats were hyperactive for 2 to 3 weeks whether activity was measured by running wheel revolutions or open field observations. Assays of brain catecholamines revealed 30 percent reductions of norepinephrine in the injured and uninjured cortex and locus coeruleus and a 20 percent reduction of dopamine in the substantia nigra. In contrast, rats with left middle cerebral artery ligations did not become hyperactive and did not show any significant change in catecholamines in any of the brain areas studied. Right and left hemispheric infarctions were comparable in their locations and extent of tissue damage. This lateralization of behavioral and biochemical response to cerebral infarction may be the consequence of anatomical or physiological asymmetries in the brain.*

Ligation of the right middle cerebral artery in the rat leads to a reduction in norepinephrine (NE) concentration in the ipsilateral and contralateral cortex and brainstem and during a 40-day postoperative period there is a complete or partial return of NE to control levels (*1*). Fluorescence microscopic studies revealed a decrease in the number of fluorescent varicosities or intensity of fluorescence in both the ipsilateral and contralateral cortex and brainstem concomitant with the decrease in catecholamine concentrations (*2*). These changes in catecholaminergic neurons have been accompanied by several transient alterations in behavior, including increased spontaneous activity and a biphasic change in both shock-induced aggression (*1*) and intracranial self-stimulation (*3*). These behavioral changes were related to the biochemical changes by the finding that the period of spontaneous hyperactivity could be blocked pharmacologically either by postoperative daily treatment with the NE uptake blocker desmethylimipramine or by preoperative destruction of NE neurons with 6-hydroxydopamine (*4*).

Since all of the previous behavioral, anatomical, and biochemical experiments were done on animals with right middle cerebral artery ligation, and recent studies have reported asymmetries in brain catecholamine content (*5, 6*), the present study was undertaken to determine the behavioral and biochemical effects of left middle cerebral artery ligation.

Sprague-Dawley male rats (approximately 300 g) were housed individually in cages with food and water freely available and a regular schedule of 12 hours of light, 12 hours of dark. The cages (*7*) consisted of a stationary compartment and a running wheel with free access to either compartment. The running wheel could move freely in either direction and was connected to a cyclometer which was read at 24-hour intervals. Under chloral hydrate anesthesia (350 mg per kilogram of body weight, intraperitoneally), either the right or left middle cerebral artery was ligated under a dissecting microscope with a 6-0 ophthalmic suture. The surgical approach was made through a frontoparietal craniotomy extending from the coronal suture posteriorly to the periorbital area arteriorly and from the protrusion of the zygomatic arch inferiorly to the ridge separating the dorsal and lateral aspects of the skull superiorly. The suture was passed through the dura, behind the vessel, and out through the dura again, and the vessel and overlying dura were tied together; the artery was then severed distal to the tie with dural scissors. The ligature site, which was just above the rhinal fissure, varied slightly from animal to animal, but the surgical approach was the same on each side; there appeared to be no anatomical differences in the size or course of the artery on the two sides.

Two series of experiments were done to determine the effect of left middle cerebral artery ligation on spontaneous activity. In the first series, 33 rats were placed in the activity cages and allowed to acclimatize for 3 weeks before being

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