tomatine in larval tissues of the parasites indicates that antibiosis against the parasites is a result of absorption of the α-tomatine from the hosts.

Our studies demonstrate the potential incompatibility of biological control with HPR programs based on chemical antibiosis. The partial alleviation of α-tomatine antibiosis by the addition of β-cholesterol to the diet suggests that the presence of sterols or steryl esters in tomato plants may mask tomatine toxicosis to insects. However, the prolonged larval period, reduced pupal eclosion, smaller size, and shortened adult longevity of a parasite (*H. exiguae*) resulting from toxicosis by a plant antibiotic (α-tomatine) presents an enigma to integrated pest management in breeding high concentrations of a particular plant toxin to inhibit insect pests. This incompatibility may be exacerbated even further if the pest population evolves tolerance to the antibiotic, while the parasite population remains sensitive; the analogous problem is commonly encountered today with chemical insecticides (*15*). Alternatively, there may be compatibility if biological control agents that contact fewer plant antibiotics via the "physiological filter" of the host are able to evolve tolerance to these antibiotics more readily than their hosts, which must evolve tolerance to a multitude of plant chemicals while facing mortality induced by the parasite. Furthermore, according to present theory insects should be at a disadvantage while feeding on plants containing antibiotics (*16*). However, our results suggest that this putative disadvantage may be outweighed by the ability of these herbivorous insects to serendipitously utilize these antibiotics as prophylactics against their parasites.

BRUCE C. CAMPBELL
SEAN S. DUFFEY
Department of Entomology, University of California, Davis 95616

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10. The excised parasites were ground in boiling methanol. The homogenate was filtered, diluted to 25 percent methanol in H_2O, acidified to *p*H 2, and extracted once with petroleum ether and once with ethyl ether. The aqueous solution was readjusted to *p*H 8 and extracted three times with ethyl ether; the residue was dried under nitrogen gas and redissolved in 2 ml of 50 percent methanol. The sample was stored at 0°C until used for hemolytic (*12*) and GLC (*11*) assays.
11. A 100-μl portion of partially purified parasite extract (*10*) was hydrolyzed with 2*N* HCl at 100°C for 2 hours to liberate tomatidine. The solution was then adjusted to *p*H 8 and extracted three times with ethyl ether. The ether fractions were pooled and dried under vacuum. Prior to GLC analysis, tomatidine was derivatized by Tri-Sil 'Z' (Pierce Chemical Co., Rockford, Ill.). β-Cholesterol was added as an internal standard. The GLC analyses were performed on a Varian 3700 GC equipped with a coupled flame ionization and thermionic specific detector (for N); we used a stainless steel column (0.5 m by 2.2 mm) containing 5 percent OV-101 on Chromsorb W; the temperature program was 200° to 300°C at 10° per minute; the carrier gas was helium, at 30 ml/min.
12. For the hemolysis assay, the indicator system consisted of 3 ml of washed sheep red blood cells (5 percent) in normal saline plus tris buffer (*p*H 8). Microliter portions of standard α-tomatine solution were compared with parasite extract (*10*) for hemolytic activity. The portions were added to the indicator system, incubated at room temperature for 30 minutes, and centrifuged at 3000 rev/min for 10 minutes. Released hemoglobin was measured colorimetrically at 542 nm.
13. β-Cholesterol (10 μmole) was added to hemolysis system. The β-cholesterol was stored as a 50 percent methanol solution; it precipitates α-tomatine and abolishes hemolytic activity.
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Sexual Difference in Pattern of Hormone Accumulation in the Brain of a Songbird

Abstract. *After adult zebra finches (*Poephila guttata*) received injections of tritiated testosterone, fewer hormone-concentrating cells were found in females than in males in two brain regions involved in song: hyperstriatum ventrale pars caudale and magnocellular nucleus of the anterior neostriatum. In some other regions, no sexual difference was detected. It is, therefore, possible that sex differences in the sensitivity of specific neural populations to hormones underlie the striking anatomical dimorphism observed in neural regions controlling song.*

Sex steroids act on the brain to bring about a number of behavioral and neuroendocrine effects. One step in the mechanism of steroid action includes limited-capacity binding of steroids by certain neurons, binding that can be detected by autoradiography (*1*). Numerous autoradiographic studies have allowed generalizations about the topography of cells that concentrate steroids in the vertebrate brain. One is that the distribution of steroid target neurons has been fairly stable throughout vertebrate evolution, since labeled cells are found in homologous brain regions in fish, amphibians, reptiles, birds, and mammals (*2*) [although there are exceptions to the general pattern (*3, 4*)]. A second generalization, that the distribution of labeled cells is similar in males and females of any given species (*5*), implies that the distribution of hormone-concentrating cells is not changed by whatever processes determine the sexual differentiation of the brain. We now report a definite sex difference in the numbers of cells accumulating hormone in specific brain regions of a songbird, the zebra finch (*Poephila guttata*), after injection of tritiated testosterone.

Male zebra finches sing a short courtship song learned in early life from the father (*6*). Castrated adult zebra finches sing much less frequently than intact birds, and testosterone propionate injections reverse the effects of castration (*7*). Autoradiographic evidence in the adult male (*4*) indicates that after tritiated testosterone is injected, labeled neurons are found in several brain regions implicated in the control of song or other vocalizations. These include the caudal nucleus of the hyperstriatum ventrale (HVc), magnocellular nucleus of the anterior neostriatum (MAN), nucleus intercollicularis of the midbrain (ICo), and the tracheosyringeal motoneurons (nXIIts), which are the hypoglossal motoneurons innervating the vocal organ, or syrinx. Behavioral and anatomical evidence in the canary indicates that these brain regions are interconnected and that they

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control song (*8*); evidence points to the same conclusion for zebra finches (*9*).

In contrast to males, female zebra finches do not sing, even when given androgen injections in adulthood (*10*). The volumes of brain regions involved in song control are much smaller in females than in males, which corresponds to the female's inability to sing (*11*). This neural dimorphism is extreme, making this system interesting as a model of sexual differentiation of the brain in general. With this in mind, we compared the distribution of steroid target cells in female and male brains.

The gonads of seven adult female and four adult male zebra finches were removed (*12*) 2 or 3 days before the birds received an injection of 25 to 200 μCi of tritiated testosterone (specific activity, 85 or 152 Ci/mmole; New England Nuclear) (*13*) in 95 percent ethanol. The animals were decapitated 1$^1/_2$ hours after the injection, and the brains were quickly removed and frozen with powdered Dry Ice. Preparation of the autoradiograms followed published procedures (*14*). Coronal sections, 6 μm thick, were cut from the brains in a cryostat at −19°C and then applied to microscope slides previously dipped in nuclear track emulsion (Kodak NTB-3). The slides were stored in lightproof, desiccated boxes at about 4°C for 10 to 49 weeks, then developed (Kodak D-19) and fixed; the tissue was stained with cresyl violet or thionine and dehydrated in a series of alcohols. Control autoradiograms were prepared from two ovariectomized females injected with ethanol only. These controls established that labeled cells found in brains injected with testosterone were not a result of positive or negative chemography (*15*).

The autoradiograms were analyzed under a bright-field microscope. Initially, three female brains were scanned systematically, detailed maps were made showing positions of labeled cells, and these maps were compared with those of males in a previous study (*4*). Cells were considered labeled if the grain density over the cell equaled or exceeded five times the background density seen over adjacent unstained neuropil. As in the male, labeled cells were found in the medial preoptic area (POA), periventricular magnocellular nucleus (PVM) of the anterior hypothalamus, infundibular region (IF) of the posterior hypothalamus, ICo, and nXIIts (Fig. 1, a and b) (*16*). Unlike the male, however, the female had very few labeled cells in HVc and MAN (Fig. 1, c and d).

In the second phase of analysis, we examined in all 11 brains, the topographical distribution of labeled cells in POA, PVM, IF, HVc, MAN, ICo, and nXIIts. We measured the positions of these fields of labeled cells relative to anatomical landmarks (*17*). We detected no sex difference in the distributions of labeled cells in POA, PVM, IF, ICo, and nXIIts. This analysis of each brain also confirmed the relative absence in all of the females and presence in the males of well-labeled cells throughout HVc and MAN.

The third phase of analysis was to compare the numbers of labeled cells in five brain regions (HVc, MAN, ICo, nXIIts, and PVM) in males and females. For this type of analysis, a simple multiplicative criterion of cell labeling (such as five times background used above) is probably not suitable. In our autoradiograms, the number of grains over unlabeled cells appears to be distributed in a Poisson fashion (*18*). The shape of the Poisson distribution varies with the mean. One result is that there is a reasonable chance that an unlabeled cell may reach five times background if the mean grain density is low (less than one grain per cell-sized area), whereas there is an extremely small probability of finding an unlabeled cell with five times background if the grain density is higher. Within any one field of cells, small cells will have low mean grain counts and large cells will have large mean counts. Therefore, a simple multiplicative criterion tends to overestimate the relative number of small labeled cells. This problem may be important in comparing numbers of labeled cells in male and female HVc, MAN, and nXIIts, since the average cell size in these brain regions is smaller in females than in males (*19*). To avoid this problem, we used a Poisson criterion of labeling. The mean background density of grains over unstained neuropil was counted with the aid of an ocular micrometer grid. For each cell analyzed, the area of the cell was estimated by comparing it with the grid, and the expected grain count was calculated. For example, a cell with an area of two units would be expected to have six grains over it, if it were unlabeled in a field with a background density of three grains per unit. Using this expected count as the mean of the Poisson distribution, we considered the cell labeled if the actual

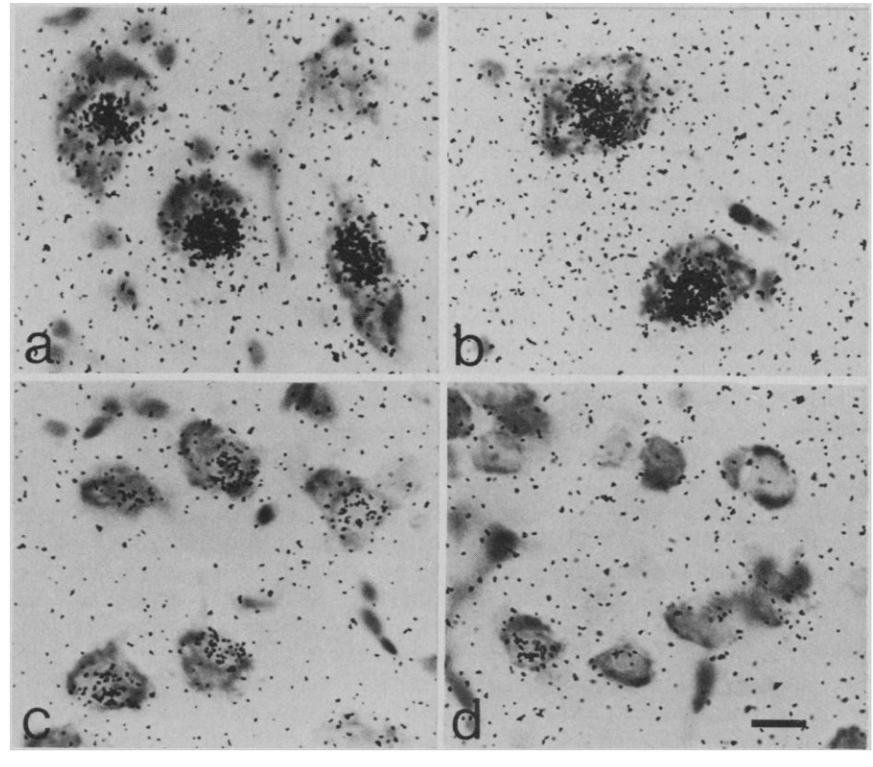

Fig. 1. (a and b) Autoradiograms of motoneurons in the tracheosyringeal portion of the hypoglossal nucleus in an adult male zebra finch (a) and an adult female (b), showing no sexual difference in accumulation of testosterone or its metabolites in the relatively unstained nuclei of the motoneurons. These neurons innervate the syrinx, which produces song. (c and d) Autoradiograms from the same animals in the magnocellular nucleus of the anterior neostriatum of the male (c) and female (d). Only one cell appears to be labeled in the female. This reflects the paucity of hormone-accumulating cells in female MAN. The dose of testosterone was 27.7 ng/g for the male and 34.2 ng/g for the female. The autoradiograms were exposed for 101 days, developed, and stained with thionine. Calibration bar, 10 μm.

number of grains equaled or exceeded the 99 percent confidence interval limit (one-tailed) derived from the Poisson distribution for that mean value.

The percentages of labeled cells in male HVc and MAN were significantly larger than in the corresponding female nuclei [t (4) $\geq$ 5.18, $P < .01$], whereas we detected no significant difference in percentages of labeled cells in the other nuclei ($P > .5$) (Fig. 2) (*20–21*). Since the volume of the male HVc is more than five times as large than that of the female, there are probably fewer cells in the female HVc. Even among this smaller population of female cells, a smaller percentage of cells is labeled, which indicates that the sexual difference in the absolute numbers of labeled cells may well be greater than the percentage of difference and certainly exceeds any sex difference in total cell numbers in HVc. This may also be true for MAN cells, but this cannot be assessed at present since we have no data on the relative volumes or cell numbers in male and female MAN (*22*).

Are there fewer hormone-accumulating cells in adult female HVc and MAN because these cells differ in their intrinsic properties from cells in the corresponding male nuclei? Or perhaps corresponding male and female cells have identical properties, but fewer female cells show accumulation because of a sex difference extrinsic to HVc and MAN in those processes which affect the availability of hormone. For example, it could be argued that testosterone or its metabolites cross the blood-brain barrier less easily in females. This is unlikely, since neurons of both sexes accumulate hormone in other brain regions. Another "extrinsic" hypothesis is that cells in male and female HVc and MAN accumulate a metabolite of testosterone that males but not females produce outside of HVc and MAN. Although we cannot absolutely exclude this possibility, it too seems unlikely. Available evidence suggests that the cells in male HVc, MAN, and nXIIts have a similar profile of affinities for various sex steroids, since they accumulate radioactivity after injections of testosterone and 5α-dihydrotestosterone, but little after estradiol injection (*23*). Therefore, if the female lacks an important metabolite that is produced outside of these three brain regions in males and hence available to all of them, all three would be expected to show less accumulation. Since female cells in nXIIts are heavily labeled and those in HVc and MAN are not, we suspect that the sex difference is intrinsic to the cells of MAN and HVc (*24, 25*).

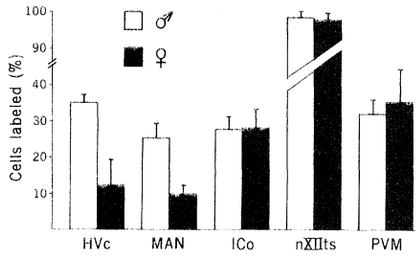

Fig. 2. This histogram shows the means and standard deviations of percentages of cells labeled in five brain regions in three male and three female brains. Four of these regions (HVc, MAN, ICo, nXIIts) are thought to be involved in the control of song or other vocalizations, and PVM is in the anterior hypothalamus. Statistically significant differences are found between the sexes in HVc and MAN but not in the other three brain regions. Abbreviations: *HVc*, caudal nucleus of the hyperstriatum ventrale; *MAN*, magnocellular nucleus of the anterior neostriatum; *ICo*, nucleus intercollicularis; *nXIIts*, tracheosyringeal motoneurons; and *PVM*, periventricular magnocellular nucleus.

An intrinsic sex difference in these cells in adult zebra finches implies that, during the sexual differentiation of the brain, (i) hormone-concentrating cells in HVc and MAN of females fail to develop or are lost, (ii) the ability of female HVc and MAN cells to accumulate testosterone or its metabolites fails to develop or is lost, or (iii) both (*25*). Even if the sex difference is extrinsic to HVc and MAN, at some point in development, hormones probably begin to have less effect on female than male cells in HVc and MAN (*26*). If this difference appears early in the process of sexual differentiation (during periods of neuronal growth and synaptogenesis) a differential sensitivity of these neurons to hormones could result in differential growth (*27*) and synaptogenesis of these brain regions and of brain regions which synapse on them or receive inputs from them (*28*). Thus, the differential hormone sensitivity could be part of the developmental basis for the marked sexual difference in volumes of areas that control song and the sexual difference in singing ability (*29*).

An alternative hypothesis is that the difference in steroid accumulation is not a cause of other anatomical dimorphisms, but is one of a number of concomitant changes produced by the unknown factors controlling sexual differentiation of the brain. Although our data do not allow us to choose between these hypotheses, they do constrain our thinking about possible mechanisms of sexual differentiation. For example, it is unlikely that there is a simple proportional loss during development of all cell types in HVc in females because the sex difference in number of hormone-accumulating cells is greater than any sex difference in total cells in HVc. This lends some weight to the hypothesis that lack of steroid sensitivity in HVc or MAN may precede and contribute to other dimorphisms.

ARTHUR P. ARNOLD
ALBERT SALTIEL
Department of Psychology and *Brain Research Institute, University of California, Los Angeles 90024*

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12. In females, the single left ovary was removed. To check the success of the ovariectomies, we stained serial sections of the tissue surrounding the former point of attachment of the ovary with hematoxylin and eosin. We found small remnants of ovarian tissue in every female, but in most cases they consisted almost entirely of necrotic tissue that had been electrically cauterized during the ovariectomy. Therefore, it is unlikely that they secreted significant amounts of hormone. That well-labeled cells were found in a number of brain regions confirms this fact.
13. Three of the females and two of the males received injections of [1,2,6,7,16,17-^{3}H]testosterone, the rest with [1,2,6,7-^{3}H]testosterone. The dose range was 5.3 to 18.0 μCi per gram of body weight or 9.8 to 34.2 ng/g for the females, and 1.9 to 14.6 μCi/g or 6.5 to 27.7 ng/g for the males.
14. The method was slightly modified from that used by D. W. Pfaff and M. Keiner [*J. Comp. Neurol.* **151**, 121 (1973)].
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16. See (*4*) for maps of positions of labeled cells in these regions. Labeled cells were also found in

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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