References and Notes

1. D. Hunter, *Am. Sci.* **66**, 551 (1978).
2. A. R. Ford, *U.S. Geol. Surv. Bull. 1405 D* (1976), p. 35; _____, D. L. Schmidt, W. W. Boyd, Jr., *U.S. Geol. Surv. Map A-10* (1978).
3. J. C. Behrendt, J. R. Henderson, L. Meister, W. L. Rambo, *U.S. Geol. Surv. Prof. Pap. 844* (1974).
4. J. C. Behrendt, L. Meister, J. R. Henderson, *Science* **153**, 1373 (1966).
5. J. C. Behrendt, *ibid.* **144**, 993 (1964); _____ and C. R. Bentley, *Antarctic Map Folio Series Folio 9* (American Geographical Society, New York, 1978).
6. G. Grikurov, personal communication.
7. A. B. Ford and S. W. Nelson, *Antarct. J. U.S.* **7**, 147 (1972).
8. M. E. Beck, Jr., *Geophys. J. R. Astron. Soc.* **28**, 49 (1972).
9. A. B. Ford and R. W. Kistler, *N.Z. J. Geol. Geophys.*, in press.
10. We used a proton precession magnetometer operated by Applied Physics Laboratory, Johns Hopkins University, in a C-130 aircraft owned by the NSF and operated by the U.S. Navy. Ice-sounding was done with equipment owned and operated by the Scott Polar Research Institute and the Technical University of Denmark. An inertial navigation system was used for positioning, with guidance provided by USGS topographic maps of the area.
11. D. J. Drewry, *Polar Rec.* **17**, 359 (1975).
12. J. C. Behrendt, *J. Geophys. Res.* **67**, 221 (1962).
13. M. E. Beck, Jr., A. B. Ford, W. W. Boyd, Jr., *Nature (London)* **217**, 534 (1968).
14. J. W. Cady, *Geophysics*, in press.

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Gonadal Steroids: Effects on Excitability of Hippocampal Pyramidal Cells

Abstract. *Electrophysiological field potentials from hippocampal slices of rat brain show sex-linked differences in response to 1 × 10^{-10}M concentrations of estradiol and testosterone added to the incubation medium. Slices from male rats show increased excitability to estradiol and not to testosterone. Slices from female rats are not affected by estradiol, but slices from female rats in diestrus show increased excitability in response to testosterone whereas slices from females in proestrus show decreased excitability.*

Sex steroids bind to and affect both morphological and functional properties of the hypothalamus and closely related diencephalic structures (*1*). In the hippocampus, the binding of tritiated estradiol and testosterone does not approach that in diencephalic structures, nor does the hippocampus bind gonadal steroids with the same degree of affinity as it does adrenal coricosteroids (*2*). Naturally occurring changes in estradiol during the estrous cycle affect the excitability of brain tissue (*3*), and experimental administration of estradiol (*4*) and testosterone (*5*) alters electrical activity in the limbic system.

To further define the effects of gonadal steroids on neuronal excitability in the rodent hippocampus, we applied testosterone and 17β-estradiol to hippocampal slices (*6*) from female and male rats. Not only was the excitability of hippocampal pyramidal cells affected by these gonadal steroids, but the response profiles were different for hippocampal slices from male and female rats.

Slices of the hippocampus were obtained from adult male and female rats by standard procedures (*7*). Stage of estrus in females was determined by microscopic analysis of vaginal smears (*8*). Stimulating electrodes were placed in the Schaffer collateral pathway (see Fig. 1A), which is afferent to the CA1 pyramidal cells, from which field potential responses were recorded in the cell body layer (*9*). Figure 1B shows representative monosynaptic field potentials prior to and 20 minutes after steroid administration (*10*). All measurements of population spike responses (*7*) obtained after administering steroids were expressed as a percentage of the corresponding control (before steroid administration) value. Responses qualitatively identical to the response at 20 minutes could be observed as soon as 5 minutes after steroid administration. Steroids were added to the media in a concentration of $1 \times 10^{-10}M$ as an aqueous suspension with polyvinylpyrrolidone (*11*).

The addition of 17β-estradiol and testosterone affected the excitability of the CA1 neurons to suprathreshold afferent stimulation. The steroids had no effect on the population excitatory postsynaptic potential threshold. Hippocampal slices from male rats exposed to estradiol exhibited the greatest change in excitability; the CA1 population spike was increased in amplitude by an average of 72.6 percent. In contrast, a small and inconsistent increase (13.6 percent) in population spike amplitude was observed when slices from male rats were treated with testosterone.

Statistical tests (*t*-test) indicated that the spike amplitudes in slices from male animals given estradiol were reliably higher than those of corresponding slices treated with testosterone ($P < .05$) and that increases produced by testosterone were not significantly different from those observed in the control experiments. Control experiments with slices from male animals placed in medium either with or without the polyvinylpyrrolidone vehicle yielded no consistent change (*12*).

When hippocampal slices from diestrous female rats were treated with testosterone, the spike amplitudes increased by an average of 21.7 percent. A consistent slight depression in pyramidal cell population spike amplitude (8.1 percent) was obtained in slices from diestrous animals after administration of estradiol.

In slices from proestrous animals, estradiol produced an insignificant increase (2.3 percent) in the mean amplitude of pyramidal cell responses. A much larger effect was produced by the same concentration of testosterone which attenuated synaptic activity by 22.2 percent in slices from proestrous females. The difference between mean relative amplitudes for slices from diestrous and proestrous animals treated with testosterone was statistically significant ($P < .01$). The results are summarized in Fig. 1C.

These findings may be examined in terms of a classical receptor binding mechanism. In that mechanism, the ability of a steroid hormone to affect cell function depends on an interaction of the steroid with an unoccupied cytosol receptor protein and subsequent migration of the steroid-receptor complex to the nucleus where RNA and protein synthesis may be affected. Separate receptor proteins for estradiol and testosterone have been characterized, with both types of receptor being found in both genders (*13*). The changes in excitability that we observed in slices from male rats are difficult to attribute to increased protein synthesis resulting from estrogen-triggered new messenger RNA production, since the effects are seen with virtually no lag phase. Moreover, the results obtained for slices from female animals appeared to be independent of stage within the estrous cycle. Since estradiol occurs in highest concentrations during proestrus and lowest during diestrus, the level of endogenous estrogen or free receptor does not seem to be a response-determining factor. We observed no significant response to estradiol in slices from diestrous or proestrous females despite the large effect of this steroid in male tissue. In addition, testosterone was excitatory in slices from diestrous females, but inhibitory in slices from proestrous animals. The changes in excitability produced by both steroids were

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asymptotic in 20 minutes and most of the effect had occurred by 10 minutes after their administration. These short-latency changes in neuronal activity imply a cellular effect at some level other than new RNA synthesis.

An alternative explanation involves the biotransformation of estradiol to catecholestrogen in brain tissue (*14*). These catecholestrogens inhibit tyrosine hydroxylase activity resulting in depressed levels of norepinephrine. Thus the observed effects of estradiol, and perhaps testosterone as well, may be the result of the interaction of their catechol derivatives with neurotransmitter or neuromodulator metabolism (*15*). There is evidence, however, that the Schaffer collateral-CA1 synapse we have studied is glutaminergic rather than catecholaminergic (*16*). Thus, this mechanism may be unlikely.

Some of the phenomena we observed might be attributed to membrane effects. This explanation is particularly attractive in view of the rapid time course of the responses. Indeed, testosterone alters the refractory period of certain neurons, suggesting a direct membrane effect (*17*). The steroids might alter membrane transport mechanisms or physical properties such as fluidity or solubility. However, such hypothetical effects do not immediately suggest a basis for the opposite pattern of responses to the steroids in slices from male and female animals. Nor could they account for the apparent reversal of testosterone effects in slices from proestrous females.

A more satisfactory model would require a class of hormone-specific, high-affinity steroid receptors in the hippocampal cell membrane. External membrane-binding sites for estradiol on endometrial cells have been demonstrated (*18*). Similar hippocampal receptors could mediate rapid changes in excitability and at the same time allow for sex-linked and endogenous hormone-dependent effects of the type that we obtained.

Our results also suggest that the action of testosterone on brain is not mediated exclusively by its aromatization to estradiol (*19*), since there are marked differences in the response profile of testosterone and estradiol in both males and females.

This study provides some intriguing data concerning gender-related differences in function of the hippocampus. It appears that this forebrain structure, which has been implicated in a variety of endocrinological, psychological, and neurobiological processes, responds differently in males and females to endogenous steroid hormones. Such differences may reflect hippocampal sexual differentiation similar to that seen in other brain regions (*20*).

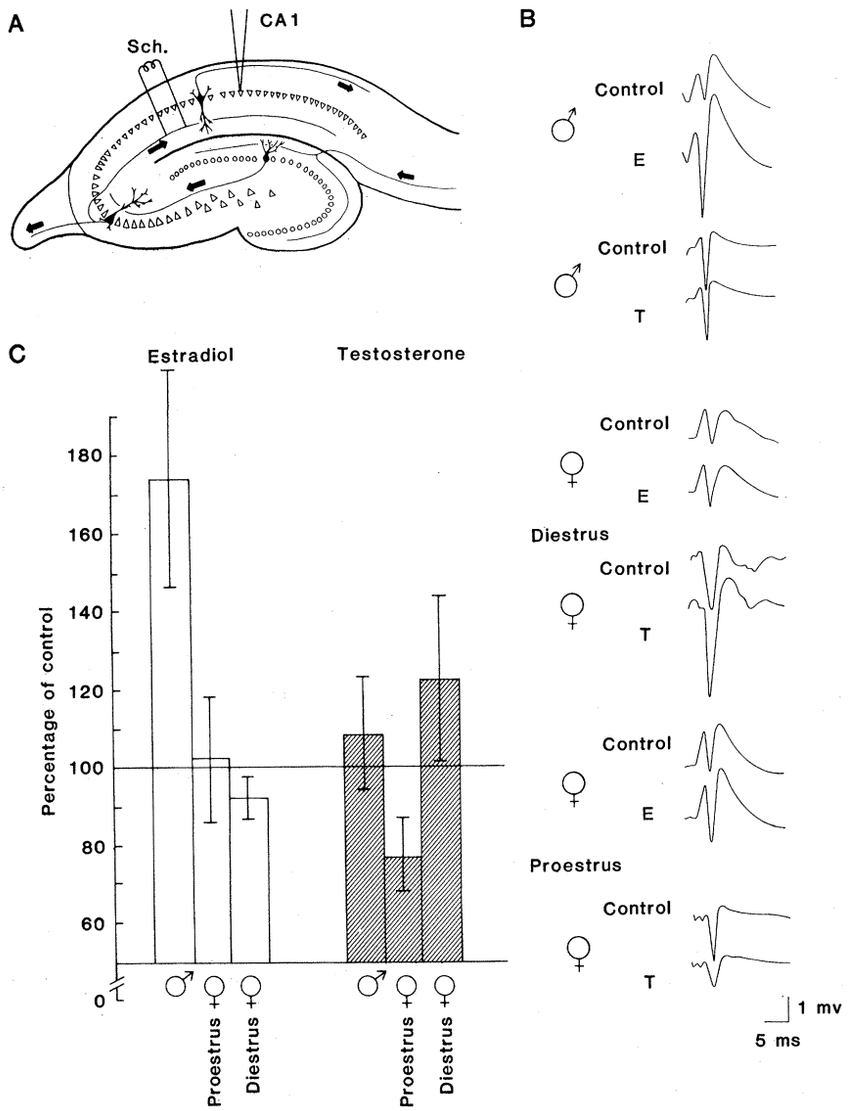

Fig. 1. (A) Diagram of a transverse hippocampal slice. Stimulating electrodes were located in the afferent pathway, which contains the Schaffer (*Sch.*) collaterals. Recording micropipettes were situated in the pyramidal cell body layer in CA1. Cells of this subfield receive monosynaptic input from the CA3 pyramids via the Schaffer collateral system. (B) Representative field potentials from slice preparations in the various experimental conditions. Extracellular population spike responses to a given stiumulus intensity are shown from the control period (before steroid administration) and after the administration of $1 \times 10^{-10} M$ 17β-estradiol (*E*) or $1 \times 10^{-10} M$ testosterone (*T*). Potentials from slices obtained from males and from proestrous and diestrous females are shown for purposes of comparison. All potentials are single sweeps recorded at the same voltage and time scales. (C) Bar graph summarizing the major experimental outcomes. Values on the ordinate are mean percentages of spike amplitudes after steroid administration. Data for each condition are from six to ten animals, each contributing one slide. Cursors representing magnitude of variability (standard error of the mean) are shown for each bar. See text for results of statistical tests.

TIMOTHY J. TEYLER
Neurobiology Program, Northeastern Ohio Universities College of Medicine, Rootstown 44272
RICHARD M. VARDARIS
Psychology Department, Kent State University, Kent, Ohio 44240
DEBORAH LEWIS
Neurobiology Program, Northeastern Ohio Universities College of Medicine
ALLEN B. RAWITCH
Department of Biochemistry, University of Kansas Medical Center, Kansas City 66103

References and Notes

1. B. Dufy, C. Partouche, D. Poulain, L. Dufy-Barbe, J. Vincent, *Neuroendocrinology* **22**, 38 (1976); G. Meyer, R. Ferres-Torres, M. Mas, *Brain Res.* **155**, 108 (1978); D. Pfaff, in *The Regulation of Mammalian Reproduction*, S. Segal, Ed. (Thomas, Springfield, Ill., 1972), p. 5; R. Zigmond, in *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, S. Snyder, Eds. (Plenum, New York, 1975), vol. 15, p. 239; B. McEwen, I. Liberburg, N. Maclusky, L. Plapinger, *Ann. Biol. Anim. Biochim. Biophys.* **16**, 471 (1976); K. Fuxe, T. Hökfelt, O. Nilsson,

Neuroendocrinology **5**, 107 (1969); C. Toran-Allerand, *Brain Res.* **106**, 407 (1976).
2. B. McEwen, J. Gerlach, D. Micco, Jr., in *The Hippocampus*, R. Isaacson and K. Pribram, Eds. (Plenum, New York, 1975), vol. 1, p. 285.
3. P. Timiras, in *Influence of Hormones on the Nervous System* (Karger, Basel, 1971), p. 242; K. Kubo, R. Gorski, M. Kawakami, *Neuroendocrinology* **18**, 176 (1975); M. Kawakami, E. Terasawa, T. Ibuki, *ibid.* **6**, 30 (1970); R. Moss and O. Law, *Brain Res.* **30**, 435 (1971); J. Bueno and D. Pfaff, *ibid.* **101**, 67 (1976); H. Carrer, D. Whitmoyer, C. Sawyer, *ibid.* **142**, 363 (1978).
4. E. Terasawa and P. Timiras, *Endocrinology* **83**, 207 (1968); D. Innes and E. Michal, *J. Exp. Zool.* **175**, 487 (1970).
5. R. Dyer, N. Macheod, F. Ellendorff, *Proc. R. Soc. London Ser. B* **193**, 421 (1976).
6. K. Skrede and R. Westgaard, *Brain Res.* **35**, 580 (1971); G. Lynch and P. Schubert, *Annu. Rev. Neurosci.* **3**, 1 (1980).
7. Tissue preparation was as reported by T. J. Teyler [*Brain Res. Bull.*, in press]. Population responses were measured as reported by B. E. Alger and T. J. Teyler [*Brain Res.* **110**, 463 (1976)]. Briefly, the population excitatory postsynaptic potential amplitude was measured from baseline to the point of maximum amplitude. The population spike was measured as the means of two values; the initial positivity to the initial negativity and the initial negativity to the second positivity. Slices were allowed to equilibrate for 60 minutes before we obtained recordings. The temperature was held at 34°C.
8. Daily smears were taken from females to determine their cycling pattern. In most instances two cycles were followed before we used an animal in the study.
9. Recording electrodes for extracellular population responses were glass micropipettes (1 to 5 μm tip diameter filled with 2*M* NaCl). Stimulation was by concentric, bipolar stainless steel electrodes placed in the afferent fiber path (Schaffer collateral system). The stimulation-evoked, monosynaptic population potentials were displayed on an oscilloscope and recorded for later analysis.
10. Field potentials were elicited by stimulation at 0.2 per second with intensities from below threshold to response maxima. Stimulus duration was 0.1 msec. Shown are data from stimulation intensities chosen to elicit approximately a 1-mV population spike in the control period before steroid treatment. We used the same stimulus protocols before and after steroid administration.
11. The normal incubation medium (Earle's solution) in the slice chamber was replaced with medium containing steroid by means of a push-pull syringe system. Undisturbed electrophysiological recordings can be maintained during and after the 5-minute period required for medium exchange. The dose was chosen to approximate physiological concentrations of steroid.
12. Medium exchange in the controls yielded either no change or a transient increase (10 to 12 percent) in population spike amplitude that recovered to preexchange values within 10 minutes. The transient enhancement of excitability in control experiments may be attributed to the effect of perfusion with fresh medium or the elimination of metabolic products.
13. F. Stormshak, J. Harris, R. Gorski, in *Receptors and Hormone Action*, B. O'Malley and L. Birnbaumer, Eds. (Academic Press, New York, 1978), p. 63; I. Lieberburg and B. McEwen, *Endocrinology* **100**, 588 (1977).
14. T. Lloyd and J. Weisz, *J. Biol. Chem.* **253**, 4841 (1978).
15. I. Kamberiand and Y. Kobayashi, *J. Neurochem.* **17**, 261 (1970); V. Luine *et al.*, *Brain Res.* **86**, 293 (1975); J. D. Barchas, H. Akil, G. R. Elliott, R. B. Holman, S. J. Watson, *Science* **200**, 964 (1978).
16. P. Schwartzkroin, *Brain Res.* **85**, 423 (1975).
17. K. M. Kendrick and R. F. Drewett, *Science* **204**, 877 (1979).
18. R. Pietras and C. Szego, *Nature (London)* **265**, 69 (1977).
19. F. Naftolin and K. Ryan, *J. Steroid Biochem.* **6**, 993 (1975).
20. G. Raisman and P. Field, *Brain Res.* **54**, 1 (1973); B. McEwen, I. Liberburg, N. Maclusky, L. Plapinger, *Ann. Biol. Anim. Biochim. Biophys.* **16**, 471 (1976); F. Nottebohm and A. P. Arnold, *Science* **194**, 211 (1976); A. P. Arnold and A. Saltiel, *ibid.* **205**, 702 (1979).
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Ferritin Synthesis by Human T Lymphocytes

Abstract. *Mononuclear cells from peripheral blood of normal humans, unselected spleen cells from patients with Hodgkin's disease, and selected T and non-T lymphoid cells from normal peripheral blood and from the spleens of Hodgkin's disease patients were examined for de novo synthesis and secretion of ferritin. After precipitation of labeled lysates and supernatants from unseparated and selected T cells with antiserum to human liver ferritin, two bands were visible on sodium dodecyl sulfate–polyacrylimide gel analysis. The two bands were detected in molecular weight regions 19,000 and 21,000, which are thought to represent the L and H subunits of the ferritin molecule, respectively. The slower band (subunit H) was more radioactive than the faster band (subunit L). The H subunit is found in greater amounts in the serum of some tumor patients, but its cellular origin has not been established. The present findings indicate that cells of the immune system contribute to the synthesis and secretion of a ferritin molecule with a high proportion of H subunits.*

It has been suggested that cells of the immune system participate in the recognition and binding of iron as part of their postulated role in surveillance (*1*, *2*). We have examined several aspects of the interaction of iron and iron-binding proteins with lymphoid cells (*3*-*6*) and macrophages (*7*, *8*). In a recent immunofluorescence study of the distribution of transferrin, ferritin, and lactoferrin in selected populations of human peripheral blood lymphoid cells, we observed an association of transferrin and ferritin with T lymphocytes (erythrocyte rosette-forming cells) but not with B lymphocytes (surface immunoglobulin-bearing) (*9*). The presence of intracytoplasmic transferrin and ferritin in T lymphocytes could constitute a basis for the control exerted by T lymphocytes on a number of other biological systems known to require iron for their normal function, such as erythropoiesis (*10*), cell division (*11*), collagen synthesis (*12*), and bacterial and tumor cell growth (*13*-*16*). However, detection of proteins in cells by immunofluorescence does not discriminate between uptake and de novo synthesis of these products by the cells. By using a sensitive immunoprecipitation assay developed to measure the synthesis of murine terminal deoxynucleotidyltransferase (*17*), we demonstrated that human T lymphocytes (*18*) synthesize and secrete ferritin.

Table 1. Summary of experimental data.

Source of cells (*18*)	Experiment	Cell fraction	ERFC* (%)
Peripheral blood	1	PBM	
		T	99
	2	T	96
	3	T	98
	4	T	97
	6	T	94
Spleen	7	Unseparated	
		T	
		Non-T	
	8	Unseparated	
		T	
		Non-T	
	9	Unseparated	36
		T	82
		Non-T	8
	10	Unseparated	33
		T	87
		Non-T	3

*Sheep erythrocyte rosette-forming cells.

Table 1 shows the cell populations analyzed. Synthesis and secretion of ferritin were observed in unseparated peripheral blood mononuclear (PBM) cells and spleen cells and in T lymphocytes separated from both sources (Fig. 1). After precipitation of labeled cell lysates and supernatants with antiserum to human liver ferritin and reduction of the resultant precipitates, two bands were visible in sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE). The two bands migrated to the molecular weight regions to which subunits of the human ferritin molecule were previously found to migrate (*19*, *20*). There is considerable evidence that these bands represent de novo synthesis of ferritin. First, purified, unlabeled ferritin isolated from human spleen and heart (*21*, *22*) and electrophoresed under the same conditions showed two bands in the same molecular weight regions (Fig. 1, J and K). Second, identical bands were observed with purified iodinated ferritin isolated from spleen of Hodgkin's disease patients (Fig. 1, H and I). Third, precipitation of T cell lysates with antiserum previously absorbed with purified human spleen ferritin resulted in disappearance of the bands (Fig. 1, F and G). Finally, these bands were not detectable in the same material after precipitation of control samples with normal rabbit serum (Fig. 1A).

In all metabolically labeled preparations of unseparated PBM and purified T cells, the slower band (the H subunit) was more radioactive than the faster band (the L subunit), as illustrated in the representative sample (Fig. 1, B and C). A similar pattern is also seen with Coo-

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