Telemetered Recording of Hormone Effects on Hippocampal Neurons

Abstract. *Frequency-modulated telemetry was used to record the effects of hormones on single-unit activity in the brains of freely moving rats. Corticosterone decreased unit activity in the dorsal hippocampus. Adrenocorticotrophic hormone had the opposite effect.*

Recording neuronal activity from a freely moving animal eliminates the effects of anesthesia and permits concurrent observations of behavior. For such recording telemetry is convenient because it eliminates electrical artifacts due to direct wire connections and minimizes restrictions of the animal's movement. Since [^{3}H]corticosterone is preferentially bound in the hippocampus (*1*), we have telemetered single-unit activity from the dorsal part of that structure in female rats and have discovered opposite effects of corticosterone and adrenocorticotrophic hormone (ACTH) on hippocampal neurons.

Female rats, either hypophysectomized or intact, were prepared initially under anesthesia for telemetered recording. Several electrodes made from Nichrome wires (62 μm in diameter) were lowered through a hole in the skull to the dorsal hippocampus or other selected structures. Conventional methods of recording were used during placement of the electrodes so that sites with good neural activity might be selected. Each bipolar electrode was made of two Nichrome wires twisted around each other and cut across at their tips, which were separated by about ½ mm in length. The wires were led to an Amphenol plug which was cemented to the skull (*2*).

After the animal's recovery from the operation, each electrode was tested every 2 or 3 days to see if neural activity could be recorded. When neural activity could be recorded, an experiment was conducted immediately. Recording was performed with frequency-modulated (FM) transmitters about 1 cm in diameter and weighting 2 g, including battery (*3*). The transmitter plugs onto the Amphenol connector on the skull, receiving input from the bipolar electrode and broadcasting the amplified signal to an FM tuner. The tuner's output was led directly to an oscilloscope, and from that point conventional electrophysiological displays could be used.

In other preparations, records were taken from hypophysectomized or intact female rats anesthetized with urethane (1.4 g/kg). Input from glass micropipettes filled with NaCl and used for extracellular recording was led to conventional electrophysiological amplifiers and displays (*4*). During these short-term (acute) experiments, cortical electroencephalograms (EEG) were recorded through screws in the skull and displayed on a polygraph. For both acute and long-term (chronic) recording experiments, single-unit spikes were counted by means of a Schmitt trigger and histogram-generating circuit whose output displayed on the polygraph provided an on-line quantitative record of spike discharge frequency. After all experiments, we determined electrode placements by making lesions at the site of recording and examining the electrode tracks in histological sections.

Injections of corticosterone (0.5 or 1.0 mg intraperitoneally) were usually followed by reliable decreases in telemetered hippocampal unit activity (Fig. 1, Table 1). Control injections of the vehicle (ethanol) had very small, brief effects or, more commonly, no effect at all. In contrast, injections of ACTH (10 to 50 I.U. in saline, intraperitoneally) usually gave rise to increased hippocampal unit activity. Some ACTH-induced increases were demonstrated in the same cells whose activity was depressed by corticosterone. Effects of ACTH were usually faster and shorter-lived than those of corticosterone. Corticosterone-induced decreases began 10 to 40 minutes after injection and lasted until at least 2 hours after injection, whereas ACTH-induced increases began within 3 to 10 minutes and lasted 25 to 50 minutes after injection. In two animals prepared for chronic recording the effects of both corticosterone and ACTH were repeated in different experiments on different days. Effects of corticosterone usually could not be tested twice on the same day because of the long-lasting inhibition from the first injection; characteristically the unit might for hours show only occasional "break-through" bursts of firing from the lowered rate. In contrast, effects of ACTH were often tested twice on the same unit and were more variable than those of corticosterone. When effective, injection of ACTH was often followed by bursts of activity above the control level, rather than by a smoothly increasing firing rate.

Results of conventional micropipette recording from anesthetized rats supported those from telemetered recording

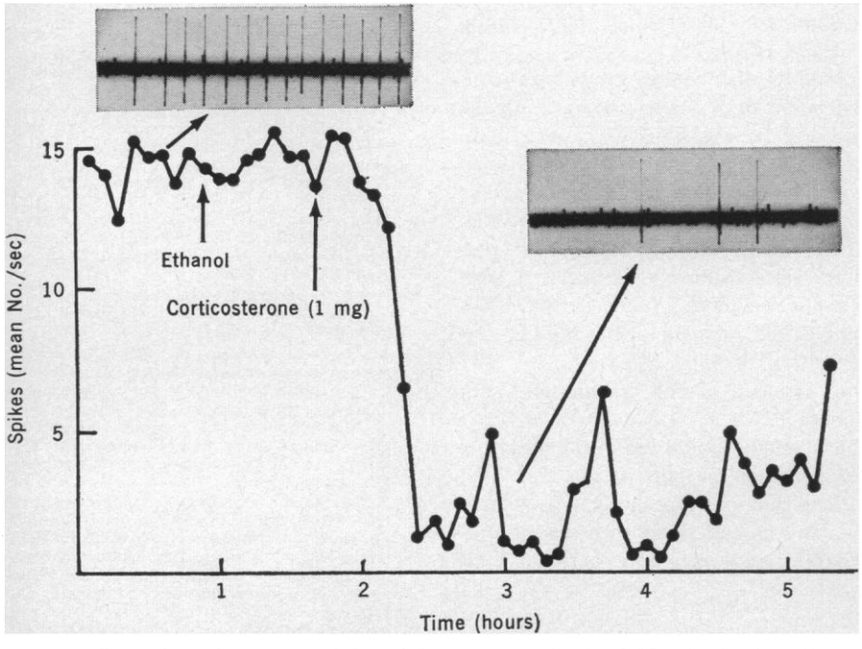

Fig. 1. Effect of corticosterone (injected intraperitoneally in 0.05 ml of ethanol) on single-unit activity in the hippocampus, recorded by telemetry from a freely moving, hypophysectomized female rat. One-second samples from the record show unit activity before (left) and after (right) injection of corticosterone.

Table 1. Effects of corticosterone and ACTH on firing rates of single units in the dorsal hippocampus of female rats.

Rats	Experiments (No.)	Effect on firing rate		
		Increase	Decrease	No change
Corticosterone				
Anesthetized	14	1	11	2
Telemetered, freely moving	9	1	7	1
ACTH				
Anesthetized	9	6	1	2
Telemetered, freely moving	8	4	0	4

Table 2. Effects of corticosterone and ACTH on cortical EEG of anesthetized female rats.

Hormone injected	Experiments (No.)	Hormone effect		
		Synchronization	Desynchronization	No change
Corticosterone	16	8	1	7
ACTH	8	1	1	6

(Table 1). Since both kinds of preparations gave similar results, it is unlikely that the effects recorded in the freely moving rats came from movement or other artifacts to which chronic recording is usually susceptible, and it is also unlikely that the effects of the hormones on hippocampal neurons are secondary to effects on behavioral activity. Histological examination showed that, in both acute and telemetered recording experiments, effects of corticosterone and ACTH were recorded from sites in the pyramidal layer and the dentate gyrus of the dorsal hippocampus.

These results indicate that ACTH and corticosterone have independent effects on hippocampal activity. Since the corticosterone effect was observed in hypophysectomized ($N = 12$) as well as intact animals ($N = 6$), it evidently was not mediated by inhibition of pituitary ACTH release. Similarly, the ACTH effect was not mediated by secretion of corticosterone because the ACTH effect was opposite to that of corticosterone, and because animals such as we used, hypophysectomized long before the experiments, secrete little steroid after a single injection of ACTH (*5*). Although steroids other than corticosterone were not used in these experiments, the pattern of uptake of radioactive hormones suggests the possibility of partial specificity: the hippocampus shows higher uptake of corticosterone than any other brain region (*1*), whereas the uptake of sex steroids is higher in the preoptic area and hypothalamus (*6*).

Our electrophysiological data fit well with the recent suggestion that ACTH has excitatory effects in the central nervous system and that corticosterone exerts the opposite influence, to counteract this excitation (*5*). We do not yet know the degree of specificity of these effects within the brain, but we have begun exploration of other anatomical locations in anesthetized animals. In nine experiments on the cerebral cortex, thalamus, and midbrain reticular formation only one corticosterone effect on unit activity has been recorded (decreased activity of a midbrain unit). However, injections of corticosterone were often followed by long periods of synchronized cortical EEG, sometimes accompanying the effect on hippocampal unit activity (Table 2).

The sensitivity of the hippocampus to hormonal influence is in keeping with other indications that this structure is affected by, and also regulates, pituitary-adrenal function. Radioactive corticosterone is concentrated more highly in the hippocampus than in other brain regions and shows nuclear uptake in hippocampal cells (*1*). Moreover, electrical stimulation of dorsal hippocampus can decrease basal concentrations of adrenal corticosteroids in the blood (*7*), whereas implantation of corticosteroids or lesions in the hippocampus are followed by increased concentrations (*8*). Although the relations between hippocampal neuronal firing and hormone secretion may not be simple or direct, these effects of stimulation, implantation, and lesions are consistent with the notion that corticosterone reduces dorsal hippocampal activity, which is in agreement with our results.

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Developing Neuromuscular Junctions: First Signs of Chemical Transmission during Formation in Tissue Culture

Abstract. *Electrophysiological study of rat neuromuscular junctions in the early stage of formation in tissue culture showed that chemical transmission begins with discrete, localized release of transmitter about the time when nerve-muscle contacts are first visible with light microscopy. Noncontractile myotubes with as few as three nuclei showed evidence of junctional transmission.*

The earliest form of chemical transmission in developing chemical synapses is unknown. In a classic case, the rat neuromuscular junction, nerves are present in developing muscle by 14 days in utero (*1, 2*), and muscle contraction in response to reflex or nerve stimulation begins at 15½ to 16 days (*3*). Studies with intracellular recording have shown that there is discrete synaptic release of transmitter at 17 days and thereafter (*4*), but there are no comparable studies before 17 days. It is unknown whether the earliest nerve-muscle contacts are functional synapses (*2, 5*) or whether the first form of neurochemical excitation of muscle occurs by long-range diffusion of transmitter (*6*). Moreover, it