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Subcortical Multiple Unit Activity and Plasma Levels of Prolactin and LH During Strychninization of the Cerebral Cortex in the Female Rat[1] (37937)

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The induction of spreading depression in the cerebral cortex of female rats is associated with changes in the multiple unit electrical activity (MUA) of preoptic and hypothalamic neurons (1) and with increments in either plasma LH (2) or prolactin (3), depending on the hormonal background. The consistent and relatively long-lasting (15–30 min) drop in subcortical MUA in response to spreading depression suggested that it might reflect a lack of stimulatory input to neurons inhibiting gonadotropin release, i.e., suppression of inhibition, resulting in elevated plasma hormone concentrations.

During recovery from spreading depression the cortical EEG record contained spikes suggestive of seizure activity. Wondering whether this aspect of the SD sequence was influencing hormonal secretion we looked for methods of duplicating it. Among convulsant drugs, the ability of topical application of strychnine to increase cortical excitability is well known. We therefore applied strychnine to the cortex of the rat brain, and we now report that strychnine-induced cortical seizure activity fails to evoke either the subcortical MUA changes or the hormonal changes accompanying cortical spreading depression.

Materials and Methods. Adult (250–300 g) Sprague Dawley (Simonsen) female rats were maintained under controlled illumination (14 hr of light and 10 hr of darkness) and fed a standard laboratory diet. Under urethane anesthesia (130–140 mg/100 g, ip) multiple unit activity (MUA) was recorded from the medial preoptic area and hypothalamic structures following the coordinates of the de Groot Atlas (4). The techniques employed were similar to those described earlier (1). After obtaining a control record we applied a 2×2 mm^2 filter paper moistened with 2% strychnine to the exposed dura for 3–5 min. At the end of each experiment pentobarbital was injected to assess the level of biological activity being recorded (5). Any record which failed to show the characteristic reduction in electrical activity in response to this barbiturate was discarded. Electrode placements were marked with the Prussian blue reaction by passing 10 μA dc for 10–15 sec and perfusing the brain with potassium ferrocyanide and formalin; it was then sectioned and strained with carbol fuchsin for histological examination.

Hormonal studies were performed in two groups of animals primed for either prolactin or LH release. The former group (PMS-hCG) were treated initially with 300 IU PMS (NIAMD-PMSG-1, NIH) and 53–56 hr later with 120 IU hCG (Pregnyl, Organon), and used 7 days after PMS administration. The latter group (OVX-E_2-P) was treated, 1–2 months postovariectomy, with a single injection of estradiol benzoate (50 μg in 0.05 ml) and progesterone (25

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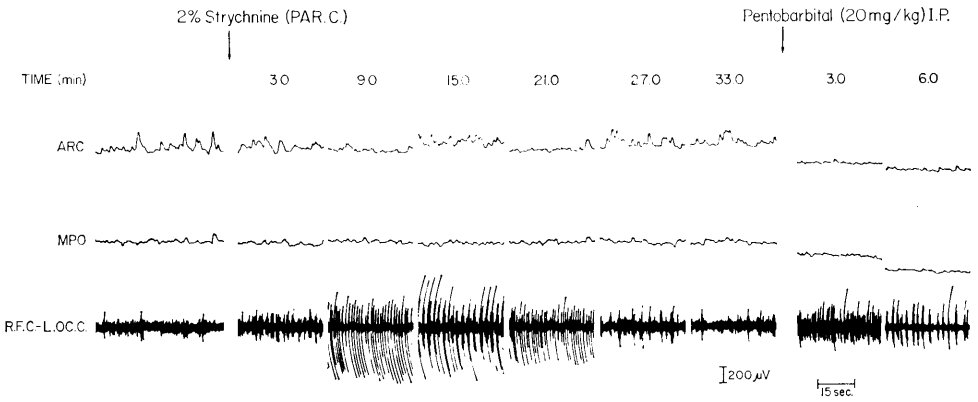

FIG. 1. Effects of strychnine applied to the parietal cortex (PAR.C.) on the MUA of the arcuate nerve (ARC) and medial preoptic area (MPO). Cortical EEG was recorded between the right frontal cortex (R.F.C.) and left occipital cortex (L.O.C.C.).

mg in 0.5 ml) and used 3 days later. Throughout the experimental period the rats were kept under continuous ether anesthesia. An opening in each frontal bone was made, avoiding damage to the dura. Three screws were fixed to the skull for EEG recordings. The right femoral artery was cannulated with polyethylene tubing containing heparinized saline for blood sampling. Forty-five to sixty minutes after completion of surgery 0.5-ml samples were taken each 20 min. Blood cells were resuspended in saline and returned to the animal after the following sampling had been taken. After two control samples, 2% strychnine was applied to the exposed dura for 10–15 min. The dura was then rinsed with normal saline, and sampling continued for another 100 min. Plasma samples were stored frozen until assay was conducted.

Luteinizing hormone and prolactin were measured by radioimmunoassay in duplicate using NIAMD Rat LH RP-1 and NIAMD Rat Prolactin RP-1 as standards. Means were compared applying Student's *t* test.

Results. Topical application of strychnine sulfate elicited patterns of cortical seizures activity within 2–3 min which lasted for periods up to 20–30 min (Figs. 1 and 2). During the periods of cortical spiking the

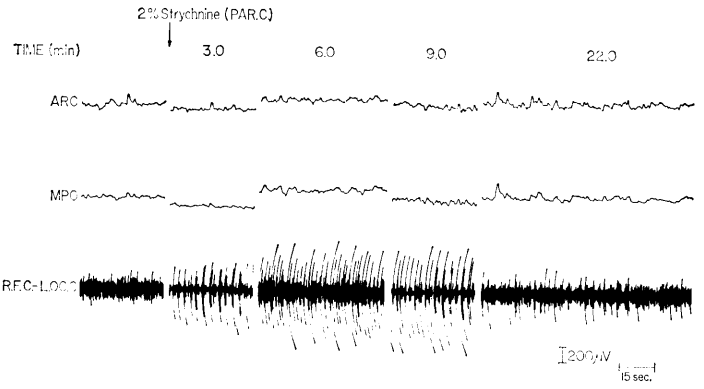

FIG. 2. Effects of strychnine applied to the parietal cortex (PAR.C.) on the MUA of the arcuate nerve (ARC) and medial area (MPO). Cortical EEG was recorded between the right frontal cortex (R.F.C.) and left occipital cortex (L.O.C.C.). Note that integrated MUA levels parallel the changes in amplitude of tonic EEG activity as reflected in the height of the solid black EEG record.

tonic EEG activity often showed changes in amplitude and such alterations as occurred in the levels of integrated MUA in the arcuate nucleus and medial preoptic area paralleled these tonic EEG changes. The cortical spiking *per se* did not appear to exert any sustained influence on subcortical integrated MUA, and the fluctuations in MUA were relatively minor.

The effects of cortical strychnization on plasma concentrations of prolactin and LH are summarized in Table I. The control levels of both hormones in PMS-hCG and OVX-E_2-P groups are comparable to those reported previously (2, 3). It is apparent that cortical seizure activity induced by the topical application of strychnine does not alter plasma levels of either of these two pituitary hormones even under optimal conditions: PMS-hCG for prolactin and OVX-E_2-P for LH.

Discussion. In contrast to the present results, the induction of cortical spreading depression by application of 25% KCl was followed by marked elevations of plasma prolactin in the PMS-hCG group and LH in the OVX-E_2-P animals, both reaching significance in 40–60 min (2, 3). We suggested that the differential responsiveness depended on the particular steroid environment fostered by the priming treatments (6, 7).

In relation to spreading depression, during recovery from the low tonic activity immediately following application of KCl there is a period in which the cortical EEG shows a pattern of grouped high amplitude spikes mounted on a low amplitude tonic activity. Concomitant with these changes in cortical EEG there appear in the MPO and other subcortical structures short-term, abrupt increases in MUA (1). The temporal relationship of these sudden increases in MPO activity with cortical spiking is difficult to ascertain with our present methods, but the indication is that they are coincident. In the present study, the cortical spiking induced by strychnine appears to be quite different from that seen in recovery from spreading depression. During the period of strychnine-induced cortical spiking, in which there was no change in tonic activity, no changes in MUA of the MPO can be seen.

Actually, the overall effect of strychnine may well be, in spite of the similarities in cortical spiking, quite the opposite of spreading depression. This is suggested by their contrasting influences on cardiorespiratory regulation (8) and subcortical thresholds of activation (9). Perhaps the effects of these two divergent experimental approaches on the hormonal responses should be subjected to a known standardized stimulus as was done effectively relative to thresholds in the amygdala and hippocampus by Kreindler and Steriade (9). Previous reports in the rabbit in which several convulsant drugs were tested in relation to the induction of ovulation (10, 11) also indicate the need for further experimentation in this area.

Summary. Under urethane anesthesia the application of 2% strychnine sulfate to the cerebral cortex of female rats induced seizure activity. Multiple unit electrical activity recorded simultaneously from the medial

TABLE I. Plasma Prolactin and LH Concentrations after Cortical Strychninization under Ether Anesthesia (ng/ml RP-1).

		Sampling time (min)						
	N	−20	0	20	40	60	80	100
Prolactin (PMS-hCG)	8	238.8 ±26.3[a]	248.1 ±24.1	236.5 ±36.9	239.3 ±34.2	203.8 ±42.0	213.8 ±37.8	226.3 ±38.5
Prolactin (OVX-E_2-P)	4	208.5 ±54.1	203.8 ±57.2	194.3 ±44.8	194.3 ±44.8	233.5 ±34.5	276.0 ±46.1	180.0 ±25.0
LH (OVX-E_2-P)	9	94.5 ±9.7	113.5 ±18.4	121.9 ±16.2	114.1 ±19.1	110.9 ±15.3	109.7 ±15.2	109.6 ±15.0

[a] Mean ± SE.

preoptic area and basal hypothalamus showed significant changes only when the amplitude of the tonic EEG activity rose or fell. This suggests that tonic cortical activity is the crucial factor relating EEG events with preoptic or hypothalamic MUA during cortical strychninization. Plasma prolactin and LH levels in PMS-hCG and OVX-E_2-P treated animals were not modified by the application of strychnine to the cerebral cortex. The results suggest that spreading depression is a more potent influence on pituitary function by virtue of its demonstrated effects on subcortical activity in preoptic and hypothalamic areas.

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