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Methohexital, succinylcholine, ECS, and the estrous cycle

MARGARET SEATON, GAIL VANCE, ELEANOR JONES, and JOEL S. MILNER
Western Carolina University, Cullowhee, North Carolina 28723

The effects of methohexital, succinylcholine, and electroconvulsive shock (ECS) on the estrous cycle were investigated. Eighty-eight rats, chosen on the basis of estrous stability, were selected from a subject pool of 106 animals and were randomly assigned to one of eight treatment conditions, with the restriction that each condition contain 11 animals. Treatments were as follows: one ECS, five ECSs, one ECS with methohexital and succinylcholine, five ECSs with methohexital and succinylcholine, one sham ECS (SECS), five SECSs, one SECS with methohexital and succinylcholine, and five SECSs with methohexital and succinylcholine. Consistent with previous reports, the five-, but not the one-, ECS treatment produced a significant delay in estrus. However, the data indicated that when five ECS treatments were given to rats that had received methohexital and succinylcholine, no significant delay in estrus was observed. No estrous delays were observed following any of the remaining treatments, nor were additional delays observed after cycling began in the five-ECS treatment conditions.

The authors of two initial studies on electroconvulsive shock (ECS) and the estrous cycle report different outcomes following ECS treatment of rats. Jensen and Stainbrook (1949) found that arrays of 3, 5, and 15 ECS treatments each produced a delay in the onset of estrus. Once estrus began following each treatment, all animals resumed their regular cyclic activity. In contrast, Woolly and Timiras (1962), as part of an investigation of the relationship between estrus and brain excitability, observed that neither ECS-induced clonic nor tonic-clonic seizures altered the regularity of estrus. Even though shock intensity and duration, rat strain, number of treatments, and interval between treatments varied between these two studies, the fact that Woolly and Timiras did not observe estrous disruption is difficult to explain.

In a more recent series of studies (Green, Seaton, Williams, & Milner, 1981; Milner, Gilbert, & Prewett, 1978; Milner & Green, 1980), multiple ECS treatments were observed to delay estrus in the rat. More specifically, although 1 or 2 ECS treatments were not found to disrupt the estrous cycle, arrays of 3, 4, 5, 10, 15, and 20 ECS treatments were found to delay estrous onset. Furthermore, the delay appears to increase as the number of ECS treatments increase. As in the Jensen and Stainbrook (1949) study, once cycling began following each treatment array, no additional delays were observed.

Although the disruption of estrus by multiple ECS treatments has been relatively well established, studies investigating ECS effects on estrus have always given ECS treatments without the concurrent administration of either an anesthetic or an anticonvulsant. The use of such drugs would produce a procedure more comparable to that typically employed in ECS treatments of humans. More important, since many drugs are known to affect the reproductive cycle and since a drug or a combination of drugs may interact with ECS to produce unique effects, the present study investigated the effects of a barbiturate (methohexital), a muscle relaxant (succinylcholine), and ECS on the estrous cycle in rats.

METHOD

Subjects

One hundred and six naive female Sprague-Dawley albino rats arrived in the laboratory at 56 days of age. These rats, which were the initial subject pool, were placed in individual stainless steel cages that measured 40 x 24 x 19 cm. Throughout the experiment, animals were maintained on an ad-lib food and water schedule.

Procedure

Seven days after arrival in the laboratory, all 106 rats were given daily vaginal smears for a period of 56 days. Eighty-eight rats, chosen on the basis of stability of estrus, were selected from the initial subject pool of 106 animals. Stability of estrus was defined as continuous cycling on a regular interval basis for at least five complete cycles immediately preceding the application of treatment.

The 88 selected rats were assigned randomly to one of eight treatment conditions with the restriction that each condition contain 11 subjects. Treatment conditions were as follows: one ECS, five ECSs, one ECS with methohexital and succinylcholine, five ECSs with methohexital and succinylcholine, one sham ECS (SECS), five SECSs, one SECS with methohexital and succinylcholine, and five SECSs with methohexital and succinylcholine.

Following placement in the treatment conditions, the rats received the assigned treatment. A Grason-Stadler shocker was employed to deliver the ECS. The ECS consisted of a 30-mA constant current .5 sec duration from a 28-V (50- to 60-Hz) power source. The ECS was administered via saline-soaked, gauze-covered ear clips and was sufficient to induce a complete tonic-clonic convulsion. The SECS consisted of attaching the saline-soaked, gauze-covered ear clips to each rat for approximately 5 sec without delivery of shock.

When appropriate, methohexital, 40 mg/kg of body weight, was administered 7 min prior to delivery of ECS or SECS, and succinylcholine, .5 mg/kg of body weight, was injected 3 min

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Table 1
Mean Number of Days Until Onset of Estrus After ECS and SECS for Each Treatment Condition

Condition	T-1	1-2	2-3
1 ECS	3.5	4.3	4.8
1 ECS + MS	2.8	6.0	4.3
5 ECSs	9.0*	4.7	4.7
5 ECSs + MS	3.7	4.5	4.9
1 SECS	3.6	5.0	4.9
1 SECS + MS	4.0	4.6	4.6
5 SECSs	3.9	4.3	5.0
5 SECSs + MS	5.0	4.6	4.2

*Note–T-1, 1-2, and 2-3 = days between treatment and first estrus, first and second estrus, and second and third estrus, respectively. MS = methohexital and succinylcholine. *p < .01.*

prior to delivery of ECS or SECS. Rats in the five-shock conditions received ECS or SECS every 48 h until treatments were completed. Daily vaginal smears were continued during and following treatments. Smears were discontinued only after each subject had completed two uninterrupted estrous cycles.

RESULTS

Two animals in the five-ECS-with-methohexital-and-succinylcholine treatment group died. One rat died from an accidental drug overdose and the other rat died from undetermined causes within 24 h following the fifth ECS drug treatment.

As expected, statistical analysis revealed no significant differences ($p > .01$) in the number of days in the estrous cycles between treatment groups for the last five complete cycles immediately preceding treatments. The mean number of days until the onset of estrus after ECS or SECS for each treatment condition are presented in Table 1. A three-factor (one or five treatment applications by ECS or SECS by drugs or no drugs) analysis of variance was employed to determine treatment differences, if any, in the number of days until onset of estrus following completion of treatments. This analysis indicated a significant difference between one and five treatments [$F(1,78) = 17.16$, $p < .01$]. The Number of Treatments by ECS/SECS [$F(1,78) = 8.78$, $p < .01$] and ECS/SECS by Drug/No Drug [$F(1,78) = 15.69$, $p < .01$] interactions were also significant. No other effects were significant ($p > .01$).

Analyses of the number of days between the first and second estrous cycles following completion of treatments showed no significant differences ($p > .01$) between treatment conditions. Finally, no significant differences ($p > .01$) were found between treatment conditions in the number of days between the second and third estrous cycles following treatments.

DISCUSSION

The results indicate that although one ECS did not produce a significant delay in the onset of estrus, five ECS treatments did. These findings are consistent with those of Jensen and Stainbrook (1949), Milner et al. (1978), and Milner and Green (1980), who reported that five ECS treatments disrupt the reproductive cycle, although one does not.

Surprisingly, when the five ECS treatments were combined with methohexital and succinylcholine, no significant delay in the estrous cycle was observed. The drugs appeared to be prophylactic to the ECS. It was hypothesized that physiological stress may be a mediating factor in the ECS-induced disruption of the reproductive cycle and that the barbiturate and muscle relaxant may have served to reduce general stress, and therefore, served as a prophylaxis to estrous disruption. In order to provide preliminary data on the possibility that rats receiving five ECS treatments experience more stress than rats receiving ECS and drugs, autopsies were performed on all animals in the five-ECS and five-ECS/drug groups in order to determine the weight of the thymus and adrenal glands, which are known to increase in size and density following severe stress. The autopsies revealed no significant difference ($p > .05$) between the two groups in weights of the thymus and the adrenal glands. Athough no differences were observed in this post hoc analysis, it must be noted that the autopsies were performed after all rats were cycling regularly. Furthermore, changes in the weights of glands are a gross measure of increased glandular activity. Serum assays conducted on serum taken during the period following ECS when estrus is disrupted would have provided more meaningful data. Thus, the possible role of stress in the ECS disruption of estrus remains to be determined.

Finally, no estrous delays were oberved as a consequence of the drug injections alone. After estrus was observed following treatments, no estrous delays were observed for any of the treatment groups after cycling began following treatment.

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