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**IBOTENIC ACID-INDUCED LESIONS
OF THE MEDIAL ZONA INCERTA
DECREASE LORDOSIS BEHAVIOR
IN THE FEMALE RAT**

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ABSTRACT

In order to examine the role of the medial zona incerta in female sexual behavior, ovariectomized estrogen and progesterone treated female rats were tested for sexual receptivity following bilateral injections of the selective neurotoxin, ibotenic acid (3 ug/0.3 ul dissolved in phosphate-buffered saline) directly into the medial zona incerta. These injections produced a significant attenuation of lordosis behavior in highly receptive females when compared to saline injected controls (shams). This decrease in sexual receptivity was also reflected in a significant increase of rejections of male mount attempts. Additionally, the frequency of hopping, darting and ear wiggling was significantly decreased in ibotenic injected females when compared to saline injected controls. Consistent with previous studies these lesions produced a transient impairment of drinking behavior (hypodipsia) typical of rats with large electrolytic lesions of the ZI. This is the first report to demonstrate that mZI neurons play a role in mediating sexual receptivity in the female rat. Collectively, these results suggest that the functional integrity of the mZI is of crucial importance for the VMH-MCG mediated expression of sexual receptivity in the female rat. Ibotenic acid induced lesions of the medial zona incerta, however, did not abolish sexual behavior.

INTRODUCTION

Sexual receptivity in the female rat (lordosis) is dependent on ovarian hormones. Moreover, the hormonal induction of lordosis behavior in the laboratory rat has been shown to be dependent, at least in part, on the integrity of projections from the ventromedial hypothalamus (VMH) and the midbrain central gray (MCG). As a result, this pathway has been implicated in the regulation of lordosis behavior in the female rat (16).

One area of the brain that has received little attention with respect to lordosis behavior in the female rat is the medial portion of the zona incerta (mZI). Recently, however, anatomical as well as electrophysiological evidence indicate that the mZI may play an important role in the neural regulation of female sexual behavior (9,12-14,19,24).

In an anterograde study using the lectin *Phaseolus Vulgaris* leuco-agglutinin (PHA-L), Ter Horst and Luiten (24) reported that following iontophoretic injections of PHA-L into the ventrolateral portion of the VMH (vVMH), extensive terminal labelling was found in the mZI. In a study by Eaton and Moss (151) they examined the electrophysiological and chemical properties of neurons located within the mZI. They found that more than half of the cells that were orthodromically excited by VMH stimulation were inhibited by exogenous administration of dopamine. In that study, substances were considered to produce an effect in the firing rate of a neuron if they caused a change in firing at least 30 % of the time. Interestingly, using this criterion, three neuropeptides (B-endorphin, substance P, and CCK-8) previously implicated in the regulation of sexual behavior in the female rat (4,5,17) had little effect on mZI neurons which were orthodromically excited by

VMH stimulation. In a subsequent study, Eaton and Moss (8) reported that orthodromic excitation of the mZI following electrical stimulation of the VMH could be completely blocked by kynurenic acid, an excitatory amino acid antagonist. The authors speculate that perhaps an excitatory amino acid projection to the mZI and the MCG from the VMH plays an important role in the neural regulation of lordosis behavior in the female rat.

Although an intriguing hypothesis, presently no published studies exist in the literature that have investigated the role of the mZI in lordosis behavior in the female rat. There is evidence, however, that strongly implicates the mZI in various aspects of reproductive behavior. For example, in a retrograde neuroanatomical tract-tracing which examined the afferent projections to the midbrain central gray, Morrell et al. (15) found numerous neurons in the mZI which projected to the dorsal midbrain central gray. Furthermore, genital stimulation of female cats has been reported to produce a high density electrical response in the mZI (19). Indeed, in a combined retrograde and anterograde study by Rogers and Cadusseau (18) they report a large somatosensory input within the mZI. In fact, although the ZI received projections from many functionally distinct areas, the somatosensory input predominated.

Therefore, the purpose of the present study is to determine if a bilateral lesions of the mZI would affect lordosis behavior in ovariectomized estrogen and progesterone treated female rats.

Recently, ibotenic acid, a structural analog of glutamate is receiving considerable attention as a compound which produces selective neurotoxic lesions. Indeed, since its introduction, numerous studies have reported that injections of ibotenic acid selectively destroys neuronal perikarya without disrupting fibers of passage (10,22,23) Accordingly,

in this study, the effects of bilateral lesions of the mZI on female sexual behavior was assessed using the selective neurotoxin, ibotenic acid.

In a study by Brown and Grossman (2) they reported that bilateral lesions of the mZI produced by iontophoretic injections of kainic acid induced an impairment of drinking behavior. These animals typically consumed 20-30 % less than normal for approximately 3-5 days after surgery. Therefore, in this study, water consumption of all animals was measured. Any deficits were used as an indication of a successful mZI lesion.

GENERAL METHODS

ANIMALS:

Adult female Long Evans rats were used in this study. They were housed individually in stainless steel wire mesh cages and maintained in a temperature controlled room with a 15 hour light / dark cycle (lights off at 9PM). All animals were ovariectomized (OVX) under ether anesthesia at least one week prior to surgery. The animals weighed 160-230g at the time of OVX and 195-260g when lesioned.

IBOTENIC ACID LESIONS:

A pilot study was conducted to determine the minimal dose of ibotenic acid that produced an effective lesion without inducing sickness in the animals. Animals were anesthetized under sodium pentobarbital anesthesia and a 2 uL Hamilton microsyringe filled with ibotenic acid was inserted into the mZI using the atlas of Paxinos and Watson as a guide (11). The actual coordinates, however, were determined experimentally (AP - 2.7; ML + - 1.5; DV -7.1). Ibotenic acid was dissolved in phosphate buffered saline at a concentration of 10 ug IBO in 1 ul saline, Ph 7). Animals were bilaterally injected with various doses of ibotenic acid (IBO) 0.5 ul, 0.4 ul, and 0.3 ul and observed periodically for twenty-four hours following the injections. The injections were given over 60-90 seconds and the syringe was left in place for five minutes to prevent leakage dorsally. Animals receiving bilateral injections of 0.5 ul (5ug), and 0.4 ul (4ug) of IBO died within two hours of injection whereas those given 0.3 uL (3ug) IBO per side survived but exhibited various

behaviors including stiffening, curling posture and stereotypy from 2-36 hours following injection. The 3 ug dose was chosen to use in this experiment. All IBO injections were administered within two weeks of mixing the IBO.

BEHAVIORAL TESTING:

Tests for sexual receptivity were conducted as follows: A female rat was placed in a three-paneled wood and front-paneled clear plastic arena with a vigorous Long Evans male rat that had been adapted to the chamber. All females were injected with 10ug of estradiol and 1mg of progesterone 53 and 4 hours before behavioral testing respectively. Two behavioral indices of receptivity were used. The Lordosis Intensity Scale (LIS) and the lordosis quotient (LQ) (11). To establish the LIS, the female was placed with a sexual vigorous male until the male mounted, with pelvic thrusting ten times. The response to a mount was rated on a 0 - 3 point scale (2) as follows: 0 = no lordosis, 1 = marginal lordotic reflex, 2 = normal lordotic reflex, and 3 = exaggerated lordotic reflex. The LQ was calculated as the number of lordotic responses divided by the total number of mounts. A value for the LIS was obtained by totalling the LIS ratings and dividing by the number of mounts. A female who is not maximally receptive will display rejection behavior (11) where the female will fend off an approach of the male by rolling over on the back or kicking the male. This can be quantified by calculating the rejection quotient which is the total number of rejections divided by ten male mounts. A female displaying an RQ of 0 is maximally receptive (See Fig. 5). Observation and recording was terminated after ten male copulatory mounts.

HISTOLOGICAL ANALYSIS:

Following two behavioral tests, all animals were anesthetized with an overdose of sodium pentobarbital and perfused transcardially with 0.9 % saline followed by 4 % paraformaldehyde. The brains were then removed and stored in sucrose solution for a minimum of 24 hours. Sections were taken of the lesioned area and stained using crystal violet. The location and size of the lesion were determined and recorded without reference to the data.

RESULTS:

Microscopic examination of the lesioned animals revealed that 8 out of 10 had extensive bilateral cell loss along with glial cell proliferation confined to the mZI. In one animal there was no obvious cellular destruction. In another, extensive cell loss ventral lateral to the mZI which encroached on the lateral hypothalamus was observed. As a result, these two animals were eliminated from the data analysis. Seven out of eight sham injected controls had injection sites located within the medial zona incerta. One animal, however, had an injection site immediately ventral to the mZI. That left seven animals for data analysis. As illustrated in figure 2, post-operatively, animals that had confirmed lesions of the mZI were hypodipsic compared to sham injected controls ($F(3,18) = 5.67$, $p < 0.01$). Post hoc examination of the data revealed that the significant main effect corresponded to significant difference between sham and IBO lesioned animals on days 1 2 and 3.

The mean lordosis quotient (LQ) along with the mean lordosis intensity scale for two behavioral tests are presented in Figures 3 and 4. It can be seen in these figures that

bilateral injections of IBO into the mZI (3ug / cannula) induced an significant attenuation of lordosis behavior in ovariectomized estrogen and progesterone treated females when compared to sham injected animals (test 1: $t(18) = 6.56$, $p < 0.05$; test 2: $t(18) = 4.21$, $p < 0.05$). As illustrated in Fig. 5, the mean rejection quotient was also significantly higher in the lesioned females compared to saline injected controls ($t(18) = 4.34$, $p < 0.05$). These lesions, however, did not abolish sexual receptivity (Fig. 3 and 4).

DISCUSSION

The major result of this study is that cellular damage of the mZI produced by the selective neurotoxin, ibotenic acid, induced a significant attenuation of lordosis behavior in ovariectomized estrogen treated female rats. This was revealed by significant decreases in the LQ and LIS scores in ibotenic acid lesioned animals when compared to saline injected controls. In addition, a significant increase in rejecting behavior (RQ) on test 2, along with decreases in hopping and darting behavior, suggest that the mZI also has a role in regulating proceptive behaviors in the female rat. This is the first report to demonstrate that mZI neurons may play a significant role in mediating sexual receptivity in the female rat.

Studies using intracerebral implants show that estrogen action restricted to the ventromedial nucleus of the hypothalamus is sufficient to induce receptivity when this treatment is combined with systemic injections of progesterone (21). Increasing evidence suggests that estrogen acting within the VMH leads to increases in specific neuronal proteins, some of which have been shown to affect female sexual behavior (3,17). Based on numerous anatomical as well as behavior studies, one circuit that clearly regulates lordosis behavior in the female rat is the functional interaction between the VMH and the dorsal midbrain central gray (16).

Anatomical studies have shown that neurons of the VMH project to the mZI (18,24). Eaton and Moss (7,8) have reported that electrical stimulation of the medial ZI produces orthodromic excitation of VMH neurons. Although the VMH-to mZI pathway has not been considered to play an important role in the regulation of sexual behavior, Collectively, our

results strongly suggest that estrogen activates cells in the VMH, which excites neurons within the mZI. Although speculative, it can be postulated that the culmination of input of the midbrain central gray from the mZI and the VMH is of crucial importance in the neuroregulation of lordosis behavior in the female rat. Presently, it is not known what neurotransmitter(s) is used to temporally coordinate estrogen action within the VMH-mZI MCG triad and lordosis behavior in the female rat. Consequently, future studies should be done to address this question. Two candidates of immediate interest would be sP or an excitatory amino acid. Substance P has now been implicated in both male and female sexual behavior (4,5) (see (6) for review). Numerous sP cell bodies and receptors have been reported to be found within the mZI (20). Additionally, studies by Beart et al. (1) along with Eaton and Moss (7) strongly implicate the importance of an excitatory amino acid projection from the VMH to the mZI and MCG in the regulation of female sexual behavior.

In conclusion, the results of this study indicate that neuron within the medial zona incerta play an important role in the mediation of sexual receptivity in the female rat. The exact mechanism through which this the mZI exerts this control remains to be elucidated.

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FIGURE 1 Is an illustration of a coronal section of the rat brain depicting the medial zona incerta.

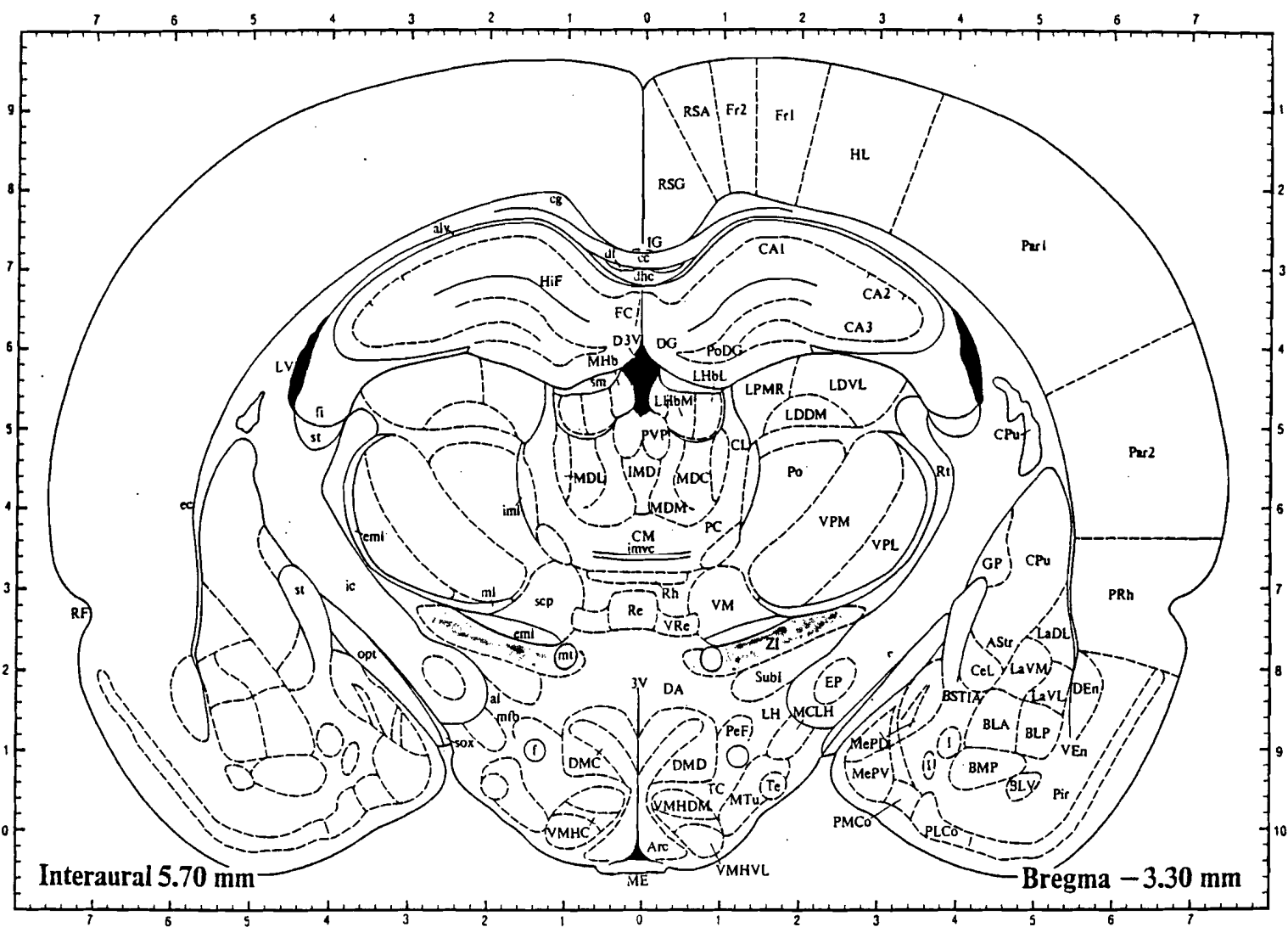


FIGURE 2 Mean daily water consumption for both groups one day before and three days after surgery.

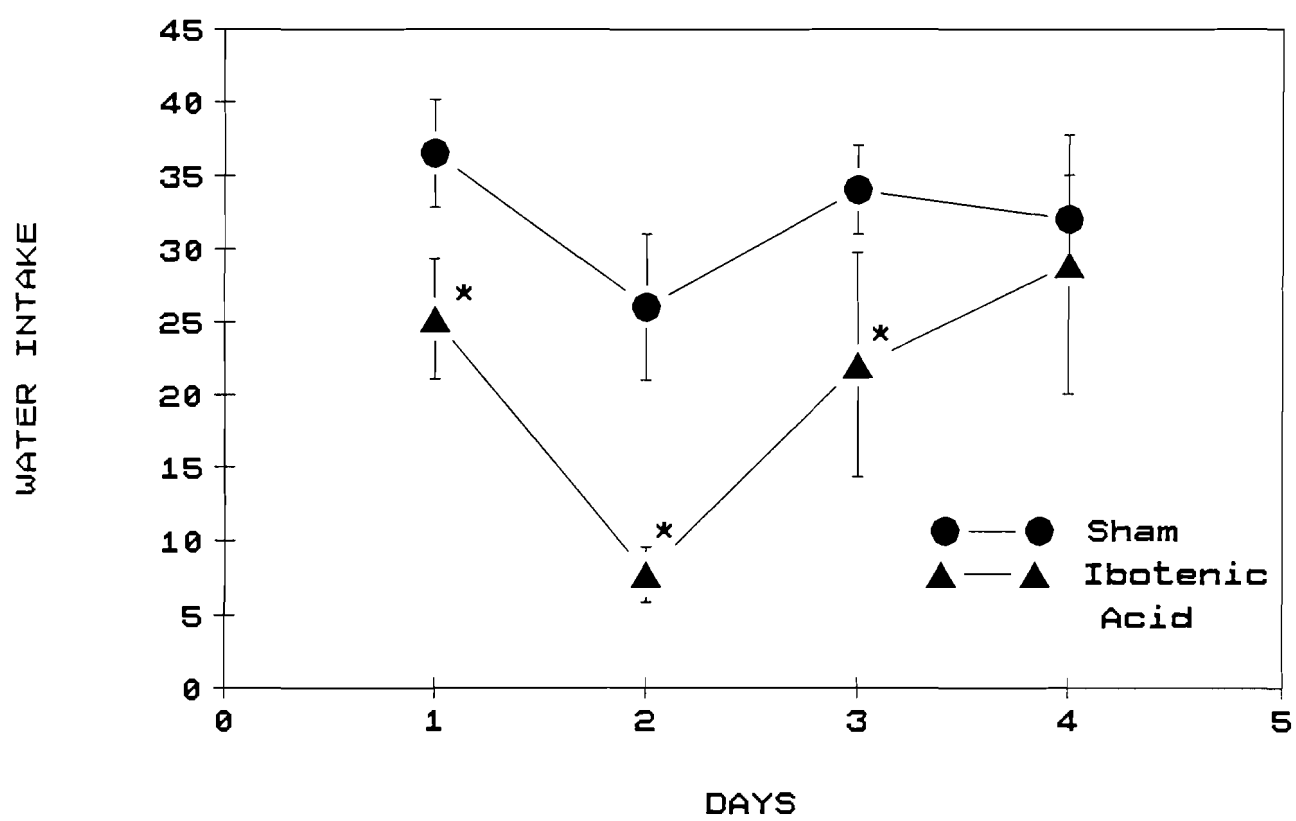


FIGURE 3 Mean lordosis quotient in response to mounting by males during 2 mating tests in females which received bilateral injections of ibotenic acid (3ug/cannula) or phosphate buffered saline (sham) into the medial zona incerta. All females were E+P primed. Vertical lines represent \pm standard errors of the mean.

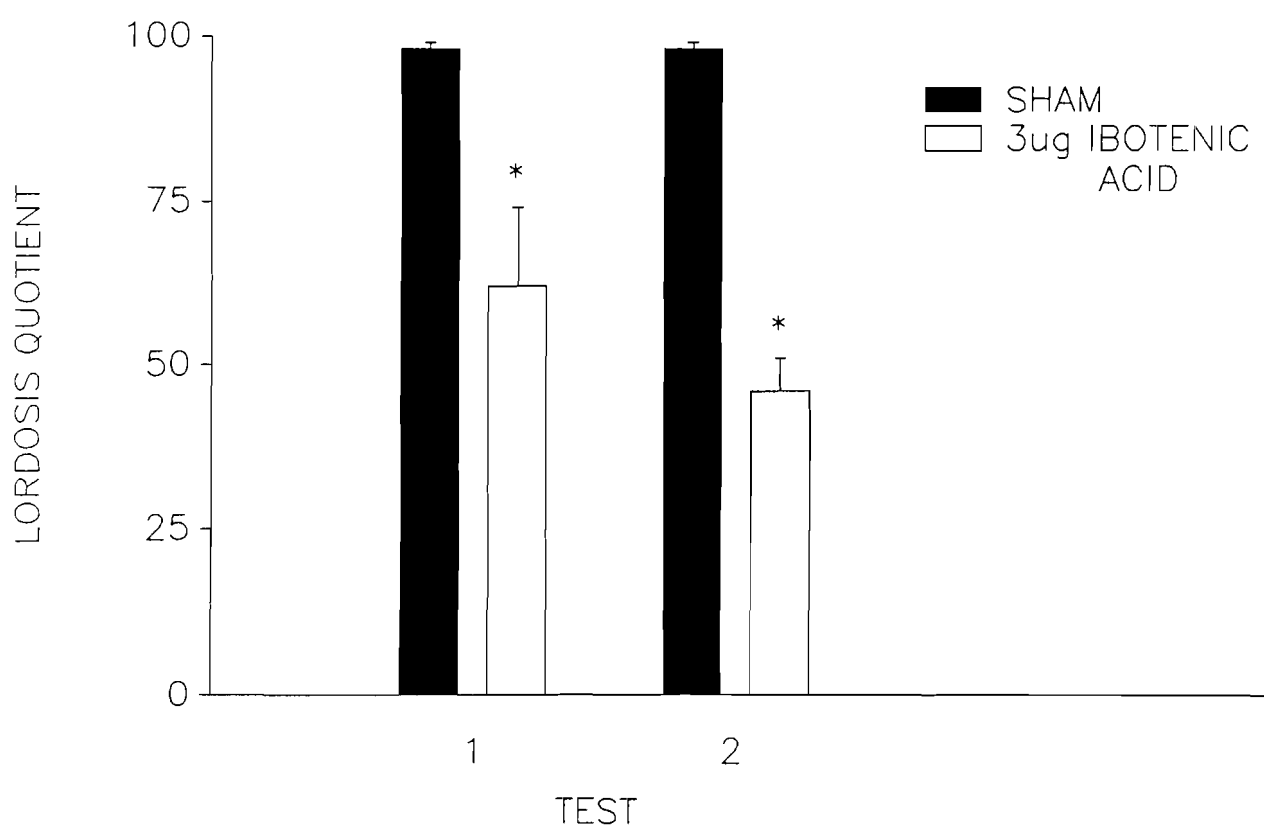


FIGURE 4 Mean lordosis intensity scores in E+P primed females reveal the effects of ibotenic acid induced lesions of the medial zona incerta on lordosis behavior during 2 mating tests. Vertical lines represent \pm standard errors of the mean.

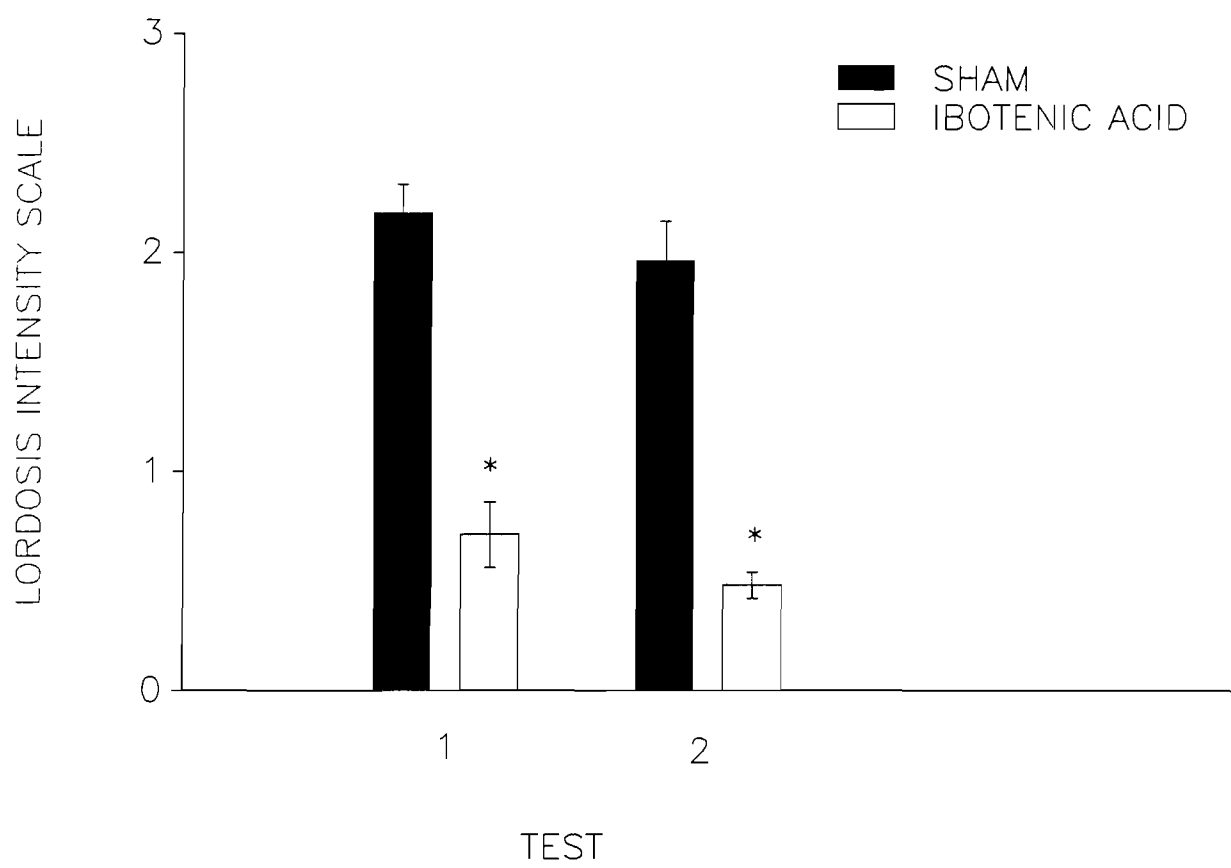


FIGURE 5 The effects of ibotenic acid induced lesions of the mZI on the mean rejection quotient during 2 mating tests. A female displaying maximal receptivity would have a rejection quotient equal to 0. Vertical lines represent \pm standard errors of the mean.

