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Long-term kindling of the basolateral amygdala impairs copulatory behavior in male rats

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Abstract

This experiment examined the consequences of long-term kindling of the basolateral amygdala on male sexual behavior and the frequency of both spontaneous wet dog shakes (WDS) and those induced by the 5-HT_{2A} receptor agonist DOI. Results demonstrated that following 60 stimulations of the left basolateral amygdala over a 4-week period, male Long–Evans rats exhibited decrements in every aspect of sexual behavior. Specifically, latencies to mount, intromit and ejaculate were all prolonged following long-term kindling, and ejaculation frequencies were significantly reduced. Furthermore, spontaneous peri-copulatory WDS were increased in kindled rats, suggesting a possible role of the 5-HT_{2A} receptor. However, countering this suggestion, there were no differences between sham and kindled rats on WDS induced by the 5-HT_{2A} receptor agonist DOI. These results suggest that kindled rats may exhibit elevated levels of endogenous serotonin during exposure to a female rat, which would attenuate copulatory behavior, while concurrently increasing WDS expression.

Keywords: Kindling; Basolateral amygdala; Sexual behavior; 5-HT_{2A}; Wet dog shakes

Kindling of structures in the limbic system, especially the basolateral amygdala (BLA), is a valid and reliable animal model of temporal lobe epilepsy [4]. Briefly, this paradigm consists of intermittent stimulations of the basolateral amygdala, which initially do not elicit any observable response, but following successive stimulations begin to elicit prolonged tonic-clonic seizures [4]. This model has proved to be fruitful on two fronts of epilepsy research: first, it is sensitive to anti-convulsant agents, suggesting its potential in examining pharmacotherapeutic regimens for the treatment of temporal lobe epilepsy [11]; second, it reliably induces inter-ictal emotional changes, such as increased fear and anxiety-like behavior, which are commonly seen in temporal lobe epileptics [7]. These findings suggest that the validity of this model may extend beyond examining the neuroplastic phenomenon associated with epileptogenesis, to include being a useful tool for examining emotional and motivational changes seen in epileptics [7].

In addition to the enhanced emotionality and increased anxiety typically seen in temporal lobe epilepsy, hyposexuality is one of the most prevalent inter-ictal disturbances seen in this population [2]. As many as two thirds of epileptic patients have been shown to display compromised sexual activity [9]. This attenuated sexual activity appears to be due to both reductions in sexual motivation [17] and impairments in the sexual arousal process [9], with some patients displaying only one of the aforementioned etiologies. Because this behavioral disturbance is so commonly associated with temporal lobe epilepsy, it is pertinent to the validation of the kindling model of epilepsy that all behavioral alterations in humans be quantified in an animal model. This experiment examined the effect of 60 successive stimulations of the BLA over a 4-week period on multiple parameters of sexual activity in sexually trained and proficient male rats.

Wet dog shakes (WDS) are a shuddering behavioral stereotypy induced by increases in 5-HT neurotransmission [3] and activation of serotonin 2A (5-HT_{2A}) receptors [19]. Given that male sexual activity is inversely correlated with WDS [18], and regulated by 5-HT_{2A} receptors (e.g., [5,6]), we also assessed the occurrence of peri-copulatory WDS, to deter-

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mine if any behavioral changes may be attributable to changes in 5-HT neurotransmission.

Twenty Long–Evans male rats (Charles River Canada Inc., Quebec) were obtained at 6 weeks of age. They were screened for sexual proficiency, and 16 animals displaying consistently vigorous sexual activity were selected for the study and housed in groups of two or three (500–700 g). In addition, 20 sexually experienced female Wistar rats were used to elicit copulatory activity in males. Females had been previously ovariectomized at 3 months of age under a combination of ketamine HCl (75 mg/kg ip) and xylazine (7 mg/kg ip) using standard surgical procedures. Females were housed in groups of three or four. All rats were housed in standard, triple wire mesh cages, in a housing environment maintained at $21 \pm 1\,^{\circ}C$. Purina Rat Chow and tap water were provided ad libitum, and colony lights were set on a 12 h/12 h light cycle with the lights off at 09:00 h. Stimulation and behavioral testing occurred during the dark cycle in which the animal is in its behaviorally active phase.

Sixteen rats were implanted at 20 weeks of age with single bipolar electrodes (MS-303-2; Plastic Products Company) in the left BLA under ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) anesthesia following standard stereotaxic protocols. The electrode tip was aimed 2.8 mm posterior, 5.0 mm left, and 9.0 mm ventral to the skull surface at bregma. The incisor bar was set at −3.3 mm, and all coordinates were derived from [13]. The electrode was secured to the skull with four stainless steel screws and dental acrylic. During a postsurgery recovery period of 10 days, each rat was habituated to the stimulation lead and handled for 2 min. Following recovery, animals were randomly divided into two groups: sham ($n = 8$) and kindled ($n = 8$). The kindled animals were stimulated two to three times a day, averaging 15 stimulations a week over a 4-week period. On each stimulation trial, the rat was removed from its cage, carried to the apparatus, attached to the stimulation lead and allowed to move freely around the test chamber for 30 s. After 30 s, the experimenter pressed the button on the stimulator to deliver a brief amygdalar stimulation (1 s, 60 Hz, 400-μA rms, sine wave). After stimulation offset, the stimulation lead was promptly removed, and the rat remained in the test chamber for an additional 120 s before it was returned to its home cage. Each convulsive response was rated according to Pinel and Rovner's extension (1978) [14] of Racine's [15] widely used five-class scale of limbic convulsion severity (Class 1: facial movements only; Class 2: facial movements and head nodding; Class 3: facial movements, head nodding, and forelimb clonus; Class 4: facial movements, head nodding, forelimb clonus, and rearing; Class 5: facial movements, head nodding, forelimb clonus, rearing, and falling once; Class 6: presence of Class 5 criteria along with multiple rearing and falling episodes; Class 7: presence of Class 6 criteria along with running fits; Class 8: running fit with periods of tonus). Seizure class was monitored to ensure that all subjects were progressing at the same rate. Sham stimulations were performed identically, and stimulation leads were attached for comparable durations; however, no current was conducted through the lead.

Following 60 stimulations, males were tested for copulatory behavior and WDS, 24 h following the final stimulation. Females were injected with 10 μg estradiol benzoate 48 h prior, and 500 μg progesterone 4 h prior to copulatory testing to induce estrus. All testing occurred in Plexiglas chambers (30 cm × 30 cm × 45 cm in height) covered with contact bedding. Males were given a 5-min habituation time before presentation of sexually receptive females. Testing occurred over a 30 min period during the middle third of the dark cycle and the parameters of sexual behavior that were assessed were mount, intromission, and ejaculation latencies as well as ejaculation frequency. In addition, the frequencies of WDS were tallied for the 30-min testing period. Following the sexual behavior testing, males were given an injection of saline and placed alone in observation bins for 15 min to permit scoring of the occurrence of spontaneous WDS in the absence of a female. Following this test, males were injected intraperitoneally with 1 mg/kg of the 5-HT_{2A} agonist (+/−)1-(2,5 dimethyl-4-iodophenyl)-2-aminopropane (DOI; Research Biochemicals International), and monitored again for the next 15 min on the occurrence of WDS.

At the conclusion of the experiment, all rats were killed with CO_2 according to the Canada Council on Animal Care guidelines. Their brains were removed and preserved in formalin for 24 h. The brains were then frozen and sectioned along the coronal plane through the amygdala. Each section was 35 μm thick, and every fourth section was mounted on a slide and stained with cresyl violet. The position of each electrode tip was estimated from the stained slides through the use of a light microscope and the stereotaxic atlas [13].

Histological analysis verified the placement of electrodes within the BLA. However, two animals from the kindled group and two animals from the sham-kindled group had electrodes that were in the boundaries of the central nucleus of the amygdala and were removed from any further analysis.

Results from this experiment demonstrated that long-term kindling of the BLA resulted in reduced copulatory behavior. Specifically, it was found that animals that had been kindled displayed longer latencies to engage in mounting behavior [$t\ (10) = -3.75$, $p < .005$] and intromitting behavior [$t\ (10) = -3.39$, $p < .01$], and to achieve ejaculation [$t\ (10) = -2.59$, $p < .03$] compared to sham stimulated animals. Furthermore, kindled rats exhibited a lower frequency of ejaculations than sham stimulated rats [$t\ (10) = 2.86$, $p < .02$]. WDS that were scored during the peri-copulatory period were also found to be elevated in kindled animals [$t\ (10) = -3.46$, $p < .01$]. Data representing copulatory behavior are presented in Table 1. Since WDS reflect 5-HT_{2A} receptor activity [19], subsequent testing examined kindled verus sham-stimulated rats in their WDS response to administration of the 5-HT_{2A} receptor agonist DOI. While it was found that DOI significantly increased WDS frequency [$t\ (22) = -3.07$, $p < .01$],