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Prenatal stress suppresses the prefrontal and amygdaline EEG changes associated with a sexually-motivated state in male rats

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ABSTRACT

The medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) participate in the modulation of several motivated behaviors, such as the sexual behavior. Both structures are sensitive to stress when it is experienced mainly in critical periods of the life-cycle, such as the prenatal period. This study evaluated the effects of prenatal stress on electroencephalographic activity (EEG) of the mPFC and BLA during sexual motivation. EEG was recorded in the mPFC and BLA of male rats assigned to either a prenatally-stressed group (SG, dam immobilized from days 14 to 21of pregnancy), or a control group (CG), during the following conditions: awake-quiet state without sexual motivation, and awake-quiet state with sexual motivation. Compared to CG, fewer SG subjects presented copulatory responses and their levels of sexual motivation were lower. The CG subjects with sexual motivation showed a higher absolute power (AP) of the 14–30 Hz band in the left mPFC and BLA than those without sexual motivation. The SG showed a lower AP of the 4–7 and 8–13 Hz bands in the left BLA. Thus, prenatal stress suppressed the prefrontal and amygdaline EEG changes associated with a sexually-motivated state. EEG data show that stress affects the functioning of these two brain structures and so could interfere with the adequate processing of sexual stimuli. These findings contribute to understanding the brain mechanisms that underlie the effect of prenatal stress on the processing of sexual stimuli in male rats.

1. Introduction

Stress affects the expression of several physiological and behavioral indices, though its effects vary depending on the nature, intensity and temporality of the stressors involved [1–3], as well as of the period of the life-cycle during which it is experienced. When stress is felt during critical periods, such as the prenatal and pubertal stages, its effects can last into adulthood, altering behavioral, hormonal, neural and motivational aspects [4–9]. For example, it has been reported that prenatally-stressed rats present reduced levels of *N*-acetyl-aspartate [10] and alterations in the concentration of dopamine receptors in the medial PFC (mPFC) [11], as well as reduced excitability in the neurons of the basolateral amygdala (BLA) [12]. These two structures (mPFC and BLA) have also been implicated in the regulation of sexual behavior, since they participate in detecting and processing sexually-related stimuli to induce sexual motivation and arousal [13,14]. One study that reveals the role of the mPFC in sexual motivation was performed by Ågmo et al. [13], who made ablations of medial and dorsal PFC in male rats, and observed an increase in mount and intromission latencies (> 60 min) in the presence of a receptive female. Another study, in which EEG activity in the mPFC was evaluated in males during a sexually-motivated state (i.e., when exposed to a receptive female after an intromission), showed an increased absolute power (AP) of the low frequencies (7–12 Hz), suggesting that during the state of sexual motivation, this structure could be processing information related to the sexual stimulus [15]. The participation of the basolateral amygdala (BLA) has also been demonstrated; for example, in a study in which the BLA of male rats was inactivated with tetrodotoxin, was founded that the rats showed decreased sexual motivation, as reflected in higher mount, intromission and ejaculation latencies [14].

It has been reported that prenatal stress alters the functionality of cerebral structures implicated in regulating sexual behavior, and that sexual performance in adult male rats is highly-sensitive to prenatal stress, as this induces increased mount and intromission latencies while

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decreasing the proportion of subjects that copulate [4–8]. Moreover, several studies support the notion that subjecting the mother to stress does indeed affect fetal development [16,17], and that the effects on the cytoarchitecture and functionality of certain brain structures can persist into adulthood [10,11,18,19], thus altering sexual behavior [5,9,20]. One of the methods most often used to induce prenatal stress in rats is immobilization, which has been shown to produce impairments in sexual behavior [9]. In fact, a previous study demonstrated that rats prenatally-stressed by immobilizing the dam exhibit low frequencies of genital grooming and spontaneous penile erections during puberty, with increased mount and intromission latencies in adulthood [20].

Considering that the mPFC and BLA play a crucial role in the correct detection and processing of sexually-relevant stimuli –elements essential for inducing a sexually-motivated state– and that sexual behavior and brain functionality are affected by prenatal stress, the aim of this study was to determine the effects of prenatal stress on the EEG activity of the mPFC and BLA during a sexually-motivated state in adult male rats. We hypothesized that the prenatally-stressed rats will present a different EEG functionality in the mPFC and BLA, and that these EEG changes will be associated with decreased sexual motivation.

2. Method

2.1. Animals

Twelve pregnant Wistar rats were obtained from a colony bred at the Institute of Neurosciences at the University of Guadalajara. These dams were maintained under a 12/12-h light/dark cycle (lights on at 08:00 h) at 22 ± 1 °C, with food and water ad libitum during gestation and lactation. On days 14–21 of pregnancy, 6 of the dams were randomly assigned to a stress group (SG), and the other 6 to a control group (CG). On the experimental days, the dams from both groups were kept for two hours in a soundproofed room, but only the SG females were placed in Plexiglas animal holders (17 × 7.5 × 5.8 cm) once per day for two hours (11:00 h–13:00 h), following the protocol for immobilization-induced stress modified by Velázquez-Moctezuma, et al. [9]. The control dams remained in their home cages in the soundproofed room, also for two hours.

All the male rats used in this study were the progeny of these 12 females. At 22 days of age (day of birth = 1), all pups were weaned and sexed. Both groups were nourished with food and water ad libitum throughout the experiment. Animal care and all procedures involving the rats were approved by our Institutional Animal Care and Use Committee, in accordance with NIH specifications.

2.2. Sexual interaction tests

The pregnant females used in this study gave birth to 49 male pups, which made up the control group (CG), and 56 more male pups that were assigned to the stressed group (SG). At the age of 3 months (approximate weight = 250–350 g), all 105 male rats were submitted to three copulatory tests to select the sexually-experienced individuals that would participate in the study. All behavioral tests were conducted between 10:00 and 13:00 h; i.e., during the dark phase. The following copulatory parameters were measured: 1) mount latency (ML): time in seconds from the entrance of the sexually-receptive female into the observation box to the first mount with pelvic thrusting but no vaginal penetration; 2) intromission latency (IL): time in seconds from the entrance of the sexually-receptive female into the observation box to the first mount with pelvic thrusting and behavioral signs of vaginal penetration; 3) ejaculation latency (EL): time in seconds from the first intromission to the behavioral pattern indicative of ejaculation; and 4) inter-intromission period (IIP): mean time in seconds that elapsed between each intromission. Only those subjects that reached ejaculation in at least two of the three tests, and that presented intromission in all three tests were considered sexually-experienced.

2.3. Stereotaxic surgery

Only the sexually-experienced male rats of each group were bilaterally implanted (CG, n = 27; SG, n = 20). For the surgical procedure, they were first injected with atropine sulfate (0.1 mg/kg) to block the depressant effects of barbiturates on the heart, and then anaesthetized with sodium pentobarbital (47 mg/kg i.p.). Stainless steel electrodes (0.2 mm in diameter) were implanted bilaterally in both hemispheres, specifically into the prelimbic area (mPFC) (3.2 mm anterior to bregma, 1 mm lateral to midline, and 4.0 mm below the duramater), and the basolateral amygdala (ABL) (2.8 mm posterior to bregma, 5 mm lateral to midline, and 8.4 mm below the duramater), while the incisor bar was set at − 3.3 mm, in accordance with Paxinos and Watson's stereotaxic atlas (2007). Two stainless steel screws were placed in the anterior and posterior parts of the skull to serve as reference and ground electrodes, respectively. All electrodes were attached to a miniature connector fixed to the skull with stainless-steel hooks and acrylic cement. Adequate care was taken to minimize pain and discomfort in the animals throughout the experiment. After surgery, all rats were housed in individual cages (15 × 15 × 30 cm) with food and water ad libitum. The post-surgical recovery period was 7 days.

2.4. Recording conditions

For EEG recording, a transparent Plexiglas testing chamber (64 cm × 40 cm × 34 cm) divided in two equal compartments by means of a transparent acrylic partition with several 2-mm diameter holes was used. Each compartment measured 32 cm × 40 cm × 34 cm. A male was placed in one compartment, while a sexually-receptive female was placed in the other. Both rats could see, hear and smell each other, but no direct contact between them was possible.

After the post-surgical recovery period, and one day before the experiment, the rats were taken to the recording room, which was soundproofed, semi-dark and at ambient temperature. There, they were connected to the polygraph for adaptation during 30 min.

During the experiment, each male rat was placed in one compartment of the testing chamber and the EEG was recorded during 5 min (awake-quiet state without sexual motivation). After that, a sexually-receptive female was placed in the same compartment with the male. The male was allowed to have one intromission with her (to induce the sexually-motivated state). Immediately afterwards, the same female was moved to the adjacent compartment and EEG was recorded during the awake-quiet state with sexual motivation, also during 5 min.

2.5. EEG recording

Each rat was connected to a Model 7B GRASS polygraph with a recording window of 3–30 Hz. The polygraph was attached to an analogue-digital converter (CAD, Advantech Co., Mod. PCL-812). The sample rate was set at 1024 Hz, and all EEGs were stored on a PC for analysis off line. EEG was recorded specifically during the two following stages: (1) awake-quiet state without sexual motivation, and (2) awake-quiet state with sexual motivation.

2.6. EEG analysis

EEG signals were carefully inspected before analysis to discard all segments that contained noise artifacts or were contaminated by movement. Only EEG recordings that were free of noise artifacts or movement were included in data analysis, such that the total number of subjects included was as follows: for CG, n = 16, and for SG, n = 14.

Absolute power [AP, defined as the power density of each frequency expressed in microvolts^2] was calculated using the Fast Fourier Transformation (FFT) in all frequencies of the power spectrum (4–30 Hz). Results were then grouped according to the traditional EEG bands for rats (4–7, 8–13 and 14–30 Hz).To approximate a normal