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ENHANCED SYNAPTIC RESPONSES IN THE PIRIFORM CORTEX ASSOCIATED WITH SEXUAL STIMULATION IN THE MALE RAT

J. G. PFAUS, T. L. M. TSE, C. M. WERK, M. L. CHANDA, A. LEBLONDE, V. L. HARBOUR AND C. A. CHAPMAN*

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC, H4B 1R6 Canada

Abstract—Male rats that copulate to ejaculation with female rats bearing an odor show a learned preference to ejaculate selectively with females that bear the odor. This conditioned ejaculatory preference reflects an association between the odor and the reward state induced by ejaculation. Although little is known about the neuronal mechanisms that mediate this form of learning, convergence of genitosensory and olfactory inputs occurs in both hypothalamic and cortical regions, notably within primary olfactory (piriform) cortex, which may be involved in the encoding or storage of the association. The present study contrasted the ability of genital investigations, mounts, intromissions, ejaculations, and a sexually conditioned olfactory stimulus, to enhance evoked synaptic field potentials in the piriform cortex. Rats in the Paired group underwent conditioning trials in which they copulated with sexually receptive females bearing an almond odor. Rats in the Unpaired control group copulated with receptive females bearing no odor. Responses in the piriform cortex evoked by electrical stimulation of the olfactory bulb were recorded in male rats as they engaged in different aspects of sexual behavior, and were also recorded after conditioning, during exposure to cotton swabs bearing the almond odor. The monosynaptic component of responses was increased during intromission and ejaculation, and the late component of responses was increased during anogenital sniffing and mounting (with or without intromission). However, no differences in the amplitudes of evoked responses were found between the Paired and Unpaired groups, and no differences in synaptic responses were found during presentation of the odor after conditioning. These data indicate that short-term alterations in synaptic responsiveness occur in piriform cortex as a function of sexual stimulation in the male rat, but that responses are not significantly altered by a conditioned odor.

Key words: sex, field potential, piriform, olfactory learning, male rat.

Learning modifies sexual responses and mate preferences in the male rat (Ismail et al., 2008, 2009; Kippin et al., 1998; Pfaus et al., 2001, 2003). For example, genitosensory stimulation from penile intromission and ejaculation crystallizes copulatory patterns (Whalen, 1961), whereas ejaculation is a necessary unconditional stimulus for sexual reward as measured by maze-learning (Drewett, 1973; Eliasson and Meyerson, 1975; Hetta and Meyerson, 1978; Kagan, 1955; Meyerson and Lindström, 1973; Warner et al., 1991; Whalen, 1961), conditioned anticipatory responses (Mendelson and Pfaus, 1989; Van Furth and Van Ree, 1996), and conditioned place preference (Ågmo and Berenfeld, 1990; Camacho et al., 2004; Hughes et al., 1990; Martinez and Paredes, 2001; Mehrara and Baum, 1990; Miller and Baum, 1987; Tenk et al., 2009). Innately attractive estrous odors stimulate mounting behavior in male rats (Beach, 1942; Lopez et al., 1999), and intermittent exposure to these odors sensitizes dopamine release in the nucleus accumbens (Mitchell and Gratton, 1991). We have also shown previously that male rats allowed to copulate to ejaculation with estrus females bearing an artificial odor (e.g., almond) come to prefer to ejaculate with sexually receptive females bearing that specific odor relative to unscented receptive females (Ismail et al., 2008, 2009; Kippin et al., 1998, 2001; Kippin and Pfaus, 2001a,b). Thus, the association of a neutral odor with the sexual reward state induced by ejaculation gives that odor sufficient conditioned incentive value to modulate partner preference and mate choice. Investigating this form of learning can provide insights into the neural mechanisms that control experience-dependent modifications in sexual behavior, and may also clarify how cortical plasticity contributes to learning.

The primary olfactory or piriform cortex (PIR) may play a central role in learning of the odor-conditioned mate preference. The PIR receives direct inputs from the olfactory bulb and has extensive reciprocal connections with other limbic and neocortical regions (Johnson et al., 2000). The PIR has been heavily investigated for its role in olfactory discrimination, and several studies have observed synaptic and neuronal alterations in PIR associated with learning olfactory discrimination tasks or tasks in which electrical stimulation of the olfactory inputs is used as a discriminative stimulus for positive reward (Roman et al., 1987, 1993; Saar et al., 1999). Cell firing in the PIR during an eight-odor discrimination task is also influenced by whether odors were associated with reward (Schoenbaum and Eichenbaum, 1995). Levels of Fos protein have been used to characterize brain regions associated with olfactory discrimination (Datiche et al., 2001) and sexual activity (Pfaus and Heeb, 1997). Fos is a marker of neuronal activation, and is elevated dramatically in the PIR of male rats exposed to bedding scented with an almond odor previously paired with sexual reward, compared to unconditionally rewarding estrus odors or the almond odor in unconditioned males (Kippin et al., 2003). Fos in the PIR is

*Corresponding author. Tel: +1-514-848-2424, +1-514-848-2220; fax: +1-514-848-2817.
E-mail address: andrew.chapman@concordia.ca (C. A. Chapman).
Abbreviations: ANOVA, analysis of variance; PEI, postejaculatory interval; PIR, piriform cortex.

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also activated dramatically and selectively by odors associated with sexual reward in female rats (Coria-Avila and Pfaus, 2007). Given the role of Fos and other immediate-early genes in the signal transduction cascade that leads to long-term changes in neuronal sensitivity to stimulation (Hughes and Dragunow, 1995), its induction suggests that the PIR may be part of a distinct neural pathway for the learning of contingencies between neutral odors and rewarding sexual stimulation (Pfaus et al., 2009).

The present experiments had two major goals. The first was to determine if the induction of a conditioned ejaculatory preference is associated with long-lasting changes synaptic responses in the PIR. Single electrical stimuli delivered to the olfactory bulb result in simultaneous activation of a large number of synaptic inputs to the piriform cortex, and the resulting extracellular field potential can be used effectively to monitor changes in the strength of synaptic transmission within the piriform cortex (Ketchum and Haberly, 1993; Chapman et al., 1998a). Changes in synaptic responses associated with development of a conditioned ejaculatory preference would imply that the PIR contributes to the formation of an association between odor and sexual reward. The second goal was to determine if different types of sensory stimulation during sexual activity are associated with changes in the excitability of PIR neurons. Male sexual behaviors include anogenital sniffing and chasing the female, mounting, intromission, ejaculation, and a postejaculatory refractory period of behavioral quiescence. Odor-conditioned ejaculatory preferences occur only if male rats are in close proximity of the scented female during the postejaculatory period (Kippin and Pfaus, 2001b), and if they experience sufficient sexual arousal prior to ejaculation (Ismail et al., 2009). Elevated excitability in the PIR could contribute to postsynaptic depolarization that contributes to synaptic modifications (Jung et al., 1990; Kanter and Haberly, 1990). Thus, increased excitability during a specific stage of sexual behavior would suggest that that stage contributes to the formation of the association. We investigated this possibility by stimulating the olfactory bulb with single stimulation pulses during different types of sexual stimulation and by evaluating the associated changes in the amplitude of the evoked synaptic responses in the PIR.

EXPERIMENTAL PROCEDURES

Animals and surgery

Methods for surgical implantation of stimulating and recording electrodes were similar to those used previously (Haykin et al., 1996; Chapman et al., 1998a). Male Long-Evans hooded rats (150–200 g; Charles River Canada, St-Constant, QC, Canada) were treated with atropine sulfate (0.02 mg/kg, i.p., Sigma, Oakville, ON, Canada), anesthetized with sodium pentobarbital (65 mg/kg, i.p.), and placed in a stereotaxic apparatus. Two bipolar, Teflon-coated stainless steel, twisted-wire electrodes were implanted. The tips of each electrode were 125 μm in diameter, and 0.5 mm apart. The stimulating electrode was placed in the right olfactory bulb (A 6.6 mm, L 1.2 mm, V 1.2 mm relative to Bregma) and the recording electrode was placed in the anterior PIR (A 3.6 mm, L6.5 mm, V 8.5 mm relative to Bregma). Final vertical positions of electrodes were adjusted to maximize the amplitude of differentially recorded synaptic responses. Electrode leads were mounted in a nine-pin connector and embedded in dental cement that was anchored to the skull by four jeweler's screws. A screw in the left parietal bone served as a ground electrode. Buprenorphorine HCL (0.02 mg/kg) was given as a post-surgical analgesic. Rats were housed in groups of four prior to surgery, and then singly following surgery, under a reversed 12 h light-dark cycle at 21 °C with *ad lib* access to food and water (lights off at 8:00 h). To verify the location of electrodes after completion of testing, animals were given an overdose of sodium pentobarbital (120 mg/kg) and perfused intracardially with ice cold saline followed by 10% formalin. Brains were extracted and post-fixed in 10% formalin followed by 30% sucrose and frozen before being cut on a cryostat into 40 μm sections and stained with Cresyl Violet.

Female Long-Evans rats (200–250 g) from the same breeder were used as sexual partners for the males. Females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), mixed at a ratio of 4:3, respectively, and injected ip in a volume of 1 ml/kg of body weight. Females were then ovariectomized bilaterally via lumbar incisions and housed subsequently in groups of four. Females were administered estradiol benzoate (Steraloids; 10 μg in 0.1 ml sesame oil, s.c.) 48 h and progesterone (Steraloids; 500 μg in 0.1 ml sesame oil, s.c.) 4 h before each copulatory test. Tests were conducted at 4 day intervals during the middle third of the rats' dark cycle in bilevel chambers with sexually vigorous males. Females received five tests prior to the experiment in order to acquire baseline rates of appetitive and consummatory sexual responding (Pfaus et al., 1999).

Stimulation and recording

Experiments were conducted in plastic "racetrack" chambers ($48\times26\times21$ cm^3) with a Plexiglas partition in the middle that enabled animals to engage in precopulatory chasing, and for the female to pace the rate of copulatory contact (Fig. 1). The cage floor was covered with wood chip bedding and wire mesh. Electrical stimuli delivered to the olfactory bulb were 0.1 ms biphasic constant current pulses generated using a digital-analog channel (50 kHz) and a stimulus isolation unit (A-M Systems, Model 2200). Interpulse interval was always at least 10 s. Evoked responses in the PIR were filtered (0.1 Hz to 5 kHz bandpass), amplified (A-M Systems and Model, 1700), displayed on an oscilloscope, and digitized at 10 kHz using a 16 bit A/D board for storage on computer hard disk. The software program Experimenters' Workbench (Datawave Tech) was used to control data acquisition and to measure field potential amplitudes.

Input-output tests were used to monitor the amplitude of synaptic responses in the PIR evoked by a range of stimulation pulse intensities delivered to the olfactory bulb. During each test, 10 field potentials were recorded and averaged at each of six pulse intensities (100, 200, 400, 600, 800, and 1000 μA). One pulse was delivered every 10 s. Results were used to confirm the stability of baseline responses, and to determine the amplitude of single stimulation pulses to be used in subsequent tests. The intensity used was the one that produced a response 50% of the response evoked by the largest pulse (100–400 μA).

Effects of sexual behavior on evoked responses

Responses recorded during copulatory trials were evoked during the performance of different types of sexual contact. The recordings allowed amplitudes of responses to be compared between groups, and also allowed short-term changes in evoked responses associated with ongoing behavior to be monitored. After an initial input-output test, responses were recorded during a 5 min baseline period using mid-intensity stimulation pulses at a rate of once every 10 s. A sexually receptive female was then intro-