Physiology & Behavior 133 (2014) 39–44

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Brain activation by an olfactory stimulus paired with juvenile play in female rats ☆

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HIGHLIGHTS

- An odor paired with juvenile play (CS+) increase FOS-IR in mPFC, OFC, DS and VTA.
- CS+ plus Sex increase activity in Pir Ctx, DS, LS, accumbens, BNST and amygdala.
- One single sex experience modifies the neuronal response to the CS+.
- CS+ fails to increase FOS-IR in the VTA, mPFC and OFC after sexual experience.

ARTICLE INFO

Article history:
Received 6 December 2013
Received in revised form 6 March 2014
Accepted 8 May 2014
Available online 13 May 2014

Keyword:
Social play
FOS immunoreactivity
Olfactory stimulus
Conditioning stimulus
Partner preference
Sexual behavior

ABSTRACT

We have previously shown that reward experienced during social play at juvenile age can be paired with artificial odors, and later in adulthood facilitate olfactory conditioned partner preferences (*PP*) in female rats. Herein, we examined the expression of FOS immunoreactivity (FOS-IR) following exposure to the odor paired with juvenile play (CS+). Starting at day P31 females received daily 30-min periods of social play with lemon-scented (paired group) or unscented females (unpaired group). At day P42, they were tested for *play-PP* with two juvenile males, one bearing the CS+ (lemon) and one bearing a novel odor (almond). Females were ovariectomized, hormone-primed and at day P55 tested for *sexual-PP* between two adult stud males scented with lemon or almond. In both tests, females from the paired group displayed conditioned *PP* (play or sexual) toward males bearing the CS+. In the present experiments females were exposed at day P59 to the CS+ during 60 min and their brains processed for FOS-IR. One group of female rats (Play + Sex) underwent *play-PP* and *sexual-PP*, whereas a second group of females (Play-only) underwent exclusively *play-PP* but not *sexual-PP*. Results showed that in the Play-only experiment exposure to the CS+ induced more FOS-IR in the medial prefrontal cortex, orbitofrontal cortex, dorsal striatum, and ventral tegmental area as compared to females from the unpaired group. In the Play + Sex experiment, more FOS-IR was observed in the piriform cortex, dorsal striatum, lateral septum, nucleus accumbens shell, bed nucleus of the stria terminalis and medial amygdala as compared to females from the unpaired group. Taken together, these results indicate mesocorticolimbic brain areas direct the expectation and/or choice of conditioned partners in female rats. In addition, transferring the meaning of play to sex preference requires different brain areas.

☆ The experimental protocols in this study were approved by a committee of the graduate program in the Centro de Investigaciones Cerebrales de la Universidad Veracruzana, Mexico, following the Official Mexican Standard NOM-062-ZOO-1999 (Technical Specifications for the Production, Care and Use of Laboratory Animals).

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http://dx.doi.org/10.1016/j.physbeh.2014.05.008
0031-9384/

1. Introduction

Sexual partner preferences can be shaped by learning via Pavlovian conditioning [1–8]. Rats display conditioned partner preference toward individuals that bear odor-based conditioned stimuli (CS) previously paired with an unconditioned rewarding stimulus (UCS), such as positive social interaction and sexual reward [1,2,5,7–9]. Accordingly, a CS modifies the sexual incentive for a potential mate. For example, a male rat prefers to copulate with a female that bears an odor paired with a reinforcer during infancy such as lactation and maternal care [10], or paired with previous rewarding sexual experiences [1,2,5]. Interestingly, conditioned sexual partner preference in adulthood can be evoked by odors associated with non-sexual rewarding stimuli as well. For instance, we previously showed that female rats exhibit a sexual partner preference for a male bearing an olfactory cue associated with juvenile play [8] or tickling by a human hand [7], which mimics several components of the somatosensory reward of social play in rats [11–14]. In our studies, female rats showed their preference displaying more visits, play and sexual solicitations (hops and darts), and allowed more intromissions and ejaculations from males scented with the paired odor. Those results indicated that juvenile play is highly rewarding because it acts as a reinforcer and facilitates the development of conditioned preference for play, as well as for sex [8].

During copulation animals respond to both internal and external signals which facilitate the activation of neural pathways involved in motivation toward a potential mate [15]. However, there are no studies on the brain areas involved in the acquisition and expression of conditioned partner preference induced by juvenile play. FOS immunoreactivity (FOS-IR) provides an index of neural activity in areas activated by conditioned odors associated with rewarding stimuli [15–19]. Coria-Avila and Pfaus showed that exposure to an odor alone previously associated with the reward state induced by paced copulation, where the female can regulate the rate of sexual stimulation received from the male, induced more FOS-IR in brain areas such as the piriform cortex (Pir Ctx), medial preoptic area (mPOA), and ventral tegmental area (VTA) [17]. Consequently, these three regions might be involved in the facilitation of conditioned partner preference induced by juvenile play. However, other brain areas such as orbitofrontal cortex (OFC) and prefrontal cortex appear to be required for normal play to occur [20–25].

The probability of juvenile play to be experienced as rewarding might depend on how the brain computes defensive strategies in response to the characteristics of the play partner [24,26,27]. For example, a male rat playing with a dominant partner responds with complete body rotations. In contrast, when the same rat plays with a subordinate male or a female, he is more likely to perform partial body rotations [27]. Rats with lesions in the OFC do not express this partner-related modulation of social play behavior [24]. Similarly, juvenile rats are more likely to respond with complete body rotation, whereas adult rats change to partial rotation. Juvenile mPFC-ablated rats [25] and socially deprived rats exhibit the adult pattern of defensive behavior, showing only partial body rotations [28,29]. This suggests the cortex may not be necessary for the production of social play but it is essential for the correct development and complexity of its social fine-tuning.

On the other hand, subcortical brain areas seem to be more related to the generation and control of social play behavior. For example, lesions in the septal area increase the amount of social play in rats [30] but lesions on the dorsomedial, posterior and parafascicular regions of the thalamus reduce play [31]. Moreover, lesions to the amygdala eliminate sex differences in the frequency of juvenile social play [32]. Likewise, the size of the amygdala and striatum are correlated with the presence of social play behavior in nonhuman primates with a larger size associated with more time spent on play behavior [33,34]. Social play behavior is important in the neurodevelopment of mammals and it is key in the diagnosis of human disorders as autism. Thus, it is important to understand what brain areas are involved in the generation of social play, and what others are implicated in the detection and processing of stimuli that facilitate the motivation for play and that can be transferred to attraction and desire for a particular partner.

The goal of the current study is to increase our understanding about the brain areas involved in the expectation of social play and its transference toward a conditioned sexual partner preference. Toward that end, in this study we examined the pattern of FOS-IR in adult females rats exposed to a conditioned odor associated with social play in the juvenile period. Furthermore, we examined the same pattern in females after they have confirmed their sexual preference by a male bearing the paired odor.

2. Methods

2.1. Animals

Groups of juvenile experimental female rats (5/group), as well as juvenile and adult male rats were used. Starting at postnatal day P31 female rats were individually housed in small cages containing aspen chip bedding (Rismart, Mexico) and kept in a room maintained at room temperature on a reversed 12 h light/dark cycle at the Centro de Investigaciones Cerebrales, Universidad Veracruzana, at Xalapa, Mexico. Rodent chow (Rismart, Mexico®) and drinking water were available ad libitum. An Admission Review Committee of a graduate program in Universidad Veracruzana Mexico, approved the experimental protocols following the Official Mexican Standard NOM-062-ZOO-1999 (Technical Specifications for the Production, Care and Use of Laboratory Animals).

2.2. Odor conditioning

Experimental females (P31) received a total of 10 conditioning trials. As described by Paredes-Ramos et al., [8], animals were allowed to play for 30 min with another female every 24 h. After the daily period of play each female was returned to its individual cage. In the "paired group" females that served as stimulus play partners were lemon-scented, whereas in the "unpaired group" the play partners were unscented during the conditioning trials. The odor (Deiman, Mexico ®, artificial extracts) functioned as a conditioned stimulus (CS+) and was applied on the back and neck of stimulus females 1 min before social play started.

2.3. Play partner preference test

One day after the 10th conditioning trial, female rats were tested for play partner preference with two unfamiliar age matched males. The test occurred in a three-compartment ($40 \times 30 \times 20$) chamber connected by a T-shaped tunnel. The experimental female was placed in the central compartment, and the two juvenile males were placed in the lateral compartments. One male was lemon-scented and the other was almond-scented. These odors functioned as Paired or Novel olfactory stimuli, respectively. The results of the play partner preference were previously published in Paredes-Ramos et al., [8], and indicated that female rats from the Paired group displayed a play partner preference toward juvenile males bearing the conditioned odor.

2.4. Re-training and ovariectomy

Female rats from the play partner preference test received two more conditioning trials in which they associated the odor with social play. After the re-conditioning trials they were ovariectomized (OVX) via a lumbar incision. The females were anesthetized with a mixture of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (4 mg/ml), via intraperitoneal injection. After surgery all animals received three daily subcutaneous injections of enrofloxacine (5 mg/kg) and flunixin meglumine (2.5 mg/kg), as antibiotic and analgesic treatment, respectively.