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DYNAMIC CHANGES IN EXTRACELLULAR RELEASE OF GABA AND GLUTAMATE IN THE LATERAL SEPTUM DURING SOCIAL PLAY BEHAVIOR IN JUVENILE RATS: IMPLICATIONS FOR SEX-SPECIFIC REGULATION OF SOCIAL PLAY BEHAVIOR

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Abstract—Social play is a motivated and rewarding behavior that is displayed by nearly all mammals and peaks in the juvenile period. Moreover, social play is essential for the development of social skills and is impaired in social disorders like autism. We recently showed that the lateral septum (LS) is involved in the regulation of social play behavior in juvenile male and female rats. The LS is largely modulated by GABA and glutamate neurotransmission, but their role in social play behavior is unknown. Here, we determined whether social play behavior is associated with changes in the extracellular release of GABA and glutamate in the LS and to what extent such changes modulate social play behavior in male and female juvenile rats. Using intracerebral microdialysis in freely behaving rats, we found no sex difference in extracellular GABA concentrations, but extracellular glutamate concentrations are higher in males than in females under baseline conditions and during social play. This resulted in a higher glutamate/GABA concentration ratio in males vs. females and thus, an excitatory predominance in the LS of males. Furthermore, social play behavior in both sexes is associated with significant increases in extracellular release of GABA and glutamate in the LS. Pharmacological blockade of GABA-A receptors in the LS with bicuculline (100 ng/0.5 μl, 250 ng/0.5 μl) dose-dependently decreased the duration of social play behavior in both sexes. In contrast, pharmacological blockade of ionotropic glutamate receptors (NMDA and AMPA/kainate receptors) in the LS with AP-5 + CNQX (2 mM + 0.4 mM/0.5 μl, 30 mM + 3 mM/0.5 μl) dose-dependently decreased the duration of social play behavior in females, but did not alter social play behavior in males. Together, these data suggest a role for GABA neurotransmission in the LS in the regulation of juvenile social play behavior in both sexes, while glutamate neurotransmission in the LS is involved in the sex-specific regulation of juvenile social play behavior.

Key words: GABA, glutamate, lateral septum, microdialysis, sex difference, social play.

INTRODUCTION

Social play is a highly motivated and rewarding behavior (Trezza et al., 2010, 2011) predominantly displayed between juveniles of both sexes across many mammalian species (Bekoff and Byers, 1998; Pellis and Iwaniuk, 2000; Burghardt, 2005). Studies in humans, non-human primates, and rodents suggest that social play activities among peers contribute to the development of social and emotional competence (Suomi and Harlow, 1972; Pellegrini, 1988; Hol et al., 1999; Sigman and Ruskin, 1999; Guralnick et al., 2006; Cordoni and Palagi, 2011). Social play deficits are observed in children diagnosed with neurodevelopmental disorders such as autism spectrum disorders (ASD), early-onset schizophrenia, and attention-deficit/hyperactivity disorder (Alessandri, 1992; Moller and Husby, 2000; Jordan, 2003). Therefore, a better understanding of the neurobiological regulation of social play behavior may help to gain insights into normal as well as impaired expression of this behavior.

The lateral septum (LS) plays a critical role in modulating social, motivational, and rewarding behaviors (Clarke and File, 1982; Goodson et al., 1997; Beiderbeck et al., 2007; Scotti et al., 2011; Luo et al., 2011; McDonald et al., 2012; Veenema et al., 2012; Lukas et al., 2013; Harasta et al., 2015). This is likely mediated through extensive connections of the LS with regions important for social information processing (i.e., the bed nucleus of the stria terminalis and medial amygdala) as well as with regions that play a role in motivation and reward (i.e., the ventral tegmental area and nucleus accumbens; Sheehan et al., 2004). As such, the LS has been proposed to be a key node in the social decision-making network (O'Connell and Hofmann, 2011). Not surprisingly then, the LS has been shown to be involved in the regulation of social play behavior in juvenile rats and juvenile hamsters (Beatty et al., 1982; Cheng and Delville, 2009; Veenema et al., 2013; Bredewold et al., 2014). Interestingly, while lesioning of various brain areas resulted in decreased expression of social play behavior (Meaney et al., 1981; Beatty and Costello, 1983; Bell et al., 2009), electrolytic lesioning of the septum increased social play behavior in juvenile male and

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Abbreviations: ANOVA, analysis of variance; ASD, autism spectrum disorders; LS, lateral septum.

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female rats (Beatty et al., 1982). This increase could be due to the disinhibition of output regions of the LS, since LS output is mainly GABAergic (Risold and Swanson, 1997a; Zhao et al., 2013).

GABA and glutamate, the principle inhibitory and excitatory neurotransmitters in the brain, largely modulate LS neuronal activity (Joëls and Urban, 1984a, b; Allaman-Exertier et al., 2007). GABA released in the LS likely derives from local LS neurons sending recurrent axon collaterals to neighboring neurons (Onteniente et al., 1987; Phelan et al., 1989; Jakab and Leranth, 1990; Risold and Swanson, 1997b), thereby inhibiting intraseptal neuronal activity. GABAergic input may also originate from sources projecting to the LS such as the nucleus accumbens (Zahm et al., 2013). Glutamate released in the LS derives from multiple brain regions (Leranth et al., 1999; Riedel et al., 2008, 2013; Chee et al., 2015) with the most prominent source to the LS coming from the hippocampus (Zaczek et al., 1979; Gallagher et al., 1995). Given the modulatory role of GABA and glutamate on LS neuronal activity, we hypothesized that GABA and glutamate released in the LS are involved in the regulation of social play behavior.

The current study aimed to characterize the role of GABA and glutamate neurotransmission in the LS in the regulation of social play behavior. We used intracerebral microdialysis in freely behaving male and female juvenile rats to determine whether extracellular release of GABA and glutamate in the LS are altered during social play behavior. Using acute pharmacological manipulations, we then determined the involvement of GABAergic and glutamatergic signaling in the regulation of male and female social play behavior. Because the expression of social play behaviors is similar between male and female juvenile rats when tested in dyads (Veenema et al., 2013; Bredewold et al., 2014), we predicted that both sexes will show similar changes in extracellular GABA and/or glutamate release during the display of social play behavior. Since electrolytic lesioning of the septum ablates any LS output and increased social play behavior (Beatty et al., 1982) likely by eliminating inhibitory LS output, we further predict that blocking LS-GABA receptors will decrease, while blocking LS-glutamate receptors will increase social play behavior in both sexes.

EXPERIMENTAL PROCEDURES

Animals

Male and female Wistar rats (23 days of age) were obtained from Charles River (Raleigh, NC, USA) and maintained under standard laboratory conditions (12 h light/dark cycle, lights off at 14:00 h, 22 °C, 50% humidity, food and water *ad libitum*). Rats were housed in same-sex groups of four in standard rat cages (48 × 27 × 20 cm) unless otherwise mentioned. The experiments were conducted in accordance with the guidelines of the NIH and approved by Boston College Institutional Animal Care and Use Committee.

Social play test

During the beginning of the dark phase (between 14:00 h and 15:00 h) and under red light conditions, each experimental rat was exposed to an age- and sex-matched unfamiliar rat for a period of 10 min. All tests were videotaped for subsequent analysis of behavior by a researcher blinded to the treatment conditions using JWatcher (http://www.jwatcher.ucla.edu/). The duration of social play was scored according to Veenema and Neumann (2009) and consisted of the total amount of time spent in playful social interactions including nape attacks (the experimental rat displays nose attacks or nose contacts toward the nape of the neck of the unfamiliar rat), pinning (the experimental rat holds the unfamiliar rat on its back in supine position), and supine poses (the experimental rat is pinned by the unfamiliar rat). In addition, the number of nape attacks, pins, and supine poses as well as the duration of social investigation (the experimental rat is sniffing the anogenital and head/neck regions of the unfamiliar rat), the duration of allogrooming (the experimental rat is grooming the unfamiliar rat), and the duration of non-social exploration were scored.

Probe implantation and microdialysis procedure

Experimental rats were housed individually at 28 days of age and were exposed to the social play test one day thereafter to familiarize the rats to the test procedure. At 30 days of age, rats were anesthetized with isoflurane (Butler Schein Animal Health, Dublin, OH, USA) and mounted on a stereotaxic frame with the tooth bar set at −4.5 mm. The u-shaped microdialysis probes (Brainlink, Groningen, The Netherlands) were unilaterally implanted into the LS according to Lukas et al. (2011) at coordinates 0.4 mm caudal to bregma, −1.5 mm lateral to the midline, 5.6 mm beneath the surface of the skull at an angle of 10° to avoid damage to the sagittal sinus. The probes were filled with sterile Lactated Ringer's solution (pH 7.4) and fixed to the skull with three stainless steel screws and dental cement. Two 5-cm-long pieces of polyethylene tubing (PE 20, Plastics One, Roanoke, VA, USA) filled with Ringer's solution were connected to the inflow and the outflow of the probe and fixed with dental cement. One day after surgery, probes were flushed with Ringer's and rats were familiarized to the microdialysis sampling procedure while undergoing a social play test. Two days after surgery, probes were connected via PE20 tubing to a syringe mounted onto a microinfusion pump and perfused with Ringer's (3.0 μl/min, pH 7.4) to establish equilibrium between the inside and outside of the microdialysis membrane. Two hours later, five consecutive 10-min dialysates were collected: dialysates 1 and 2 were taken under baseline (undisturbed) conditions, dialysate 3 was taken during the 10-min social play test, and dialysates 4 and 5 were taken thereafter. Microdialysates were collected in 0.5-ml Eppendorf tubes and were immediately frozen on dry ice and subsequently stored at −45 °C until quantification for glutamate and GABA using LC–MS/MS. Rats were killed with CO_2 and proper probe