*Neuroscience* 359 (2017) 172–182

# SOCIAL INSTABILITY STRESS IN ADOLESCENT MALE RATS REDUCES SOCIAL INTERACTION AND SOCIAL RECOGNITION PERFORMANCE AND INCREASES OXYTOCIN RECEPTOR BINDING

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**Abstract—Social experiences in adolescence are essential for displaying context-appropriate social behaviors in adulthood. We previously found that adult male rats that underwent social instability stress (SS) in adolescence had reduced social interactions with unfamiliar peers compared with non-stressed controls (CTL). Here we determined whether SS altered social recognition and social reward and brain oxytocin and vasopressin receptor density in adolescence. We confirmed that SS rats spent less time interacting with unfamiliar peers than did CTL rats ($p = 0.006$). Furthermore, CTL rats showed a preference for novel over familiar conspecifics in a social recognition test whereas SS rats did not, which may reflect reduced recognition, impaired memory, or reduced preference for novelty in SS rats. The reward value of social interactions was not affected by SS based on conditioned place preference tests and based on the greater time SS rats spent investigating stimulus rats than did CTL rats when the stimulus rat was behind wire mesh ($p = 0.03$). Finally, oxytocin receptor binding density was higher in the dorsal lateral septum and nucleus accumbens shell in SS rats compared with CTL rats ($p = 0.02$, $p = 0.01$, respectively). No effect of SS was found for vasopressin 1a receptor binding density in any of the brain regions analyzed. We discuss the extent to which the differences in social behavior exhibited after social instability in adolescence involve changes in social salience and social competency, and the possibility that changes in oxytocin signaling in the brain underlie the differences in social behavior.** 

**Key words: adolescence, social stressors, social recognition, conditioned place preference, oxytocin receptor, vasopressin receptor.**

## INTRODUCTION

The ability to display context-appropriate affiliative and aggressive behaviors is necessary for animals to adapt to a changing social environment (reviewed in O'Connell and Hofmann, 2011, 2012). The adolescent brain undergoes several morphological and functional changes in brain regions involved in social behavior and the salience of social cues (reviewed in Spear, 2000; O'Connell and Hofmann, 2011). Social experiences, in turn, may influence ongoing development and thereby alter future social behavior, because of the enhanced plasticity of the brain during the adolescent period (reviewed in Buwalda et al., 2011; McCormick et al., 2015). Indeed, social experience in adolescence in rats is crucial to the development of appropriate social behavior (reviewed in Buwalda et al., 2011; Pellis et al., 2014; Pellis and Pellis, 2017). For example, social deprivation during the adolescent period in rats reduced their approach behavior to pro-social 50-kHz ultrasonic vocalizations in adulthood compared with non-isolated controls (CTL) (Seffer et al., 2015). Rats isolated in early-adolescence spend less time in contact with unfamiliar conspecifics both in late-adolescence (van den Berg et al., 1999a) and in adulthood (Hol et al., 1999; Lukkes et al., 2009). After social isolation in adolescence, rats are able to perform socially appropriate behaviors, but these are often misapplied (reviewed in Pellis et al., 2014; Pellis and Pellis, 2017).

Although there is much evidence that the absence of social experience in adolescence impairs social behavior, the quality of social interactions in adolescence may also influence social development. Rats that underwent repeated social instability stress (SS) in adolescence (daily one-hour isolation and return to an unfamiliar cage partner from postnatal days 30–45) spent less time in social interaction with unfamiliar peers (Green et al., 2013), demonstrated greater aggression with a conspecific in food competition (Cumming

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*Abbreviations:* ANOVAs, analysis of variances; BLA, basolateral amygdala; CPP, conditioned place preference; CTL, control; dlBNST, dorsolateral bed nucleus of stria terminalis; dLS, dorsal lateral septum; lpBNST, posterolateral bed nucleus of stria terminalis; MeAV, anteroventral medial amygdala; MePV, posteroventral medial amygdala; mpBNST, posteromedial bed nucleus of stria terminalis; MPOA, medial preoptic area; nACC, nucleus accumbens shell; OTR, oxytocin receptor; SS, social instability stress; V1AR, vasopressin 1a receptor; vLS, ventral lateral septum; VMH, ventromedial hypothalamus.

http://dx.doi.org/10.1016/j.neuroscience.2017.07.032
0306-4522/

et al., 2014), and had impaired mating behavior (McCormick et al., 2013) that depended on the individual's social status (McCormick et al., 2017). Here, we investigated the influence of SS on social recognition and social reward (social conditioned place preference (CPP)) in addition to social interactions. Whereas our previous studies of social behavior (social interaction, food competition, sexual behavior) described in this paragraph were conducted in adulthood ( > postnatal day 60) a minimum of two weeks after the SS procedure, here tests were conducted within days of the SS exposures while the rats were mid- to late-adolescent (tests began on postnatal day 46). Although some effects of SS are known to be long-lasting (reviewed in McCormick et al., 2015), the effects of SS on social behavior may be more evident soon after the procedure in the adolescent period, a time in which social interaction, preference for social novelty, and social reward is typically higher than in adulthood (e.g., Douglas et al., 2004; reviewed in Doremus-Fitzwater et al., 2010). In addition, in the test of social interaction, we compared social interactions with an unfamiliar peer relative to that with a cage mate in the test arena to determine whether any decrease in social interaction is limited to novel peers.

We also investigated whether SS and CTL rats differed in oxytocin receptor (OTR) and vasopressin 1a receptor (V1AR) binding in brain regions that are relevant for social behavioral function and social salience. The neuropeptides oxytocin and vasopressin are involved in the regulation of appropriate social responses to social stimuli (reviewed in Veenema and Neumann, 2008). Oxytocin and vasopressin regulate social interaction (Bredewold et al., 2014; Dumais et al., 2016a), social reward (Dölen et al., 2013), and social recognition (Dantzer et al., 1988; Veenema et al., 2012; Bychowski et al., 2013; Lukas et al., 2013; Dumais et al., 2016b) in rodents by acting on oxytocin and vasopressin receptors (reviewed in Stoop, 2014). Further, gonadal hormones, the release of which increases in adolescence, regulate the expression of OTRs and both oxytocin and vasopressin in brain regions involved in social behaviors (e.g., De Vries et al., 1984; Champagne et al., 2001; Somponpun and Sladek, 2002; see Albers, 2015 for a review). In addition, there are changes in OTR binding density and V1AR binding density from early-adolescence to adulthood in rats (Tribollet et al., 1991; Lukas et al., 2010; Smith et al., 2017b). Thus, any effects of SS on social behavior may be accompanied by changes in these receptor densities.

## EXPERIMENTAL PROCEDURES

### Animals

The experiments involved male Long-Evans rats (test rats, $n = 171$; stimulus rats for social recognition and CPP testing, $n = 20$) obtained from Charles River, Kingston, New York, on postnatal day (PND) 22 and given a week to acclimate to the animal colony. Rats were housed in pairs and maintained under a 12-h light–dark cycle (lights on at 05:00 h) with food and water available ad libitum. Use of animals in these experiments was approved by the Brock University Institutional Animal Care Committee (ACC) and was carried out in adherence to the Canadian Council on Animal Care guidelines.

### SS procedure

Rats were randomly assigned to the adolescent social instability stress (SS, $n = 83$) group or to the non-stressed control (CTL, $n = 88$) group. The SS procedure was as described previously (reviewed in McCormick, 2010; McCormick et al., 2015). Beginning on postnatal day (PND) 30, SS rats were isolated in a 12-cm × 10-cm ventilated plastic container in a room separate from the colony for 1 h each day until PND 45. Immediately after isolation each day, SS rats were returned to the animal colony and housed in a new cage with a new cage partner that had also undergone the 1-h isolation. The SS procedure was conducted at various times during the lights on phase of the light–dark cycle to minimize habituation to the procedure. Although this procedure may result in some sleep restriction, which can also have consequences for development (da Silva Rocha-Lopes et al., 2017), we have shown that the effects of daily change of cage partners after daily isolation are greater than those of daily one hour isolation and return to the familiar cage partner (McCormick et al., 2007; McCormick and Ibrahim, 2007; Hodges and McCormick, 2015), which is consistent with our definition of the procedure as a social stressor. On PND 45, after the final isolation, SS rats remained with the same cage partner and were left undisturbed except for cage maintenance or test procedures. CTL rats remained undisturbed in their home cages except for cage maintenance from time of arrival at the colony until the test procedures (see Fig. 1 for experimental design and timeline of procedures).

### Social interaction test

The social interaction test was conducted on PND 46 in a plastic arena (61 cm × 30 cm × 53 cm). A video-camera mounted on the ceiling above the test apparatus was used to record the task. The apparatus was cleaned before each session with 70% ethanol. For the social interaction test, CTL ($n = 32$) and SS ($n = 32$) rats were either (1) paired with their cage partner (Familiar; CTL w/familiar CTL, $n = 16$; SS w/familiar SS, $n = 16$) or (2) paired with an unfamiliar peer of the same treatment condition (Unfamiliar partner; CTL w/unfamiliar CTL, $n = 16$; SS w/unfamiliar SS, $n = 16$). Total time spent in social interaction (including social play, anogenital sniffing, allogroom, following, investigation; as defined in Cirulli et al., 1996 and as in our previous research, Green et al., 2013) during the 15-min test session for each pair of rats was measured by an experimenter blind to both the Stress Group and Familiarity conditions. The pair of rats was treated as a unit of analysis and the total score for the pair was used. Social interaction test sessions were conducted in dim red light between one and five hours after the onset of the dark phase.