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Stress alters social behavior and sensitivity to pharmacological activation of kappa opioid receptors in an age-specific manner in Sprague Dawley rats

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ABSTRACT

The dynorphin/kappa opioid receptor (DYN/KOR) system has been identified as a primary target of stress due to behavioral effects, such as dysphoria, aversion, and anxiety-like alterations that result from activation of this system. Numerous adaptations in the DYN/KOR system have also been identified in response to stress. However, whereas most studies examining the function of the DYN/KOR system have been conducted in adult rodents, there is growing evidence suggesting that this system is ontogenetically regulated. Likewise, the outcome of exposure to stress also differs across ontogeny. Based on these developmental similarities, the objective of this study was to systematically test effects of a selective KOR agonist, U-62066, on various aspects of social behavior across ontogeny in non-stressed male and female rats as well as in males and females with a prior history of repeated exposure to restraint (90 min/day, 5 exposures). We found that the social consequences of repeated restraint differed as a function of age: juvenile stress produced substantial increases in play fighting, whereas adolescent and adult stress resulted in decreases in social investigation and social preference. The KOR agonist U-62066 dose-dependently reduced social behaviors in non-stressed adults, producing social avoidance at the highest dose tested, while younger animals displayed reduced sensitivity to this socially suppressing effect of U-62066. Interestingly, in stressed animals, the socially suppressing effects of the KOR agonist were blunted at all ages, with juveniles and adolescents exhibiting increased social preference in response to certain doses of U-62066. Taken together, these findings support the hypothesis that the DYN/KOR system changes with age and differentially responds and adapts to stress across development.

1. Introduction

The dynorphin/kappa opioid receptor (DYN/KOR) system has been identified as a potential target for the treatment of various disorders associated with stress, including anxiety, depression, and alcohol/substance use disorders (Anderson and Becker, 2017; Chavkin and Ehrich, 2014; Crowley and Kash, 2015; Knoll and Carlezon, 2010; Schwarzer, 2009; Tejeda et al., 2012; Van't Veer and Carlezon, 2013). Numerous studies in both humans and animals have demonstrated that activation of the DYN/KOR system is associated with behavioral alterations, including increased aversion, dysphoria, and anxiety, that resemble the effects of stress (Hang et al., 2015; Van't Veer and Carlezon, 2013). Additionally, blockade of DYN/KOR signaling, either through administration of KOR antagonists or through genetic or viral down-regulation/knock-down of DYN and/or KORs, attenuates stress-associated behavioral changes (McLaughlin et al., 2006; McLaughlin et al., 2003) and molecular adaptations within various brain structures known to be affected by stress (Bruchas and Chavkin, 2010; Crowley and Kash, 2015; Lemos et al., 2012).

Despite an extensive literature demonstrating dysphoric/aversive effects following activation of the DYN/KOR system, several studies have reported either opposite effects or a lack of response/sensitivity to KOR agonists (Hang et al., 2015). For example, systemic administration of KOR agonists produced anxiolytic effects on the elevated plus maze (Alexeeva et al., 2012; Braida et al., 2009; Kudryavtseva et al., 2006; Privette and Terrian, 1995). Similarly, microinjections of a KOR agonist into the infralimbic cortex also produced an anxiolytic effect (Wall and Messier, 2000b), while microinjection of the KOR antagonist, norbinaltorphimine (nor-BNI), resulted in anxiogenesis (Wall and Messier, 2000a). While these paradoxical effects of manipulations of the DYN/KOR system have been largely attributed to procedural and methodological differences across studies, a common factor that these studies

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share is that animals were tested early in life (Diaz et al., in press). Surprisingly few studies, however, have directly compared responses to manipulations of the DYN/KOR system between younger and older animals. For instance, adolescent rats were found to be insensitive to conditioned place aversion associated with systemic administration of a KOR agonist, an effect that was observed in adult rats (Anderson et al., 2014). Another study reported that KOR activation in pre-weanlings increased appetitive responding for water (Petrov et al., 2006), opposite to what had been shown in adults (Bals-Kubik et al., 1989). Moreover, a selective KOR antagonist, nor-BNI, attenuated ethanol-induced taste aversion only in stressed adults, with stressed adolescents being insensitive to the effects of nor-BNI (Anderson et al., 2013). In support of these notably different behavioral effects associated with pharmacological activation and/or suppression of the DYN/KOR system across ontogeny, we recently found that KOR activation in the basolateral amygdala (BLA) of adolescent rats increased GABA transmission, without having an effect in the adult BLA (Przybysz et al., 2017). An age-dependent increase followed by a decrease in DYN-mediated hyperpolarization of neurons within the paraventricular nucleus of the thalamus has also been shown (Chen et al., 2015). Hence, there is clear behavioral and cellular evidence consistent with age-dependent differences in the functional role of the DYN/KOR system (Diaz et al., in press).

Although the DYN/KOR system has been demonstrated to be both engaged in and altered by stress, resulting in enhanced anxiety (Crowley and Kash, 2015; Schwarzer, 2009; Tejeda et al., 2012; Van't Veer and Carlezon, 2013), the influence of age and sex has not been carefully examined. Anxiety-like behavior in rats has been extensively assessed using the social interaction test [see (File and Seth, 2003) for references and review]. In the conventional social interaction test, two rats are placed into a testing arena, and overall time spent in social interactions is generally used as a dependent measure (File and Hyde, 1978). However, this approach combines together the discrete behavioral acts (e.g., sniffing of a partner, social grooming, following, chasing, pinning, etc.) that reflect behaviorally distinctive and differentially regulated forms of social behavior, including social investigation and play fighting. These social behaviors are characterized by distinguishable developmental patterns (Vanderschuren et al., 1997; Varlinskaya and Spear, 2008; Varlinskaya et al., 1999) and differential responsiveness to anxiety-provoking manipulations (Doremus-Fitzwater, Varlinskaya and Spear, 2009b). For example, play fighting – an adolescent-typical form of social behavior – shows an inverted U-shaped ontogenetic pattern, peaking around postnatal day (P) 30–35 and gradually decreasing to reach adult levels (Vanderschuren et al., 1997). Play fighting has a rewarding value and is crucial for development of the ability to express and understand intraspecific communicative signals (Vanderschuren et al., 2016; Vanderschuren et al., 1997). In contrast, social investigation increases with age, representing a more adult-typical form of social behavior (Vanderschuren et al., 1997; Varlinskaya et al., 1999). Play fighting, but not social investigation, is drastically increased by isolate housing throughout the juvenile and adolescent periods (Vanderschuren et al., 1997; Varlinskaya and Spear, 2008) as well as by juvenile stress (Varlinskaya et al., 2013). In contrast, social investigation is exclusively decreased by prior history of exposure to non-social stressors during adolescence and in adulthood (Doremus-Fitzwater et al., 2009b; Varlinskaya et al., 2010), with no changes in social investigation evident following juvenile stress (Varlinskaya et al., 2013). Together, these findings suggest that play fighting and social investigation may be differentially affected by pharmacological activation of the DYN/KOR system. Our modification of the social interaction test (Varlinskaya et al., 1999) allows an experimental animal to freely move toward or away from a non-manipulated social partner in a 2-compartment testing apparatus, thereby permitting assessment of social preference and/or avoidance in addition to measurement of the frequencies of play fighting and social investigation (Varlinskaya et al., 1999). Using this modified social interaction test, we have found decreases in social preference and/or social investigation to reflect anxiety-like alterations (Doremus-Fitzwater et al., 2009b; Morales et al., 2013; Varlinskaya et al., 2010; Varlinskaya and Spear, 2012).

Given the mounting evidence demonstrating age-related differences in vulnerability to and outcomes of stress exposure (Enoch, 2011; Romeo, 2017; Tottenham and Galvan, 2016) as well as in responsiveness to the aversive effects of the DYN/KOR system activation (Anderson et al., 2014), the present study was designed to systemically assess the effects of pharmacological activation of the DYN/KOR system on social investigation, social preference, and play fighting across ontogeny in non-stressed males and females as well as males and females with a prior history of repeated exposure to restraint.

2. Methods

2.1. Subjects

Juvenile, adolescent and adult Sprague-Dawley male and female rats bred and reared in our colony at Binghamton University were used. A total of 72 litters provided 360 male and female offspring to serve as experimental subjects and 360 to serve as partners. Animals were housed in a temperature-controlled (22 °C) vivarium, and maintained on a 12:12 h light:dark cycle (lights on at 0700 h) with ad libitum access to food (Purina rat chow) and water. Litters were culled to 10 pups (five males and five females) within 24 h after birth on P0 and reared until weaning with their mothers in standard plastic maternity cages (47.8 × 25.4 × 20.3 cm) with pine shavings as bedding material. Rats were weaned on P21 and placed into cages (50.8 × 40.6 × 20.3 cm) with their same-sex littermates (5 animals per cage). At all times, rats used in the current study were produced, maintained, and treated in accordance with the guidelines for animal care established by the National Institutes of Health, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Experimental design

The design was a 3 (age: juvenile, adolescent, adult) X 2 (sex) X 2 (stress condition: no stress or repeated restraint) x 5 (U62,066 dose: 0, 0.1, 0.2, 0,3, and 0.4 mg/kg) factorial, with six experimental animals tested per group. Juveniles were tested on P28, adolescents on P35, and adults were tested on P70. All animals from a given litter were assigned to the same stress condition. To avoid the possible confounding of litter with the experimental variables (Holson and Pearce, 1992; Zorrilla, 1997), no more than one subject per sex from a litter was assigned to a particular U-62066 dose/stress condition, with order of testing counterbalanced across litters.

2.3. Stressor procedures

Beginning at P24 for juveniles, at P30 for adolescents, and at P66 for adults, rats from the repeated stress group were removed from their home cage between 1000 and 1200 h and then restrained in age size-adjusted (5.08 cm diameter x 12.7 cm length for juveniles, 6.35 cm diameter x 15.24 cm length for adolescents, and 8.26 cm diameter x 20.32 cm length for adults) flat-bottom restrainers (Braintree Scientific, Braintree, MA) for 90 min. The animals in their restraints were placed in a novel standard plastic holding cage similar to a maternity cage that was located in a separate holding room away from the rooms where the animals were housed or later tested for social behavior. For animals in the stress group, this restraint procedure was repeated each day for 5 days. Animals placed in the control condition were non-manipulated throughout the 5-day stressor phase until the time of testing.

As in our previous studies (Doremus-Fitzwater, Varlinskaya and Spear, 2009a; Varlinskaya et al., 2010; Varlinskaya and Spear, 2012), restraint was used as the stressor, since this stressor is primarily