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Oxytocin and vasopressin modulation of social anxiety following adolescent intermittent ethanol exposure

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Abstract

Rationale.—Adolescent intermittent ethanol exposure (AIE) produces lasting, sex-specific social anxiety-like alterations in male, but not female rats. Oxytocin (OXT) and vasopressin (AVP) brain systems play opposite roles in regulating social preference/avoidance, with OXT increasing approach to, and AVP increasing avoidance of social stimuli.

Objectives.—To test the hypothesis that social anxiety-like alterations seen in adult males after AIE are associated with a shift in the balance between OXT and AVP toward AVP, effectiveness of pharmacological activation of the OXT system and blockade of endogenous activity at AVP receptors for reversing AIE-induced social anxiety-like alterations was assessed, along with examination of the effects of AIE on OXT, vasopressin V1a and V1b receptor (OXT-R, V1a-R, and V1b-R) surface expression in the hypothalamus.

Methods.—Sprague-Dawley male and female rats were given 4 g/kg ethanol (AIE) or water intragastrically every 48 hr for a total of 11 exposures during postnatal days (P) 25–45. On P70–72, animals were given a social interaction test following administration of a selective OXT-R agonist WAY-267464, selective V1a-R antagonist SR-49059, or V1b-R antagonist SSR-149415, and hypothalamic tissue was collected.

Results.—Social anxiety-like behavior was induced by AIE in males but not females, and was selectively reversed by the selective OXT-R agonist and V1b-R antagonist, but not V1a-R antagonist. AIE was also found to decrease OXT-R, but increase V1b-R neuronal surface expression relative to water-exposed controls in the hypothalamus of males, but not females.

Conclusions.—These findings demonstrate that AIE induces changes in OXT-R and AVPR surface expression in the hypothalamus along with social anxiety-like alterations in male rats. These social anxiety-like alterations can be reversed either by activation of the OXT system or by suppression of the AVP system, data that support the hypothesis that social anxiety-like alterations induced by adolescent alcohol exposure in male rats are associated at least in part with an OXT/AVP imbalance.

Keywords

adolescent ethanol exposure; social anxiety-like alterations; sex differences; oxytocin; vasopressin; receptor surface expression; hypothalamus; WAY-67,424; SR-49059; SSR-149415

Introduction

Alcohol use is typically initiated during early adolescence (Faden, 2006). Young individuals who begin drinking at 14 years of age or earlier are four times more likely to become alcohol-dependent relative to those who started drinking at 20 years of age or later (Dawson et al., 2008; Ehlers et al., 2006). This is especially concerning given recent data from the Monitoring the Future survey reporting annual prevalence rates for alcohol use of 21%, 42% and 58% for 8th, 10th and 12th graders, respectively (Johnston et al., 2016). Over 20% of high school seniors reported consumption of 5 or more drinks per occasion (i.e., binge levels of drinking) within the past 2 weeks, with 10% endorsing consumption of 10 or more drinks, and over 5% reporting consumption of 15 or more drinks over the same time interval (Patrick et al., 2013). Binge patterns of drinking among early adolescents (ages from 10 to 14) is even more alarming, given that estimated peak blood alcohol concentrations following 5 drinks are around 280 mg/dL for 10-year-olds and about 160 mg/dL for 14-year-old adolescents (see Donovan, 2009).

The early initiation and relatively high use levels of alcohol by adolescents could potentially disrupt maturational changes occurring in the brain at this time in regions critical for cognitive control (Casey et al., 2008), motivational responding and the processing of rewarding, social and emotional stimuli (Blakemore, 2012). Indeed, early onset of drinking has been found to be predictive of alcohol-related problems later in life (Grant and Dawson, 1997; Palmer et al., 2009), with those engaging in even episodic heavy drinking at an early age being more likely to develop alcohol use (Bonomo et al., 2004; Hingson et al., 2006; Grant et al., 2001) as well as affective disorders (Fidalgo et al. 2008; Lopez et al. 2005; Schmidt et al. 2007), although these relationships are not necessarily causal.

Assessment of persisting consequences of adolescent ethanol exposure is an area of recent intensive investigation in humans and animal models (see Spear, 2018 for a review). In such studies, ethanol has been administered to adolescent rodents through a variety of routes, including intragastric (i.g.) gavage (for advantages and limitations see Knapp & Breese, 2012), with intermittent exposure in a dose range of 3.5 – 5.0 g/kg ethanol i.g. during adolescence producing average peak blood ethanol concentrations (BECs) in the range of 130–200 mg/dL (see Kim et al, in press), similar to those estimated for binge drinking in early adolescents (Donovan, 2009). Adolescent exposure to ethanol in this range in rats has been shown to induce long-lasting behavioral and neural alterations that are not evident in controls receiving water i.g (Broadwater & Spear, 2014; Liu & Crews, 2017; Vetreno et al., 2017; Risher et al., 2015a,b; Swartzwelder et al., 2017). For instance, we have shown long-lasting and sex-dependent detrimental consequences of repeated intermittent i.g. exposure to ethanol during the juvenile - early adolescent period (AIE). Male, but not female, adult rats exposed to ethanol during adolescence demonstrated social anxiety-like behavioral

alterations, indexed via significant decreases in social investigation and social preference relative to both water-exposed as well as non-manipulated controls (Varlinskaya et al. 2014, 2017). Few differences between water-exposed and non-manipulated males were evident either in terms of social behavior, sensitivity to the social effects of acute ethanol challenge or in their corticosterone (CORT) responses (Varlinskaya et al., 2014), suggesting that the gavage procedure per se does not have long-lasting consequences in males.

Although a number of neural systems might be involved in these AIE-induced social alterations in males, vasopressin (AVP) and oxytocin (OXT) brain systems are of particular interest. Both neuropeptides play important roles in complex social behaviors including maternal behavior, pair-bonding, and social memory (Bielsky and Young 2004; Bielsky et al. 2004; Ferris et al. 1992; Gabor et al. 2012; Neumann 2008). These peptides often produce similar effects on social cognition and mating behavior (see Stoop 2012, for review), but play opposite roles in modulation of anxiety-like behavior, especially anxiety evident under social circumstances (Neumann and Landgraf 2012).

The brain OXT system appears critical for social approach and social preference (Lukas et al. 2011). For instance, stress-induced social anxiety in rodents indexed via social avoidance was reversed to social preference by central administration of OXT (Lukas et al. 2011). The anxiolytic effects of OXT in a social context (Donaldson and Young 2008) are in contrast with evidence for an anxiogenic role of the brain AVP system (Mak et al. 2012; Simon et al. 2008). Anxiogenic effects of the endogenous AVP system have been confirmed by a number of studies of AVP and its V1a and V1b receptor subtypes, with decreases in anxiety-like behavior evident following central or systemic administration of AVP antagonists, local antisense nucleotides targeting V1a-R, as well as in AVP-R knockout mice (see Neumann and Landgraf 2012, for references and review). Studies have confirmed an important role for both V1a-R (Bleickardt et al. 2009; Egashira et al. 2007) and V1b-R (Litvin et al., 2011) in anxiety-related behaviors, with V1a-R perhaps more critical for non-social (Bleickardt et al., 2009) and V1b-R for social (Litvin et al., 2011) anxiety.

OXTR and V1a-R are highly expressed in a number of brain regions implicated in modulation of social behavior, including the hypothalamus, lateral septum, bed nucleus of the stria terminalis (BNST), nucleus accumbens, amygdala, and ventral tegmental area (see Dumais and Veenema, 2016 for references and review). In the brain, V1b receptors are highly expressed in the CA2 region of the hippocampus, but can also be found in areas such as the paraventricular nucleus of the hypothalamus and the olfactory bulb (Young et al., 2006).

The effects of OXT and AVP on brain, social behavior, and anxiety are sexually dimorphic (Bales and Carter 2003; Bales et al. 2004; Bielsky et al. 2005; Yamamoto et al. 2004). In males, AVP mRNA and protein levels in the bed nucleus of BNST and medial amygdala (MeA) are regulated by gonadal steroids, with adult males showing more pronounced AVP mRNA expression than young, pre-pubertal males (Pak et al. 2009). Males are more sensitive to AVP system disruptions, particularly during development, than females (Yamamoto et al. 2004). A variety of studies suggest enhanced AVP function (indexed via number of AVP-immunoreactive neurons, sizes of AVP neurons, plasma levels of AVP, etc.)

in males versus females, including humans (Ishunina and Swaab 1999), rats (Crofton et al. 1985), and prairie voles (Winslow et al. 1993). In contrast, OXT is more abundant in females (see Dumais and Veenema 2016 for references and review), and hence may potentially protect females from the emergence of social anxiety associated with AIE.

Given the sex differences in OXT and AVP brain systems and the separable roles of these peptides in modulating anxiety-like alterations in social contexts, the present study was designed to test the hypothesis (see Neumann and Landgraf, 2012) that the emergence of social anxiety-like behavioral alterations after AIE in males is associated with shifts in the balance of the OXT and AVP systems toward AVP. We further hypothesized that more typical balance could be restored either by activation of the OXT system or by suppression of the AVP system. Specifically, we assessed effectiveness of pharmacological activation of the OXT system (Experiment 1) and blockade of endogenous activity at AVP receptors (Experiments 2 and 3) for reversing AIE-induced social anxiety-like alterations observed in a modified social interaction test, as well as effects of AIE on OXT-R, V1a-R and V1b-R surface expression in the hypothalamus of adult males and females (Experiment 4). We focused on the hypothalamus, since all three types of receptors are detectable in this brain region. Furthermore, given ontogenetic changes in OXTR densities in this region (Smith et al., 2017), the OXT system of the hypothalamus may be especially vulnerable to adolescent ethanol exposure.

Materials and Methods

Subjects

Male and female Sprague-Dawley rats bred and reared at Binghamton University were used as experimental subjects (N = 352) and social partners (N = 352). All animals were housed in a temperature-controlled (22 °C) vivarium maintained on a 12:12 h light-dark cycle (lights on at 0700h) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Litters were culled to 8–10 pups within 24 hr after birth, maintaining relatively equal sex ratios whenever possible. On postnatal day (P) 21, pups were weaned and housed with same-sex littermates (n = 4/cage). To maintain population heterogeneity and to control for litter effects, only one subject per sex per litter was used in each experimental condition (Holson and Pearce 1992; Zorrilla 1997). At all times, animals were treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

Intermittent ethanol exposure

To better recapitulate ethanol pharmacokinetics such as first-pass metabolism while inducing relatively consistent ethanol exposures at BECs related to binge consumption levels, males and females were exposed to ethanol intragastrically (4 g/kg, 25% solution in tap water) every other day between 900 and 1000 hrs (11 exposures) during early-mid adolescence (P25-P45). This dose produced blood ethanol concentrations (BECs) around 200 mg/dl at P25, with a decline evident by the end of the exposure period (BECs about 130 mg/dl) at P45 (see Kim et al., in press). Controls were given an isovolumetric amount of tap water by gavage on these exposure days. It has been shown previously that adolescent rats exposed

intermittently to the dose of 4 g/kg ethanol showed only minimal signs of intoxication post-gavage such as slight motor impairment when walking, with no weight differences observed between ethanol- and water-exposed rats at any point during or after the chronic exposure period (Broadwater & Spear, 2013).

Drug administration

A selective OXT-R non-peptide agonist WAY-267464 dihydrochloride (*N*-[[4-[(4,10-Dihydro-1-methylpyrazolo[3,4-*b*][1,5]benzodiazepin-5(1*H*)-yl)carbonyl]-2-methylphenyl)methyl]-4-[(3,5-dihydroxyphenyl)methyl]-1-piperazinecarboxamide dihydrochloride; Tocris Bioscience, USA) was dissolved in 0.9% saline, whereas the V1a-R non-peptide antagonist SR-49059 ((2*S*)-1-[(2*R*,3*S*)-5-Chloro-3-(2-chlorophenyl)-1-[(3,4-dimethoxyphenyl)sulfonyl]-2,3-dihydro-3-hydroxy-1*H*-indol-2-yl]carbonyl]-2-pyrrolidinecarboxamide; Tocris, Bristol, UK) and V1b-R non-peptide antagonist SSR-149415 ((2*S*,4*R*)-1-[(*R*)-5-Chloro-1-(2,4-dimethoxy-benzenesulfonyl)-3-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-indol-3-yl]-4-hydroxy-pyrrolidine-2-carboxylic acid dimethylamide; Axon MedChem BV, Groningen, The Netherlands) were prepared as fresh solutions on each test day in 15% dimethyl sulfoxide (DMSO), 2% tween 80, and 83% physiological saline. All drugs were injected intraperitoneally (i.p.) at a volume of 2 ml/kg at doses of 0, 2.5, 5 mg/kg WAY-267464, 0, 1, 3, 9 mg/kg SR-49059, or 0, 5, 10, 20 mg/kg SSR-149415.

Experimental design

To assess the role of pharmacological activation of OXT-R (Exp.1) and pharmacological blockade of endogenous activity at AVP V1a (Exp. 2) and V1b receptors (Exp.3) on adult social behavior following AIE, animals were challenged with one of the three doses of the selective OXT-R agonist WAY-267464, or one of the four doses of the selective V1a-R antagonist SR-49059 or the selective V1b-R antagonist SSR-149415, respectively, prior to the social interaction test. Eight animals were placed into each of the 12 (Exp.1) or 16 (Exp. 2 and 3) experimental groups defined by the 2 (adolescent exposure: AIE versus water control) X 2 (sex) X (3 [Exp. 1] or 4 (Exp. 2 and 3) dose) factorial design of each study. Animals within the same adolescent exposure condition were housed together and assigned semi-randomly to one of the test drug doses.

Procedure

On P70–72, experimental animals were individually taken from their home cage and injected with the selected dose of the OXT-R agonist (Exp.1), V1a-R (Exp.2) or V1b-R (Exp.3) antagonist before being placed alone for a 30-min habituation period in a social interaction test chamber that contained clean pine shavings. The test chamber (45 × 30 × 30 cm) was composed of Plexiglas (Binghamton Plate Glass, Binghamton, NY) and was divided into two equally sized compartments by a clear Plexiglas partition with an aperture (9 × 7 cm) to allow movements of the animals between compartments. Following the 30-min habituation period, a non-manipulated social partner of the same age and sex was introduced for a 10-min social interaction test. Each apparatus contained two adjacent test units separated by an opaque wall that allowed testing of two experimental animals and their corresponding social partners, with little to no interference between the pairs. Partners were always unfamiliar

with the experimental animal and test situation, were not socially deprived prior to the test (Varlinskaya and Spear 2002; Varlinskaya and Spear 2008; Varlinskaya et al. 2014, 2017), and were experimentally and drug-naïve. Weight differences between test subjects and their partners were minimized as much as possible, with this weight difference not exceeding 20 g, and test subjects always being heavier than their partners. In order to differentiate experimental animals from their social partners during the test, each experimental animal was marked with a vertical red line across the back.

All testing was conducted under dim light (15–20 lux) between 1000 and 1400 hrs, with a white noise generator used to attenuate extraneous sounds during testing. Test sessions were recorded by an overhead camera (Sony Handycam) and scored at a later date by an experimenter blind to group assignment. Two anxiety-sensitive behavioral measures, namely social investigation and the coefficient of social preference/avoidance (Doremus-Fitzwater et al. 2009; Varlinskaya et al. 2010, Varlinskaya and Spear 2012), were scored from the video recordings and analyzed. Social investigation frequency was defined as the sniffing of any part of the body of the partner. In the modified social interaction test, social preference/avoidance is indexed via movements of the experimental animal toward or away from the non-manipulated partner and assessed via a social preference/avoidance coefficient (Varlinskaya et al. 1999). This coefficient is calculated as: (crossovers to the partner – crossovers away from the partner) / (total number of crossovers both to and away from the partner) x 100 (Varlinskaya et al. 1999); hence, lower values reflect lower social preference and are indicative of social anxiety-like alterations (Varlinskaya et al. 2014). Play fighting behavior, a characteristic form of adolescent social behavior that is under control of the OXT and AVP systems (Bredewold et al. 2014; Veneema et al. 2013) and shows adolescent-typical responsiveness to acute ethanol in AIE males tested as adults (Varlinskaya et al. 2014), was scored as the sum of the frequencies of the following behavioral acts and postures: pouncing or playful nape attack, following and chasing, and pinning (Vanderschuren et al. 1997; Varlinskaya et al. 1999). Play fighting differs from serious fighting in the laboratory rat by target of attack: during play fighting snout or oral contact is directed toward the partner's nape, while during serious fighting the object of the attack is the partner's rump (Pellis and Pellis, 1987). Experimental subjects did not demonstrate serious fighting in the present experiments. Total number of crossovers (movements between compartments) demonstrated by each experimental subject was used as an index of general locomotor activity under these test conditions in order to control for possible drug-induced general activation or suppression not related to social behavior.

In adulthood, vehicle control males from both adolescent exposure groups (ethanol vs. water) were rapidly decapitated immediately following the social interaction test for collection of hypothalamic brain tissue used for examination of receptor surface expression.

Surface Expression Analysis

Analysis of cell surface expressed proteins was conducted similarly as in our prior work (Werner et al. 2011) based on modifications of others (González et al. 2013; Grosshans et al. 2002). Briefly, following brain extraction, fresh tissue was sliced into 400µm coronal sections. The hypothalamus was microdissected and mixed with Sulfo-NHS-LC-Biotin (2

mg/ml, Thermo Scientific, Rockford IL) in ice-cold artificial cerebrospinal fluid (aCSF). Hypothalamic sections were taken immediately distal to bregma for 1.5 mm (4 slices) to include preoptic areas and the paraventricular nuclei. Unreacted biotin was inactivated with glycine and removed by washing in ice-cold aCSF. Cross-linked tissue was subsequently homogenized in RIPA buffer with protease/phosphatase cocktail inhibitors. Aliquots of total protein lysate were used to determine protein concentration via bicinchoninic acid colorimetric assay. The remainder of the sample was gently mixed overnight with avidin-conjugated magnetic beads. Beads containing the biotinylated (surface-labelled) proteins were isolated and washed with RIPA buffer. Biotinylated proteins were then released and denatured by incubating the beads in Laemmli buffer at 95°C for 7 min. Equal amounts of biotinylated proteins based on the total protein concentrations were separated by SDS polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (ThermoFisher, Waltham, MA). Membranes were probed with antibodies for OXT-R (sc-8102, SCBT, Dallas, TX), AVP V1a-R (AB3506P, EMD Millipore, Billerica, MA), or AVP V1b-R (ab104365, Abcam, Cambridge, MA). Selected primary antibodies have previously been characterized. AVP V1a-R and V1b-R have been commercially verified and share 0 – 15% sequence homology with other AVP receptors, respectively. The antibody for AVP V1a-R has been used elsewhere (Simard et al., 2015), and BLAST alignment of antigen sequences for AVP V1a-R and AVP Vab-R sequence yielded only V1a-R or V1b-R sequence homology, respectively. The OXT-R antibody has been extensively published, and has been validated elsewhere including usage of a cell line that does not express OXT-R (Gonzalez-Iglesias et al., 2015). Secondary antibodies were obtained from ThermoFisher. All bands were detected using enhanced chemiluminescence under non-saturating conditions (GE Healthcare, Piscataway, NJ), exposed to x-ray films and analyzed using NIH Image J. Molecular weights of detected bands were in the range of 45–50 kDa. Loading control proteins were not used for normalization as surface protein isolation excludes antigens typically used as loading controls. Prior surface-expressed proteins used as controls (Na-K-ATPase) are well-known to be altered by ethanol exposure (Wang et al., 2009; Santos et al., 2017). Therefore, equal amounts of loaded protein samples were similarly quantified as elsewhere (Diaz et al., 2011; Centanni et al., 2014). Optical densities for cell surface receptors were normalized to matched water controls within the same blot. Actin levels from intracellular sample elutants ran on separate membranes did not differ (*not shown*). V1a-R analysis was not conducted in females due to insufficient sample amounts.

Data Analyses

Within each experiment, social investigation, social preference/avoidance, play fighting, and total number of crossovers were analyzed using separate 2 (adolescent exposure condition) X 3 or 4 (drug dose) analyses of variance (ANOVAs) in males and females. Males and females were analyzed separately due to previous research showing AIE-induces social anxiety-like alterations in males, but not females (Varlinskaya et al. 2014, 2017). Main effects and interactions involving adolescent exposure and test dose were further explored using Fisher's planned least significant difference (LSD) post hoc tests. These planned tests focused on: a) comparisons between AIE animals and their water-exposed counterparts under basal conditions (i.e., 0 g/kg test dose); as well as b) dose-dependent responses to the

selected agonist/antagonists within each exposure/sex group. Surface expression between AIE and water exposed controls was analyzed using unpaired Student's t-tests.

Results

Experiment 1. Effects of pharmacological activation of OXT receptors on social interaction in adult males and females following AIE

In males, a 2-way ANOVA for social investigation revealed a significant interaction of the adolescent exposure and WAY-267464 dose, $F(2, 42) = 12.260$; $p < 0.001$. As in our previous studies (Varlinskaya et al. 2014, 2017), when challenged with vehicle at the time of testing in adulthood, AIE males demonstrated a significant decrease in social investigation relative to males exposed to water during adolescence (Figure 1, top left panel). The selective OXT-R agonist reversed these social alterations, with the highest dose significantly increasing social investigation in ethanol-exposed males. In contrast, both doses of WAY-267464 reduced social investigation in water-exposed males.

Similar results were evident for the coefficient of social preference, $F(2, 42) = 5.089$ $p < 0.05$. A significant decrease in the coefficient was seen in ethanol-exposed males relative to their water-exposed counterparts following 0 mg/kg dose (Figure 1, bottom left panel). The highest dose (5.0 mg/kg WAY-267464) reversed this AIE-associated decrease in social preference, with the same dose producing an opposite effect in water-exposed control males and decreasing social preference.

A significant adolescent exposure by WAY-267464 dose interaction, $F(2, 42) = 4.355$, $p < 0.05$, was also evident for play fighting (Figure 2, top left panel). The dose of 5.0 mg/kg significantly increased play fighting in ethanol-exposed males, with a trend towards a decrease in water-exposed males. Total number of crossovers differed only as a function of adolescent exposure (Figure 2, bottom left panel), with ethanol-exposed males showing greater locomotor activity during the test relative to water-exposed males, $F(1, 42) = 10.794$, $p < 0.005$, when collapsed across drug dose.

There were no significant effects in the ANOVAs of any of the social behaviors in females (see Figures 1 & 2, right panels). Like males, total chamber crossovers yielded a main effect of adolescent exposure, with ethanol-exposed females displaying greater locomotor activity relative to water-exposed females, $F(1, 42) = 13.936$, $p < 0.001$, when collapsed across drug dose.

Experiment 2. Effects of pharmacological blockade of V1a receptors on social interaction in adult males and females following AIE

In males, the ANOVA for social investigation yielded significant main effects of adolescent exposure, $F(1, 56) = 14.142$, $p < 0.001$, SR-49059 dose, $F(3, 56) = 4.103$, $p < 0.01$, as well as their interaction, $F(3, 56) = 4.400$, $p < 0.01$. As in Exp.1, ethanol-exposed males injected with vehicle demonstrated less social investigation than their water-exposed counterparts (Figure 3, top left panel). SR-49059, however, did not influence social investigation in ethanol-exposed males, although it decreased social investigation in water-exposed control males at doses of 1.0 and 9.0 mg/kg. The analysis of preference/avoidance coefficient yielded a main

effect of adolescent exposure, with ethanol-exposed males again demonstrating less social preference than their water-exposed counterparts, $F(1, 56) = 8.163$, $p < 0.01$, but no effects of SR-49059 on social preference (Figure 3, bottom left panel). The ANOVA for play fighting revealed significant main effects of adolescent exposure, $F(1, 56) = 10.915$, $p < 0.005$, and SR-49059 dose, $F(3, 56) = 3.067$, $p < 0.05$, as well as their interaction, $F(3, 56) = 3.511$, $p < 0.05$. Ethanol-exposed males displayed significantly less play fighting than water-exposed controls when both groups were injected with vehicle prior to testing in adulthood. SR-49059 did not influence play fighting in ethanol-exposed males, while water-exposed control males exhibited significantly increased play following the dose of 3.0 mg/kg and decreased play fighting at 9.0 mg/kg of SR-49059 (see Figure 4, top left panel). In males, the drug did not affect locomotor activity (Figure 4, bottom left panel).

For females, no interactions emerged. Main effects of dose were evident in females for social investigation, $F(3, 56) = 3.00$, $p < 0.05$ (see Figure 3, top right panel) and play fighting, $F(3, 56) = 3.85$, $p < 0.05$ (see Figure 4, top right panel), with both behaviors being significantly decreased by the highest dose (9.0 mg/kg) of SR-49059 regardless of adolescent exposure. Main effects of adolescent exposure were evident in females for social preference ($F(1, 56) = 4.431$, $p < 0.05$ (Figure 3, bottom right panel) and chamber crossovers, $F(1, 56) = 6.337$, $p < 0.05$ (Figure 4, bottom right panel), with ethanol-exposed females showing greater social preference but being significantly less active than water-exposed control females.

Experiment 3. Effects of pharmacological blockade of V1b receptors on social interaction in adult males and females following AIE

In males, a significant adolescent exposure by SSR-149415 dose interaction was evident for social investigation, $F(3, 56) = 5.317$, $p < 0.005$, and social preference, $F(3, 56) = 3.914$, $p < 0.05$. As in Exp. 1 and 2, levels of social investigation and social preference were significantly lower in ethanol-exposed males than their water-exposed control counterparts when challenged with vehicle prior to testing in adulthood (Figure 5, left panels). Both the 10.0 and 20.0 mg/kg doses of SSR-149415 increased social investigation in ethanol-exposed males (Figure 5, top left panel), whereas 10.0 mg/kg decreased social investigation in water-exposed control males. Post-hoc analyses of social preference revealed an increase in social preference following doses of 5.0 and 10.0 mg/kg SSR-149415 in ethanol-exposed males, whereas the 10 mg/kg dose decreased social preference of water-exposed males (Figure 5, bottom left panel). Play fighting and overall locomotor activity under social testing circumstances, indexed via total number of crossovers, were not affected by adolescent exposure or SSR-149415 (data not shown).

In females, no significant effects of AIE or V1b-R antagonist were seen for any social behavior or the activity measure.

Experiment 4. Hypothalamic OXT and AVP receptor surface expression in males and females following AIE

The brains of randomly selected male or female subjects ($n = 4-6$ per group) administered vehicle in Experiments 1 – 3 were taken immediately following the social interaction

procedure, and hypothalamic tissue was processed for analysis of OXT-R, V1a-R or V1b-R surface expression. AIE exposure in males markedly reduced OXT-R levels by 63.7% [$t(6) = 2.620$, $p < 0.05$], whereas V1b-R expression was elevated by 55.2% [$t(9) = 2.275$, $p < 0.05$] (Figure 6). Reminiscent of the lack of restorative effect of the V1a-R antagonist on social behavior in Exp.3, V1a-R surface expression did not differ between AIE and water exposed control males [$t(7) = 0.206$, $p = 0.84$]. In contrast to the significant effects observed in males, AIE exposure in females did not alter OXT-R [$t(8) = 0.549$, $p = 0.60$; water $100.0 \pm 5.6\%$, AIE = $95.9 \pm 5.9\%$], or AVP V1b-R [$t(5) = 0.263$, $p = 0.81$; water $100.0 \pm 39.5\%$, AIE $87.6 \pm 26.5\%$];.

Discussion

Similar to our previous findings (Varlinskaya et al. 2014, 2017), the results of the present experiments demonstrate reliable, sex-specific social anxiety-like alterations in adulthood following AIE exposure to 4 g/kg i.g. In all behavioral experiments, when tested under basal, 0 g/kg dose conditions, ethanol-exposed males, but not their female counterparts, showed social anxiety-like alterations indexed via decreased levels of social investigation and social preference relative to water-exposed controls. AIE-induced social suppression was not associated with decreases in overall locomotor activity, indexed via total number of crossovers under these social circumstances, and hence did not appear to reflect general behavioral inhibition. The social anxiety-like alterations seen in males were reversed either by stimulation of OXT-R or by blockade of V1b but not V1a receptors. These pharmacological findings were mirrored by hypothalamic tissue showing attenuated OXT-R and increased V1b-R (but not V1a-R) surface expression in AIE-exposed males but not their female counterparts.

The selective OXT-R agonist, WAY-267464, at a dose of 5.0 mg/kg, restored social investigation and social preference in AIE males to levels comparable with those demonstrated by water-exposed males injected with vehicle. These social effects of systemically administered WAY-267464 are reminiscent of prior work demonstrating similar effects of centrally administered OXT in male rats. For instance, administration of OXT into the central amygdala specifically reversed social suppression induced by chronic phencyclidine (PCP) exposure in adult male rats, with no anxiolytic effects evident in the open field test of anxiety (Lee et al. 2005). Likewise, social deficits associated with prenatal stress were reversed by OXT infusion into the central amygdala in adult males, with no OXT effects evident in non-stressed controls (Lee et al. 2007). Intracerebroventricular (ICV) infusion of OXT reversed stress-induced social avoidance in rats, while not affecting social preference of non-stressed animals or non-social anxiety on the elevated plus maze (Lukas et al. 2011). Thus, together with our results, these findings demonstrate that social alterations of differential genesis (e.g., prenatal stress, adolescent ethanol exposure, PCP) can be reversed by pharmacological activation of the brain OXT system, suggesting some functional suppression of this system in socially impaired and/or socially anxious individuals. This suggestion is supported by surface expression data obtained in Exp. 4 of the present experimental series, with ethanol-exposed males showing decreased OXT-R surface expression in the hypothalamus in adulthood when compared with their water-exposed counterparts.

Unexpectedly, in control males, the OXT agonist produced an opposite effect, with water-exposed males exhibiting significant decreases of social investigation at both doses of WAY-267464 and social preference at the highest dose. One possible explanation for these findings is that overstimulation of the brain OXT system with WAY-267464 in control males may result in reduced drive/need for social interaction. Indeed, under normal circumstances exposure to a conspecific in male rodents stimulates OXT release in the brain (Dumais et al. 2016), and the combination of these two sources of OXT-R stimulation may be sufficient to elevate OXT-R activation beyond optimal levels and induce suppression of social behavior, a suggestion consistent with frequent indications of inverted U-shaped functions relating activation of various peptide systems and behavior (e.g., Boccia et al. 1998).

Similarly to OXT-Rs, pharmacological blockade of endogenous activity at V1b-Rs produced social anxiolysis in ethanol-exposed males, with SSR-149415 reversing social anxiety-like decreases in social investigation and social preference. The involvement of V1b-Rs in AIE-associated social alterations is further confirmed by surface expression data, given that V1b-R expression in the hypothalamus was significantly increased in ethanol-exposed males relative to their water-exposed counterparts in Exp.4. Also similar to the OXT-R agonist, unexpectedly, generally opposite effects (i.e., decreases in social investigation and social preference) were seen in water controls after challenge with higher doses of the V1b-R. This finding contrasts with a number of studies that have reported socially anxiolytic effects of this antagonist (Griebel et al. 2002; Litvin et al. 2011; Serradeil-Le Gal et al. 2005) – at least in studies examining drug effects in stressed males who demonstrated social avoidance or decreased social interaction.

To the best of our knowledge, V1b-R antagonist has not been tested socially in non-stressed males across a relatively broad dose range as used in this study, and hence the contrasting results may reflect different roles of endogenous activity at V1b-Rs in modulation of social investigation and social preference in stressed versus non-stressed animals. It is possible that the effects of AIE on V1b-Rs may be similar to those produced by stress exposure in adult animals. Indeed, repeated ethanol exposure serves as a homotypic stressor that has the ability not only to activate the hypothalamic-pituitary-gonadal (HPA) axis following its acute administration in adolescents and adults, but also produces long-lasting effects in the brain circuits implicated in regulation of the stress response following adolescent exposure (Allen, Revier, Lee, 2011; Allen et al., 2011). It remains to be investigated, however, as to whether adolescent exposure to a different repeated homotypic stressor will produce long-lasting and sex-specific social anxiety-like alterations sensitive to reversal either by activation of the OXT system or pharmacological blockade of endogenous activity at V1b-Rs.

Finally, the social behavior of AIE exposed males was insensitive to pharmacological blockade of endogenous activity at V1a-Rs, whereas SR-49059 decreased social investigation and dose-dependently altered play fighting in water-exposed control males, suggesting that V1a-Rs may be involved in modulation of the effects of AVP on social behavior under normal circumstances (see Duque-Wilckens et al. 2016). The lack of effects of the V1a-R antagonist on social behavior in ethanol-exposed males is in accordance with the results of Exp. 4, which demonstrated no AIE-induced changes in the surface expression of V1a-Rs in the hypothalamus.

In females, AIE produced no anxiety-like social alterations, with ethanol- and water-exposed females showing no changes of social investigation and social preference following pharmacological activation of OXT-Rs or blockade of V1b-Rs. Some effects of the selective V1a-R antagonist were evident in female subjects regardless of adolescent exposure condition, with the highest dose of the drug significantly decreasing social investigation and play fighting, but not social preference in females. The lack of effects of OXT and AVP pharmacological manipulations on social preference in female rats is consistent with previously reported findings, with for instance, social preference demonstrated by adult female rats toward same-sex conspecifics not being affected by ICV administration of OXT, AVP, or OXT-R, V1a-R and V1b-R antagonists or agonists (Lukas and Neumann 2014).

The present findings further support that social preference in adulthood is strongly controlled by the brain OXT and AVP systems in a sex-specific manner. A variety of rodent studies have revealed evidence for sex-specific neural characteristics and effects of pharmacological manipulation of these systems, and such responses may be modulated by select neuronal nuclei. The lateral septum (LS), for instance, is a sexually dimorphic brain region with denser vasopressin fibers in males than females (de Vries et al. 1981) but abundant expression of V1a-R in both sexes (Veenema et al. 2012). Injections of a V1-R antagonist into this region enhanced play fighting in adolescent males and suppressed it in adolescent females (Bredewold et al. 2014; Veenema et al. 2013), whereas infusion of OXT in the LS decreased play fighting selectively in females (Bredewold et al. 2014). Also, a selective V1a-R antagonist injected ICV suppressed play fighting in adolescent males and increased it in adolescent females (Veenema et al. 2013). Given the present findings, future work would need to determine if LS selectively modulates male social responses following AIE. Nonetheless, when considered with the results of our study, these findings suggest that the central OXT and AVP systems are involved in modulation of social behavior such as play fighting in a sex- and brain region-specific fashion.

OXT and AVP brain systems play opposing role in anxiety. According to the hypothesis of Neumann and Landgraf (2012), activation of the brain OXT system shifts the OXT/AVP balance toward OXT, which results in reduced levels of social anxiety, whereas when the balance is shifted towards AVP, enhanced anxiety states occur. The results of the current experiments are consistent with this hypothesis. Although AIE-associated alterations in receptor surface expression were evident in both neuropeptide systems, pharmacological manipulations with only one of the two systems was sufficient for the reversal of social anxiety in AIE subjects. Social anxiety was ameliorated either by the selective OXT agonist or the V1b-R antagonist, suggesting that the shift in the balance between the OXT/AVP systems rather than alterations within both systems per se plays a major role in social anxiety. This balance can be restored either by activation of the OXT system or by suppression of the AVP system.

The extent to which these findings are applicable to human adolescent binge drinking and its consequences later in life is limited by the forced method of ethanol exposure. Forced ethanol exposure, giving the precise control of exposure level and timing, can produce neural adaptations different from those associated with voluntary ethanol consumptions.

Nevertheless, the reversal of social anxiety by OXTR agonists and AVP V1b-R suggests potential new translational targets for possible amelioration of the social anxiety.

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Figure 1.

Effects of the selective OXT receptor agonist WAY-267464 on social investigation (top) and social preference (bottom) in water- and ethanol-exposed male and female rats. # symbol denotes significant differences between ethanol-exposed and water-exposed rats under basal (0 mg/kg dose) conditions within sex ($p < 0.05$). * symbol denotes significant drug effects within each exposure/sex condition relative to vehicle ($p < 0.05$). Data are expressed as mean $\pm$ SEM.

Play Fighting

Crossovers

Figure 2.

Effects the selective OXT receptor agonist WAY-267464 on play fighting (top) and total number of crossovers (bottom) in water- and ethanol-exposed male and female rats. * symbol denotes significant drug effects within each exposure/sex condition relative to vehicle ($p < 0.05$). \$ symbol denotes significant differences between water and ethanol-exposed animals within each sex, with data collapsed across drug dose ($p < 0.05$). Data are expressed as mean $\pm$ SEM.

Figure 3.

Effects of the selective V1a receptor antagonist SR-49059 on social investigation (top) and social preference (bottom) in water- and ethanol-exposed male and female rats. # symbol denotes significant differences between ethanol-exposed and water-exposed rats under basal (0 mg/kg dose) conditions ($p < 0.05$). * symbol denotes significant drug effects within each exposure/sex condition relative to vehicle ($p < 0.05$). \$ symbol denotes significant differences between water- and ethanol-exposed rats within each sex, with data collapsed across drug dose ($p < 0.05$). % symbol denotes significant effects of drug dose within sex, with data collapsed across adolescent exposure ($p < 0.05$). Data are expressed as mean $\pm$ SEM.

Play Fighting

Crossovers

Figure 4.

Effects of the selective V1a receptor antagonist SR-49059 on play fighting (top) and total number of crossovers (bottom) in water- and ethanol-exposed male and female rats. # symbol denotes significant differences between ethanol-exposed and water-exposed rats under basal (0 mg/kg dose) conditions within sex ($p < 0.05$). * symbol denotes significant drug effects within each exposure/sex condition relative to vehicle. % symbol denotes significant effects of drug dose within sex, with data collapsed across adolescent exposure ($p < 0.05$). \$ symbol denotes significant differences between water and ethanol-exposed rats within sex, with data collapsed across drug doses ($p < 0.05$). Data are expressed as mean $\pm$ SEM.

Social Investigation

Social Preference

Figure 5.

Effects the selective V1b receptor antagonist SSR-149415 on social investigation (top) and social preference (bottom) in water- and ethanol-exposed male and female rats. (#) denote significant differences between ethanol-exposed and water-exposed rats under basal (0 mg/kg dose) conditions within sex ($p < 0.05$). * symbol denotes significant drug effects within each exposure/sex condition relative to vehicle ($p < 0.05$). Data are expressed as mean $\pm$ SEM.

Figure 6.

Hypothalamic OXT and AVP V1a and V1b receptor surface expression in water-exposed (H2O) and ethanol-exposed (AIE) males. # symbol denotes significant differences in expression between H2O and AIE subjects. Data are expressed as mean $\pm$ SEM, $p < 0.05$.