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Effects of AMPA receptor antagonist, NBQX, and extrasynaptic GABAA agonist, THIP, on social behavior of adolescent and adult rats

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Abstract

Adolescence is characterized by high significance of social interactions, along with a propensity to exhibit social facilitating effects of ethanol while being less sensitive than adults to the inhibition of social behavior that emerges at higher doses of ethanol. Among the neural characteristics of adolescence are generally enhanced levels of glutamatergic (especially NMDA receptor) activity relative to adults, whereas the GABA system is still developmentally immature. Activation of NMDA receptors likely plays a role in modulation of social behavior in adolescent animals as well as in socially facilitating and suppressing effects of ethanol. For instance, adolescent and adult rats differ in their sensitivities to the effects of NMDA antagonists and ethanol on social behavior, with adolescents but not adults demonstrating social facilitation at lower doses of both drugs and adults being more sensitive to the socially suppressing effects evident at higher doses of each. The roles of AMPA and extrasynaptic GABAA receptors in modulation of social behavior during adolescence and in adulthood are still unknown. The present study was designed to assess whether pharmacological blockade of AMPA receptors and/or activation of extrasynaptic GABAA receptors results in age-dependent alterations of social behavior. Adolescent and adult male and female Sprague-Dawley rats were injected with an assigned dose of either a selective AMPA antagonist, NBQX (Experiment 1) or extrasynaptic GABAA agonist, THIP (Experiment 2) and placed into a modified social interaction chamber for a 30-min habituation period prior to a 10-min social interaction test with a novel age- and sex-matched partner. Behaviors such as social investigation, contact behavior and play behavior were scored from video recordings of the interaction tests. In Experiment 1, NBQX produced similar social inhibition at higher doses in both age groups. In Experiment 2, THIP induced inhibition in adolescents, but not adults. No social facilitation was evident following low doses of either drug. Therefore, AMPA and extrasynaptic GABAA receptors appear to play little role if any in modulation of peer-directed social behavior in adolescence and adulthood and not likely to contribute to previously observed age differences in the social effects of acute ethanol.

Competing interests

The authors have no competing interests to disclose.

Keywords

Social interaction; AMPA receptor; NBQX; GABAA receptor; THIP; Age differences

1. Introduction

Adolescence is a developmental period characterized by high significance of interactions with peers [47], with human adolescents spending more time interacting with peers than children and adults [48]. Similarly, during the early adolescent age interval [postnatal day (P) 28–P35] in the rat, animals demonstrate substantial increases in social activity relative to younger or older animals, particularly the adolescent-characteristic behavior of play behavior [35,37]. Interactions with peers provide a significant source of positive experiences in humans [31] and rats [33]. Therefore, it is not surprising that socially facilitating effects of ethanol may contribute to heavy and problematic drinking during adolescence. Since adolescents strongly believe that alcohol will make them more confident and relaxed in a social setting, expectancy for social facilitation from drinking is a predictor of heavy drinking during adolescence [15,30].

Ethanol-induced social facilitation is not restricted to human adolescents but is also evident in adolescent rats [36]. Both male and female adolescent rats tested on P28–P35 under familiar, non-anxiety provoking circumstances show substantial increases in interaction with peers following acute exposure to relatively low doses of ethanol, an ethanol-induced facilitation of social behavior that is predominantly characterized by an increase in play behavior and is not normally seen in adults ([34,38,39,40,44]. Higher doses of ethanol have different social consequences, producing social inhibition, with male and female adolescent rats being less sensitive to these adverse social effects of ethanol than their more mature counterparts [40]. Social inhibition is characterized by significant decreases in social behaviors (social investigation, contact behavior, play behavior) without a suppression in locomotor activity under these social test circumstances. Therefore, social inhibition or social impairment is distinguishable from general motor impairment or sedation, especially given that the doses of ethanol that significantly suppress social behavior are typically lower than those suppressing locomotor activity under social circumstances (see [40]).

This adolescent-typical sensitivity to the social consequences of acute ethanol may be related in part to transient overexpression of NMDA receptors for the excitatory neurotransmitter glutamate in a number of brain regions [[49], [50], [51]]. Indeed, NMDA receptors have been implicated in age-specific modulation of social behavior. NMDA antagonists produce social inhibition in adult rodents [[17], [52]]. In adolescents, however, these antagonists facilitate play behavior at lower doses while suppressing social behavior at higher doses [[17], [18], [19],29] – biphasic effects on play behavior similar to those induced by ethanol [40]. The NR2B subunit of the NMDA receptor may play a particularly important role, given that a selective NR2B antagonist, ifenprodil was found to facilitate play behavior in a manner similar to that produced by low doses of less selective NMDA antagonists [18,19] as well as ethanol [40]. Together, these findings suggest that NMDA receptors may play a substantial role in both modulation of social behavior and sensitivity to ethanol-

induced social facilitation in adolescence. It is still unknown, however, whether non-NMDA glutamate receptors may also play an important role in social behavior and sensitivity to socially facilitating and/or socially suppressing effects of ethanol during adolescence. Therefore, one of the aims of the present study was to test whether effects of a selective AMPA antagonist, NBQX, are age-dependent, with low doses producing social facilitation in adolescent animals and higher doses eliciting pronounced social inhibition in adults.

The primary inhibitory neurotransmitter γ -aminobutyric acid (GABA) system also undergoes developmental changes. The most pronounced ontogenetic changes have been reported in GABAA receptor subunits, with these changes being region-dependent. For instance, expression of $\alpha 1$ subunit responsible for motor-impairing and sedative effects of ethanol [[53], [54]] is very low at birth but it is up-regulated during early adolescence [5], whereas expression of other ethanol-sensitive GABAA receptor subunits – $\alpha 2$, $\alpha 3$ and $\alpha 5$ – have been reported to be greater early in life than in adulthood in certain areas [12,23]. Adolescent-typical insensitivity to motor-impairing and sedative effects of ethanol [55] may be related to developmental changes in $\alpha 1$ subunits. Although GABAA receptors are implicated in a number of ethanol effects, no attempts have been made so far to assess whether extrasynaptic GABAA receptors may be age-dependently implicated in modulation of social behavior as well as in ethanol-associated social inhibition. Consequently, the second aim of this study was to assess effects of THIP, – a GABAA receptor agonist, with selectivity for the extrasynaptic δ -subunit. NBQX and THIP were chosen for social investigation since these drugs have been shown to be effective under different social test circumstances in adult rodents [3,13,22].

2. Method

2.1. Subjects

Male and female Sprague Dawley rats bred and reared in our colony at Binghamton University were used as experimental subjects (N=372) and social partners (N=372). All animals were housed in a temperature-controlled (22°C) vivarium maintained on a 12-/12-h light/dark cycle with lights on at 0700h and ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Litters were culled to 8–10 pups within 24h after birth and housed with their mothers in standard maternity cages with pine shavings as bedding material. Weaning occurred on postnatal day (P) 21, with experimental subjects and partners pair-housed with same-sex littermates. In all respects, maintenance and handling of the animals were in accord with 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 and drugs

The design for Experiment 1 was a 2 age (adolescent; adult) $\times$ 2 sex (male; female) $\times$ 6 dose (0.0, 1.0, 2.0, 4.0, 6.0, 8.0mg/kg NBQX) factorial with 10 animals per group (N=240). The test doses of the selective AMPA receptor antagonist, NBQX disodium salt (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt; Tocris Bioscience; half-life approximately 30min [7,27] were dissolved in 0.9% sterile saline and delivered

intraperitoneally (i.p.) at a volume of 2ml/kg body weight, with control animals receiving an equivalent volume of saline. The design for Experiment 2 was a 2 age (adolescent; adult)×2 sex (male; female)×4 dose (0.0, 1.0, 2.0, 4.0mg/kg THIP) factorial with 8–10 animals per group (N=217). The extrasynaptic GABAA receptor agonist THIP hydrochloride (4,5,6,7-Tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride); Tocris Bioscience; half-life approximately 1.5–2h [2] was also delivered i.p. in 0.9% sterile saline vehicle at doses of 0.0 (saline) 1.0, 2.0 and 4.0mg/kg in a volume of 2ml/kg body weight. In both experiments, same sex littermates were assigned semi-randomly to different drug doses to avoid the possible confounding of litter with dose effects [9,46].

2.3. Test apparatus and procedures

Social interaction chambers made of Plexiglas (Binghamton Plate Glass, Binghamton, NY) were size-proportioned for each age (adolescents: 30cm×20cm×20cm; adults: 45cm×30cm×20cm), with each separated into two compartments via a divider with an aperture in the middle that allowed movement between the two sides of the chamber. A video camera was used to record behavior during the 30min habituation period as well as the 10-min social interaction test. All testing took place under dim (15–20lx) light.

Social testing occurred on P34–36 (adolescents) or P70–72 (adults). On test day, animals were taken from their home cage, injected with saline vehicle or an assigned dose of NBQX (Experiment 1) or THIP (Experiment 2), and placed individually in the testing apparatus for 30min to habituate to the chamber. A social partner of the same age and sex was then introduced for a 10-min test period. Partners were always unfamiliar to both the test apparatus and the experimental animal, were drug-naïve and were not socially deprived prior to the test [37,38,40]. Weight differences between test subjects and their partners were minimized as much as possible, with this weight difference not exceeding 10g for adolescents or 20g for adults, and test subjects always being heavier than their partners. A marker was used on the back of each experimental animal to differentiate that animal from their social partner during the test. All testing procedures were conducted between 12:00 and 15:00h.

2.4. Behavioral measures

Social interaction data were scored for frequencies of 3 social behaviors: social investigation, contact behavior, and play behavior. A total number of crosses between the compartments was used as an index of locomotor activity under social test circumstances in order to distinguish between social facilitation and general activation as well as between social inhibition and general sedation. Social investigation was defined as frequency of sniffing of any part of the body of the partner, whereas contact behavior was characterized by the frequency of the experimental animal crawling over or under, or grooming the partner. Play behavior was assessed by the frequency with which the experimental animal displayed any of the following behaviors: pouncing or playful nape attack, following and chasing the partner, or pinning the partner [40]. Frequencies, rather than time, of each behavior were scored and analyzed, given that these elementary behavioral acts and postures (i.e., ethogram) demonstrated by experimental subjects are discrete and extremely short

lasting, especially during adolescence. All videos were scored by a trained experimenter without knowledge of the experimental condition of any given animal.

Based on initial observations that high doses of THIP induced signs of sedation, the Chandler-Crews Intoxication Rating Scale (IRS; see NADIA Consortium: <https://www.med.unc.edu/alcohol/nadiaconsortium/standardized-methods>) was added to estimate intoxication during the habituation period and to score post-test intoxication (see [6,21] for IRS applications). For the post-test analysis, animals were assigned a score from 1 (natural behavior) to 5 (severe impairment) reflected by the loss of righting reflex (LORR) and eye-blink reflex. During the habituation period, although it was not possible to score LORR and eye blink data from the videos, animals were assessed for intoxication using indices of motor impairment, crawling, and dragging of the abdomen.

2.5. Data analyses

In each study, separate 2 age×2 sex×6 NBQX dose (Exp.1) and 2 age×2 sex×4 THIP dose (Exp.2) factorial ANOVAs were used for each behavior measure to assess age, and/or sex differences in drug effects. Fisher's post hoc analyses were used to assess age, sex and dose effects. Due to violations of homogeneity of variance, IRS scores were analyzed using non-parametric Mann-Whitney U (MWU) analysis.

3. Results

3.1. Experiment 1: effects of NBQX on social interaction in adolescent and adult males and females

Data analyses revealed main effects of dose for social investigation [$F(5,216)=11.129$; $p<0.001$], contact behavior [$F(5,216)=9.039$; $p<0.001$], and play behavior [$F(5,216)=8.191$; $p<0.001$], with these social behaviors being suppressed by two highest doses of NBQX regardless of age (see Fig. 1, data collapsed across sex). Locomotor activity indexed via total number of crosses was not affected by NBQX at either age. There were also significant main effects of age for social investigation [$F(1,216)=15.739$; $p<0.001$], contact behavior [$F(1,216)=54.272$; $p<0.001$], and play behavior [$F(1,216)=65.549$; $p<0.001$], with adolescents showing higher levels of social behaviors than adults. Age effects were also evident for locomotor activity [$F(1,216)=27.538$; $p<0.001$], with adolescents showing more crosses between compartments than adults. A sex effect was seen in play behavior [$F(1,216)=5.523$; $p<0.02$], with males showing more play behavior (23.92 ± 1.48) than females (20.21 ± 1.13).

3.2. Experiment 2: effects of THIP on social interaction in adolescent and adult males and females

Significant age×dose interactions emerged with several measures, including social investigation [$F(3,116)=13.918$; $p<0.001$], contact behavior [$F(3,116)=11.868$; $p<0.001$], and play behavior [$F(3,116)=4.790$; $p<0.005$]. Adolescents given 4mg/kg THIP demonstrated significantly reduced social investigation and contact behavior relative to their saline controls, with no such effects emerging in adults (see Fig. 2, data collapsed across sex). All doses of THIP significantly reduced play behavior in adolescents, with no

suppression evident in adults. A significant age $\times$ dose [$F(3,116)=8.048$; $p<0.001$] interaction was evident for locomotor activity as well. Adolescents given 4mg/kg THIP exhibited significantly lower locomotor activity than their saline controls, with no inhibitory effects evident in adults.

Due to signs of apparent sedation caused by the highest dose of THIP (4.0mg/kg) in the initial squad of adolescents tested in Experiment 2, as discussed in the Methods section IRS scores were assessed during the habituation and post-test period for each animal. Due to non-homogeneity of variance, these data were analyzed using non-parametric MWU tests. The results revealed significantly greater intoxication rating for adolescents than for adults during both the habituation ($U=0.00$, $p<0.001$, $r=0.85$) and post-test assessments ($U=40.0$, $p<0.001$, $r=0.60$).

4. Discussion

The present study was designed to indirectly assess whether AMPA and extrasynaptic GABAA receptors contribute to adolescent-typical enhanced sensitivity to EtOH-induced social facilitation and attenuated sensitivity to EtOH's social inhibitory effects, respectively, by investigating the effects of NBQX and THIP on social behavior of adolescent and adult rats. NBQX did not produce social facilitation at either age, with higher doses of NBQX suppressing social behavior similarly in adolescents and adults. In contrast to EtOH, THIP induced social inhibition at all doses (significant suppression of play behavior) in adolescent animals, whereas the highest dose was sedative for this age group, with no social impairing or sedative effects evident in adults. Thus, the results of the current experiments suggest that age-related sensitivities to the social facilitating and inhibitory effects of EtOH are not modulated through AMPA and extrasynaptic GABAA receptors.

The selective AMPA antagonist NBQX produced significant suppression of all forms of social behavior in adolescents and adults, with no socially facilitating effects evident at either age. These effects were specific for social behavior, since locomotor activity during the social interaction test was not affected by socially suppressing doses of NBQX. These findings suggest that activation of AMPA receptors may inhibit social behavior regardless of age. The lack of age differences in the effects of the glutamate AMPA antagonist, NBQX, on social behavior differed drastically from the ontogenetic pattern of sensitivity to the social consequences of acute EtOH [40]. NBQX has primarily been investigated in the realm of the anticonvulsant literature, with little systematic investigation of the effects of NBQX on social behavior. Those studies that have examined the role of AMPA receptors in social contexts have focused on social aggression [41,42] and social memory [26]. In the social aggression study, animals given NBQX showed reduced biting of a partner while also showing increased non-aggressive behavior (such as contact behavior; [41]). Moreover, it is thought that NBQX can recover social deficits that are characteristic of autism [13]. Early life (P10) administration of NBQX has been shown to attenuate social preference deficits in adulthood that are typically characterized as autistic-like behaviors [13]. Taken together with previous findings [40], the results of the first experiment suggest that the marked difference between adolescents and adults in the social effects of EtOH is unlikely to be related to its actions as an AMPA receptor antagonist.

There is very limited evidence regarding the contributions of AMPA receptors in different effects of EtOH. Mice lacking these receptors show attenuated hypothermia in response to EtOH relative to wild type mice [4]. Yet, sensitivity to the sedative effects of EtOH did not differ between GluR1 $-/-$ and wild type mice, suggesting that EtOH effects on AMPA receptors can account for some, but not all, effects of EtOH. Moreover, EtOH tolerance can be blocked via NMDA receptor antagonists but not by non-NMDA antagonists such as NBQX [10]. In contrast to very modest involvement of AMPA receptors in EtOH effects, NMDA receptors are strongly affected by EtOH. Indeed, NMDA receptors have extremely high affinity for EtOH within the brain [11], and NMDA antagonists mimic the developmental EtOH effects in a social interaction paradigm ([29]; [[56], [17]]). That is, adolescents given low doses of NMDA antagonists demonstrate an increase in play behavior, reminiscent of the adolescent-typical increase in play behavior induced by EtOH [40]. Moreover, lower doses of the antagonists are needed to suppress social behavior in adult than adolescent rats [17], findings that again correspond with age differences in the social suppressant effects of EtOH [40]. Results of the present study further demonstrate that, in contrast to NMDA receptors ([11,29]; [[56], [17]]), AMPA receptors may not play as critical role in EtOH effects, including age differences in sensitivity to the social facilitating and/or social suppressing effects of EtOH.

Analyses of the effects of the extrasynaptic GABAA agonist, THIP, though yielding a number of age-specific effects, revealed generally opposite ontogenetic patterns to those seen with EtOH, with adolescents being uniquely sensitive to the social inhibitory effects of THIP. THIP-associated decreases in behavior at the highest dose appeared to extend beyond social measures, suggesting a general depression of locomotor activity at the highest dose, a sedative effect that emerged in adolescents only. Play behavior, however, was decreased at all doses in adolescent animals relative to saline controls, suggesting that the appearance of social suppression after lower doses of THIP may not be related to sedative effects emerging at highest dose, but rather may reflect a specific inhibitory effect on play behavior per se. The finding of enhanced sensitivity of adolescents to the sedative effects of the GABA agonist THIP is reminiscent of prior research investigating the ontogeny of the sedative effects of the general GABA agonist muscimol where adolescents showed greater sedation than adults [28]. Studies investigating the actions of THIP in social behavior have shown that THIP can relieve social anxiety when administered intraperitoneally a half hour prior to the test [3]. Moreover, THIP has been shown to restore typical GABAergic activity in rodents with fragile X syndrome that exhibit autism-like social deficits [22]. Similar to AMPA receptor antagonism, the GABA system is also thought to play a critical role in mediating social aggression [16].

The ontogenetic pattern of greater sedative effects of GABA agonists in adolescents than adults is converse from that seen with EtOH in which adolescents show attenuated EtOH-induced sedation relative to adults [1,14,32]. This discrepancy may stem from the fact that THIP is a GABAA receptor agonist with selectivity for the extrasynaptic δ -subunit, whereas GABAA receptors containing $\alpha 1$ -subunits are implicated in motor-impairing and sedative effects of EtOH [[57], [58]]. Expression and regional distribution of the $\alpha 1$ subunit is up-regulated during postnatal period, reaching its peak during early adolescence and then declining to adult levels around postnatal day 60 [5]. Given developmental changes in

expression of $\alpha 1$ subunits [45], it is not surprising that adolescents are less sensitive to the motor-impairing and sedative effects of EtOH than adults [55]. Furthermore, adolescent rats were found to be less sensitive than adults to the $\alpha 1$ -selective agonist zolpidem [20]. In contrast to $\alpha 1$ subunits that are sensitive to sedative and hence high doses of EtOH, δ -containing GABAA receptors are sensitive to low doses of EtOH [8,43]. Levels of δ -subunit mRNA and protein show a developmental increase, with the highest levels found in adults [12,25]. This ontogenetic increase can explain enhanced sensitivity to the sedative effects of THIP evident in adolescent rats (see Experiment 2). Due to the lower density of δ -subunit-containing receptors in the adolescent brain, the saturation of these receptors could be reached at lower concentrations of THIP than in adults. Our findings are in agreement with previously reported depressant effects of THIP in adolescent mice [24]. In contrast, enhanced locomotor activity has been reported in adult mice following THIP administration [59].

The present studies have some limitations that should be addressed. First, although NBQX antagonizes AMPA receptors, it also exerts similar actions upon kainate receptors. Additionally, following i.p. administration, the onset of EtOH effects may occur earlier than those of NBQX and THIP. Differences across drugs in the rapidity of drug onset could contribute to the observed differences in effects of the GABA and glutamate drugs on social behavior relative to those of ethanol. The findings from the present studies suggest that while blockade and stimulation of AMPA and extrasynaptic GABAA receptor systems, respectively, may induce socially suppressing and sedative effects, the lack of similar ontogenetic patterns with these drugs to those seen with EtOH do not support a role of these drugs in age-specific facilitating and impairing effects of EtOH.

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* Indicates significant changes at a certain dose relative to vehicle

Figure 1.
Effects of NBQX

* Indicates significant changes at a certain dose relative to vehicle

Figure 2.
Effects of THIP