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The Influence of Chronic Nicotine Administration on Behavioural and Neurochemical Parameters in Male and Female Rats after Repeated Binge Drinking Exposure

Frédéric Lallemand, Roberta J. Ward and Philippe De Witte*

Université catholique de Louvain, Laboratoire de Biologie du Comportement, 1 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

*Corresponding author: Biologie du Comportement, Université catholique de Louvain, 1 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium.

Tel: +32-10-474384; Fax: +32-10-474094; E-mail: dewitte@uclouvain.be

Abstract — Aims: The possible interaction between nicotine and ‘binge drinking’ in eliciting changes in behavioural patterns of ‘binge drinking’ rats as well as nucleus accumbens (NAc) glutamate levels has been investigated in these present studies. Methods: Adult or adolescent male and female rats received ethanol, 2 g/kg or 3 g/kg, by gavage in a ‘binge drinking’ regimen (3 times/day over a 6 h period, for 2 days followed by 5 days of abstinence) combined with or without nicotine, 0.3 g/kg, for either a 5-week (adult) or a 4-week (adolescent) period. Motor activity was then assessed for a period of 60 min after three further doses of ethanol or water. In addition, the NAc glutamate level was assayed in each group for 1 h after the first gavage regimen with ethanol, 2 g/kg or 3 g/kg, or water. Results: Adult female rats showed greater sensitivity to each ethanol dose (2 g/kg and 3 g/kg) than the adult male rats, their motor activity decreasing during the first and third ‘binge’. In contrast, in male adult rats, the sedative effects of ethanol were reduced, particularly after the third binge when no significant changes in the locomotor activity were apparent between the ethanol-administered male rats and controls. Adolescent rats did differ in their response to ethanol in comparison with adult rats. It was noteworthy that in young female adolescent rats, given 2 g/kg ethanol, motor activity was enhanced, thereby indicating that adolescent female rats are less sensitive to the sedative effects of ethanol at specific doses. In addition, male and female adolescent rats showed little change in locomotor activity in comparison with controls during the third ‘binge administration’ possibly indicating that tolerance to such alcohol doses was occurring. Nicotine administration did prevent the decrease in locomotor activity after ethanol administration during the first binge regimen in both male and female adolescents as well as adult female rats. However, after the third binge, such alcohol-induced changes in motor activity were not so well defined in the female adult rats that now showed significant decreases in motor activity. In contrast, adolescent male and female rats still showed similar motor activity to that of the controls. No clear association between the NAc glutamate extracellular content and locomotor activity was discernible in either adult or adolescent rats in these present studies. However, chronic nicotine administration markedly reduced the elevated basal glutamate content in the ‘binge drinking female’ adult rats. Conclusions: These studies have shown clear and distinct differences, with respect to both sensitivity and tolerance, in adult and adolescent male and female rats, which could be modified by supplementation with nicotine.

Introduction

In a recent report, NIAAA has highlighted the fact that there is widespread use of ethanol by adolescents, which outnumbers the use of other illicit drugs (NIAAA, 2004). Such adolescents, who commence drinking alcohol at an early age, are at a greater risk of life-time ethanol abuse and alcoholism (Duncan et al., 1997). Clinical findings have suggested that such ethanol exposure in adolescent, particularly in female subjects, during this neuro-developmental period, may disrupt normal development. Although there may be differences in ethanol pharmacokinetics, alcohol dehydrogenase genotypes, as well as presystemic alcohol metabolism in female alcoholic subjects (Ward and Coutelle, 2003), more studies are needed to elucidate whether these and other factors enhance their susceptibility to alcoholism.

A new pattern of ethanol consumption has recently emerged amongst adolescents; ‘binge drinking’ which is defined as the consumption of high doses of alcohol (at least 5 drinks) over a 2-h period, which elevates blood alcohol levels to 0.8 g/L or above (NIAAA, 2004). Such patterns of ethanol consumption in adolescents have been reported in Denmark, Ireland, Isle of Man, Malta, the Netherlands, Norway, Poland, Sweden and the United Kingdom. Brain damage and cognitive impairment occur more readily in these adolescents, particularly females, who ‘binge drink’ (Crews et al., 2000; Slawecki et al., 2004; White and Swartzwelder, 2004). In our recent studies, we have identified microglial activation exclusively in the hippocampal region of experimental rats administered a ‘binge drinking’ regimen, which may help to explain the cognitive problems encountered by ‘binge drinkers’ (Ward et al., 2009).

Alcohol abuse is often associated with a higher incidence of smoking (Hughes, 1995; Miller and Golds, 1998). The mesolimbic dopaminergic pathway is believed to play a major role in the reinforcing effects of both ethanol and nicotine. Nicotinic acetylcholine receptors (nAChR) and serotonin receptors (5-HT3) are present on the mesocorticolimbic dopamine neurons such that their stimulation by nicotine and/or ethanol will increase dopamine release into the nucleus accumbens. In addition, NMDA and GABA receptors located on these neurons could also be involved in the dopamine-mediated properties of ethanol (reviewed by Larsson and Engel (2004)). Ethanol may also increase nicotine’s affinity for certain nAChR in specific brain regions (Ward et al., 2008a). Experiments with Xenopus oocytes showed that ethanol increased nicotine activation of α2β2, α3β3 and α4β2 subunit receptors of nAChR (Larsson and Engel, 2004).

Genetic factors and gender play an important role in the metabolism and behavioural actions of ethanol, and doses of ethanol that elicit pleasurable feelings, activation and reduction of anxiety in some humans/animals may have aversive, sedative or no effect in others. The behavioural effects of moderate ethanol intake can cover a wide spectrum of effects, which can be either positive (e.g. pleasurable, activating) or negative...
Nicotine will induce changes in various behavioural characteristics. A single acute nicotine injection will initially induce a significant decrease in the locomotor activity that is followed by the dose-dependent stimulant activity. Habitual administration of nicotine will initiate a dose-dependent stimulatory action possibly indicating stimulation of nicotinic receptors. The number of brain nicotinic receptors is increased after chronic administration of nicotine (Yoshida et al., 1982), which parallels the development of tolerance to nicotine (Collins et al., 1988; Booker and Collins, 1997).

As yet there have been few studies of behavioural changes during or after ‘binge drinking’. In our recent study (Ward et al., 2009), no ethanol preference was present after withdrawal from a ‘binge drinking’ regimen. In contrast, in other ‘binge drinking’ models, physical dependence on ethanol has been reported (Crews and Nixon, 2008). Therefore, in this present study, we have studied both adolescent and adult rats, male and female, that had received a ‘binge drinking’ regimen combined with nicotine, to assess their interaction on locomotor motor activity. Measurements were made for a period of 1 hour after each of the three ethanol doses, which were administered by gavage during a 6-h period. Since glutamate has been implicated as one of the neurotransmitters involved in motor activity, its extracellular concentration was assayed in the nucleus accumbens in male and female adult and adolescent rats during the initial 60 min of the first ethanol binge.

MATERIAL AND METHODS

Male and female Wistar rats, either adult 200–250 g or adolescent 100–125 g, were individually housed in standard plastic cages and maintained in a temperature (22°C) and light-controlled environment (12 light/12 dark light cycles, light on at 8.00 am). The adolescent rats and the adult rats were placed in separate rooms. It would be expected that during the period of the experiment, the estrous cycle would be synchronized for the female rats in each group. They were given free access to commercial rat chow and tap water or nicotine solution, depending on the experimental group. All animal procedures were in strict accordance with the recommendations of EEC (86/609/CEF) and with the Belgian ‘projet de loi’ (Moniteur Belge 19.02.1992, p. 3437) on the care and use of laboratory animals.

Drugs and chemicals

Nicotine tartrate (Sigma) was dissolved in isotonic saline, (60 mg/100 mL) and diluted to a concentration of 0.0036 mg/mL (equivalent to 0.3 mg/kg) in water. The concentration of the solution was adjusted every 2 days, according to the liquid consumption and the mean body weight of the rat, to ensure that each rat received a concentration of nicotine equivalent to 0.3 mg/kg/day.

An ethanol solution 20% v/v (2 g/kg) or 25% v/v (3 g/kg) was dissolved in tap water and freshly prepared each week. An average volume of ∼3–4 mL was administered during the binge drinking regimen, such that as the weight of the rat increased the concentration of ethanol was elevated in this solution.

Experimental design

Experiment 1. An animal model of ‘binge drinking’ was used for these studies. Male and female adult rats (200–250 g) were administered ethanol by gavage three times per day with a time interval of 3 h between each gavage at concentrations of either 2 g/kg (1.27 mL ethanol) or 3 g/kg (1.52 mL ethanol) for two consecutive days. The following 5 days the rats were abstinent. A second group of rats was supplemented with nicotine. On the first day of ethanol administration, the rats commenced chronic oral administration of nicotine, 0.3 mg/kg, that continued until the end of the experiment. Appropriate control groups received water ± chronic oral administration of nicotine for a comparable time period. This cycle of 7 days was repeated four times more. The motor activity was recorded during the ‘binge drinking’ regimen on Day 56 for 1 h, on three separate occasions, after each of the ethanol administrations. At the conclusion of each of these one hourly motor activity sessions, the rat was returned to its home cage before the next ethanol dose. The time schedule is summarized in Fig. 1.

Experiment 2. Young male and female rats (100–125 g) were given ‘binge drinking’ regimen ± nicotine in a comparable fashion to that of Experiment 1. The age of the rats for the first ethanol administration by gavage was 28 days, which was then repeated for three cycles (Fig. 1). At the end of this period, motor activity was recorded for 1 h after each of the ethanol doses.

Motor activity measurement

The locomotor activity of the rats was assayed with the MacLab system. During these measurements, the rats had no access to a drinking bottle containing either tap water or the nicotine solution. The average activity was calculated during a 1 h session, with blocks of 10-min time intervals, and served as a measure of locomotor activity. The apparatus has been described in detail by Meert et al. (1992). Briefly, the apparatus consisted of plexiglas test cages (25 × 24 × 25 cm). The floor consisted of the plexiglas plate (45 × 23 × 0.6 cm) that could bend freely. Underneath the middle of the plate, two pieces of piezo-film (200 × 100 × 0.025 mm, polymeric PVF2) were glued and connected to an amplifier, such that deformations of the cage floor resulted in a piezoelectric response of each of the piezo films. One differential amplifier first summed these electric signals and the signal output was then filtered by a bandpass filter between 6 and 12 Hz (18 dB/oct slope). This end signal was sent to an eight-channel MacLab connected to a Macintosh Performa 630. A chart file enabled quantification of the frequencies of the pulse (Dalchour and De Witte, 1999).

Microdialysis experiment

In other group of rats, adult and adolescent, male and female, that had received the ‘binge drinking’ regimen (Fig. 1),
motor activity was carried out during the first 60 min of the ‘binge regimen’ when water or one of the two doses of ethanol, 2 g/kg and 3 g/kg ± chronic nicotine, was administered. Rats underwent surgical procedures, as described previously (Dahchour et al., 2005). Briefly, under general anaesthesia (chloral hydrate 400 mg/kg i.p.), the rats were fixed in a stereotaxic frame. Through a midline incision of skin and soft tissue, the skull was exposed and the Bregma point identified. A guide cannula (20 gauge stainless steel; Small Parts, Miami, FL, USA) was inserted into the nucleus accumbens (A/P 1.2 mm; M/L 1.4 mm; D/V −4.0 mm) according to the atlas of Paxinos and Watson (1986). The guide cannula was secured to the skull with two steel screws and cranioplast cement and kept patent with a 26-gauge stainless steel obturator (Small Parts Inc., FL, USA).

Dialysis experiments commenced 72 h post-operation recovery period. The dialysis probes had a molecular cut-off of 13 kDA and an inner membrane diameter of 0.2 mm (Spectrum Laboratories, Inc., USA). Microdialysis samples were collected every 20 min over a 6 h period. After the first 2 h of microdialysis, water, or one of the doses of ethanol, was administered by gavage, and samples were collected for a further 4-h period. The glutamate content was assayed by HPLC with electrochemical detection.

**Statistical evaluation**

The results are presented as mean ± standard error of the mean (S.E.M.). Data were analysed by two-way analysis of variance (ANOVA) with repeated measures on time for each treatment group (2 and 3 g/kg with or without nicotine) versus control (water) in the same sex, for each sex group (male and female, young or adult) for the same treatment (2 or 3 g/kg ethanol with or without nicotine) to assess in each study the significance of difference in motor activity after ethanol binge (2 or 3 g/kg) with or without chronic oral nicotine administration, 0.3 mg/kg/day, during the experiment. Where appropriate, post hoc pairwise comparisons were analysed by Fisher protected least significant difference test (GB-Stat 5.3 for Windows, Dynamic Microsystems, MD, USA). Criteria for significance was set at $P < 0.05$ for all tests.

**RESULTS**

(a) Effects of ethanol administered in a ‘binge type’ regimen on motor activity

Tables 1 and 2 summarize all of the results for motor activity in each of the animal groups.

(i) Adult rats. Control adult female rats exhibited a much lower motor activity than male rats (Fig. 2a and b). Analysis of the trend of decreasing activity during the 1 h period after water gavage showed similar curves for the locomotor activity in male and female adult rats. Nevertheless there were interactions between sex and time for the first and second gavage of water [respectively, $F(5, 70) = 5.654; P = 0.0002$ and $F(5, 70) = 2.685; P = 0.0281$].

After the first binge dose of 2 g/kg ethanol, there were a decrease in motor activities in both males and females compared
to controls (Fig. 2a and b) that continued throughout the period of the study for the female adult rats. In addition, there was a significantly decreased locomotor activity of female adult rats after the third gavage (Fig. 3b) compared to male \(F(1, 13) = 11.582; P = 0.0047\) and \(F(1, 13) = 8.379; P = 0.0125\), respectively. Subsequent administration of 2 g/kg in the second (data not shown) and third binge (Fig. 3a) evoked no further change in the adult male rats motor activity with respect to the controls. There were significant interactions between sex and time for the first and second gavage \(F(5, 65) = 5.544; P = 0.0003\) and \(F(5, 65) = 4.110; P = 0.0026\). The 3 g/kg ethanol gavage significantly decreased the female locomotor activity during the first gavage \(F(1, 12) = 9.269; P = 0.0102\) as well as the third \(F(1, 12) = 8.468; P = 0.0131\) compared to male (Figs. 2a and b and 3a and b).

Chronic nicotine administration had no significant effect on motor activity in either male or female adult controls, the values after 10 min being comparable to the non-supplemented controls. Chronic nicotine treatment significantly decreased male locomotor activity after the third gavage (Fig. 4a) but not the third gavage (Fig. 5a) with ethanol 2 g/kg when compared to the nicotine untreated group \(F(1, 13) = 34.303; P < 0.0001; F(1, 13) = 14.993; P = 0.0019\), but not with the second gavage (data not shown). In contrast, nicotine prevented the decreases in motor activity observed with the non-supplemented female after either doses of ethanol (Fig. 4b).

Locomotor activity in the adult female rats during the third binge regimen, which had received nicotine supplementation, showed a significant decrease in motor activity during the first 30 min after both doses of ethanol (Fig. 5b) but not male rats (Fig. 5a).

(ii) Adolescent rats. After the first and second gavage with water, there were significant decreases in motor activity in the female rats (Fig. 2d) compared to male adolescent rats (Fig. 2c) \(F(1, 26) = 4.753; P = 0.0385\) and \(F(1, 26) = 9.464; P = 0.0049\). The motor activity in adolescent males decreased significantly after each dose of ethanol in comparison with controls (Fig. 2c) while the female adolescent rats showed an increase or decrease, respectively, after 2 g/kg and 3 g/kg ethanol, compared to control (Fig. 2d).

During the first gavage, changes in the motor activity induced by the administration of 2 g/kg and 3 g/kg alone in male and female adolescent rats were not evident after chronic nicotine administration (Fig. 4c and d).

During the third gavage, nicotine supplementation did initiate some significant changes in motor activity during the initial
period of the motor activity readings in male (Fig. 5c) (for 20 min) and female (for 30 min) (Fig. 5d) adolescent rats for both ethanol doses compared to rats administered nicotine alone.

(b) Nucleus accumbens glutamate levels during the first 60 min after first administration of water or ethanol in a 'binge drinking' regimen

(i) Basal NAc glutamate content. Table 3 shows the results for the basal levels of NAc glutamate levels, for male and female adult and adolescent rats. The female adult rats, which had been administered either 2 g/kg or 3 g/kg ethanol for 56 days in a binge type regimen, showed significantly higher NAc glutamate levels when compared to the adult female controls \( F(2, 84) = 39.855; P < 0.0001 \), the adult male rats or adolescent male and female rats.

The adolescent female rats, which had received 2 g/kg ethanol in a ‘binge type’ regimen for 49 days, had significantly lower basal NAc glutamate levels than the controls \( F(2, 87) = 5.115; P = 0.0134 \) (Table 3).

Nicotine administration increased the basal NAc glutamate content in the control adult male rats, which had received water alone (Table 4). Administration of ethanol significantly decreased this basal glutamate levels \( F(2, 63) = 26.133; P < 0.0001 \). In the female adult rats that received nicotine, the basal levels of glutamate were now decreased or increased after 2 g/kg or 3 g/kg, respectively \( F(2, 63) = 93.319; P < 0.0001 \), compared to control, but significantly less than the non-supplemented rats after ethanol administration (Table 3).

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**Table 2. Number of movements at P49**

<table>
<thead>
<tr>
<th>Ethanol dose (g/kg)</th>
<th>Gavage</th>
<th>Start (0–10 min) Mean ± SE</th>
<th>10–20 min Mean ± SE</th>
<th>20–30 min Mean ± SE</th>
<th>30–40 min Mean ± SE</th>
<th>40–50 min Mean ± SE</th>
<th>End (50–60 min) Mean ± SE</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
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<tr>
<td>First</td>
<td>5397 ± 329</td>
<td>4448 ± 289</td>
<td>3937 ± 296</td>
<td>3731 ± 356</td>
<td>3450 ± 451</td>
<td>2952 ± 382</td>
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<tr>
<td>Second</td>
<td>2900 ± 412</td>
<td>2267 ± 385</td>
<td>1237 ± 325</td>
<td>1047 ± 283</td>
<td>791 ± 252</td>
<td>938 ± 280</td>
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<tr>
<td>Third</td>
<td>2556 ± 479</td>
<td>1578 ± 392</td>
<td>1372 ± 473</td>
<td>1142 ± 355</td>
<td>1109 ± 388</td>
<td>1033 ± 337</td>
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</tr>
<tr>
<td>+-Chronic nicotine 0.3 mg/kg/day p.o.</td>
<td>5817 ± 520</td>
<td>4603 ± 433</td>
<td>4117 ± 382</td>
<td>3746 ± 343</td>
<td>3638 ± 363</td>
<td>3403 ± 597</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>3232 ± 582</td>
<td>2948 ± 549</td>
<td>1838 ± 503</td>
<td>944 ± 172</td>
<td>911 ± 173</td>
<td>837 ± 239</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>4032 ± 322</td>
<td>2580 ± 360</td>
<td>1330 ± 329</td>
<td>777 ± 113</td>
<td>674 ± 95</td>
<td>583 ± 53</td>
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<tr>
<td>First</td>
<td>4032 ± 322</td>
<td>2580 ± 360</td>
<td>1330 ± 329</td>
<td>2489 ± 346</td>
<td>2025 ± 215</td>
<td>1964 ± 316</td>
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</tr>
<tr>
<td>Second</td>
<td>4868 ± 469</td>
<td>3595 ± 403</td>
<td>2964 ± 281</td>
<td>1060 ± 263</td>
<td>697 ± 163</td>
<td>685 ± 115</td>
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<tr>
<td>Third</td>
<td>2344 ± 425</td>
<td>1773 ± 311</td>
<td>1061 ± 246</td>
<td>909 ± 181</td>
<td>876 ± 202</td>
<td>938 ± 190</td>
<td></td>
</tr>
<tr>
<td>+-Chronic nicotine 0.3 mg/kg/day p.o.</td>
<td>5805 ± 557</td>
<td>4268 ± 400</td>
<td>3789 ± 410</td>
<td>2879 ± 465</td>
<td>2617 ± 492</td>
<td>2087 ± 473</td>
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</tr>
<tr>
<td>Second</td>
<td>3188 ± 535</td>
<td>1118 ± 245</td>
<td>635 ± 131</td>
<td>649 ± 149</td>
<td>747 ± 153</td>
<td>700 ± 81</td>
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<tr>
<td>Third</td>
<td>2888 ± 533</td>
<td>1063 ± 205</td>
<td>890 ± 200</td>
<td>953 ± 149</td>
<td>1002 ± 221</td>
<td>877 ± 151</td>
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<td></td>
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</tr>
<tr>
<td>First</td>
<td>4617 ± 611</td>
<td>2849 ± 391</td>
<td>1819 ± 316</td>
<td>2028 ± 205</td>
<td>1629 ± 444</td>
<td>1630 ± 362</td>
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</tr>
<tr>
<td>Second</td>
<td>2423 ± 434</td>
<td>1089 ± 301</td>
<td>793 ± 162</td>
<td>755 ± 138</td>
<td>711 ± 92</td>
<td>920 ± 214</td>
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<tr>
<td>Third</td>
<td>1487 ± 197</td>
<td>1115 ± 147</td>
<td>729 ± 72</td>
<td>552 ± 44</td>
<td>577 ± 81</td>
<td>500 ± 42</td>
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<tr>
<td>+-Chronic nicotine 0.3 mg/kg/day p.o.</td>
<td>5315 ± 595</td>
<td>4378 ± 446</td>
<td>3572 ± 430</td>
<td>3161 ± 417</td>
<td>2698 ± 311</td>
<td>2382 ± 451</td>
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<tr>
<td>Second</td>
<td>3420 ± 402</td>
<td>2441 ± 487</td>
<td>921 ± 187</td>
<td>672 ± 52</td>
<td>809 ± 166</td>
<td>829 ± 153</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>1735 ± 193</td>
<td>1066 ± 175</td>
<td>1227 ± 195</td>
<td>909 ± 164</td>
<td>1014 ± 169</td>
<td>1046 ± 159</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Basal glutamate concentrations (M) in nucleus accumbens of adult and adolescent rats after administration by gavage of either water or ethanol, 2 g/kg or 3 g/kg**

<table>
<thead>
<tr>
<th>Water</th>
<th>2 g/kg</th>
<th>3 g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>(2.84 \times 10^{-6})</td>
<td>(2.44 \times 10^{-6})</td>
</tr>
<tr>
<td></td>
<td>(\pm 1.09 \times 10^{-7})</td>
<td>(\pm 2.62 \times 10^{-8})</td>
</tr>
<tr>
<td>Female</td>
<td>(6.97 \times 10^{-6})</td>
<td>(28.6 \times 10^{-6^{**}})</td>
</tr>
<tr>
<td></td>
<td>(\pm 7.15 \times 10^{-7})</td>
<td>(\pm 1.89 \times 10^{-6})</td>
</tr>
<tr>
<td>Adolescent rats</td>
<td>(8.27 \times 10^{-6})</td>
<td>(5.73 \times 10^{-6})</td>
</tr>
<tr>
<td></td>
<td>(\pm 9.5 \times 10^{-7})</td>
<td>(\pm 7.44 \times 10^{-7})</td>
</tr>
<tr>
<td>Female</td>
<td>(6.34 \times 10^{-6})</td>
<td>(2.18 \times 10^{-6^{**}})</td>
</tr>
<tr>
<td></td>
<td>(\pm 4.81 \times 10^{-7})</td>
<td>(\pm 3.54 \times 10^{-7})</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard error. 
**P < 0.01 against the water group of each set.**
Results are presented as mean ± standard error.

**P < 0.01 against the water group of each set.

In the adolescents, nicotine administration did not have a marked effect on the basal NAc glutamate content in either male or female groups administered water or ethanol, except in adolescent female rats where 3 g/kg ethanol significantly decreased the basal NAc glutamate content.

(ii) Effect of ethanol administration on NAc glutamate levels in adult and adolescent male and female rats after administration of 2 g/kg or 3 g/kg in a ‘binge drinking’ regimen

In the adult rats, the NAc glutamate level remained significantly elevated for the first 60 min of the microdialysis experiment in the female rats (Fig. 6b), which had been administered either 2 g/kg or 3 g/kg ethanol in a ‘binge drinking regimen’ for 56 days, in comparison with its control [treatment F(2174) = 58.782; P < 0.0001; interaction NS]. In the adult male rats, only the group that was administered a further ethanol dose of 3 g/kg showed a significant increase in NAc basal glutamate levels during the initial 1-h period of the microdialysis (Fig. 6a) [treatment F(2144) = 3.339; P = 0.0551 NS; interaction F(12, 167) = 2.341; P = 0.0096].

After further administration of 2 g/kg ethanol to adolescent female rats, the NAc glutamate content (which was
Fig. 3. Locomotor activity in adult male, M (a) and female F (b), and adolescent, male, YM (c) and female, YF (d) after the third administration of ethanol 2 g/kg or 3 g/kg or water after 56 days or 49 days, respectively, of a ‘binge drinking’ regimen. For the statistical evaluation *P < 0.05, **P < 0.01.

significantly decreased initially) (Fig. 6d) gradually increased during the following 4 h of the microdialysis experiment (data not shown), although the level did not reach that of either the control or adolescent female rats administered 3 g/kg ethanol in a ‘binge drinking’ regimen [treatment F(2210) = 9.270; P = 0.0007; interaction NS]. In contrast, the male adolescent rats, administered water or ethanol 2 g/kg or 3 g/kg, showed little alteration in the NAc glutamate content during the first hour of the microdialysis experiment (Fig. 6c).

(iii) Effect of ethanol administration, 2 g/kg or 3 g/kg, in a binge drinking’ regimen on nucleus accumbens glutamate levels in adult and adolescent male and female rats supplemented with nicotine

Adult male rats, administered either 2 g/kg or 3 g/kg ethanol in a ‘binge type’ regimen, showed significantly lower NAc glutamate levels than the control adult rat initially, which then slowly increased throughout the period of the microdialysis (Fig. 7a) [treatment F(2144) = 3.339; P = 0.0551 NS; interaction F(12, 167) = 2.341; P = 0.0096]. In contrast, in the adult female rats, the NAc glutamate levels in rats administered 2 g/kg remained significantly reduced while the NAc glutamate in rats administered 3 g/kg was significantly higher [treatment F(2174) = 58.782; P < 0.0001], throughout the period of the microdialysis study (Fig. 7b).

In the adolescent rats, male and female, there were no significant changes in the NAc glutamate levels during the initial 1 h of microdialysis, after the further administration of either 2 g/kg or 3 g/kg in a ‘binge type’ regimen, when supplemented with nicotine (Fig. 7c and d).

Further, statistical analysis of the NAc basal glutamate levels and the motor activity of the rats at time 0 showed no correlation between these two parameters.

**DISCUSSION**

Adolescent and adult rats, male and female, have been utilized in these present studies to investigate whether a behavioural change, specifically locomotor activity, induced by ‘binge drinking’ could be modified by nicotine administration. Adult female rats showed greater sensitivity to ethanol, both 2 g/kg and 3 g/kg, than the adult male rats. Furthermore,
Adolescence is a critical developmental period in which the central nervous system undergoes developmental alterations including synaptic pruning, loss of glutamatergic input to the prefrontal cortex and other cortical areas. It is during this period of human development that smoking and alcohol drinking may commence. Therefore, toxicity evoked by ‘binge drinking’ during this brain maturation stage may lead to a multitude of problems including memory loss and cognitive deficits, both acutely and in later life.

A variety of behavioural tests are available to assess an animal’s response to drugs of addiction. These include tests that measure sedation, co-ordination and motor suppressant effects (Chuck et al., 2006). In this current study, motor activity in ‘binge drinking’ male and female, adult and adolescent rats has been evaluated before and after three successive ethanol doses, during a 6-h period, separated by 3 h of recovery combined with or without chronic nicotine administration. Such a ‘binge drinking’ model has not previously been assessed for locomotor activity. ‘Binge drinking’ may induce sensitization, which is defined as a situation where prior intermittent intake of ethanol leads to accentuated responses to subsequent ethanol administration. Alternatively, tolerance may develop, where the responses to successive doses of ethanol are attenuated.

Ethanol is classified as a sedative/hypnotic drug (Read et al., 1960) and is reported to have psychomotor stimulant effects at relatively low doses (Read et al., 1960; Pohorecky, 1977; Little, 2000; Correa et al., 2003a, 2003b, 2004). Higher ethanol
Motor Activity in Binge Drinking Rats Receiving Nicotine

Locomotor activity P56 after beginning of binge drinking treatment (10 min. time interval)

- M h2o _I (n= 8)
- M h2o + nic _III (n= 6)
- M etoh2g + nic _III (n= 7)
- M etoh3g + nic _III (n= 9)

Locomotor activity at P49 after binge drinking treatment (10 min. time interval)

- YM h2o _I (n= 14)
- YM h2o + nic _III (n= 12)
- YM etoh2g + nic _III (n= 10)
- YM etoh3g + nic _III (n= 10)

- F h2o _I (n= 13)
- F h2o + nic _III (n= 5)
- F etoh2g + nic _III (n= 11)
- F etoh3g + nic _III (n= 11)

Locomotor activity at P49 after binge drinking treatment (10 min. time interval)

- YF h2o _I (n= 14)
- YF h2o + nic _III (n= 10)
- YF etoh2g + nic _III (n= 9)
- YF etoh3g + nic _III (n= 10)

Fig. 5. Locomotor activity in adult male, M (a) and female, F (b), and adolescent, male, YM (c) and female, YF (d), after third administration of ethanol 2 g/kg or 3 g/kg or water after 56 days or 49 days, respectively, of a ‘binge drinking’ regimen supplemented chronically with nicotine 0.3 mg/kg. For the statistical evaluation, *P < 0.05, **P < 0.01.

doses will induce sedation, and suppress or impair motor activity (Read et al., 1960; Pohorecky, 1977; Correa et al., 2004). Although acute doses of ethanol, between 0.7 and 2.0 g/kg, enhance locomotor activity in mice (Eckardt et al., 1998), in rats acute peripheral injections of high ethanol doses tend to decrease locomotion, rearing and grooming in the open field (Masmur et al., 1986; Duncan et al., 2000; Sanchis-Segura et al., 2005), locomotion in small stabilimeter cages (Correa et al., 2003a) and in running wheels (Duncan and Baez, 1981).

Previous reports indicate that adolescents are less sensitive to the sedative effects of ethanol and show a rapid acute tolerance and/or innate low response (Silveri and Spear, 1998). Such changes of motor activity in adolescent rats after ethanol may relate to a reduced ethanol metabolism rate in these developing animals (Zorzano and Herrera, 1989; Silveri and Spear, 2000) such that they are relatively insensitive to ethanol. In another study, peri-adolescent rats were less sensitive to the sedative effects of ethanol, 4 or 5 g/kg ethanol, than adult rats (Little et al., 1996). Therefore, adolescent rats may show a greater propensity to develop acute (Silveri and Spear, 1998) and chronic (Grieve and Littleton, 1979; Swartzwelder et al., 1998) tolerance to ethanol.

Previous studies have also focused on the effect of chronic co-administration of nicotine and ethanol on behavioural characteristics, such as motor incoordination (Dar et al., 1994), impairment of aerial reflex and performance (Tracy et al., 1999) and ethanol preference and motor activity (Lallemand et al., 2007). In the latter study, we showed that the locomotor activity induced by alcohol withdrawal, ~6–7 h after cessation of chronic alcohol administration (Dahchour and De Witte, 1999), was markedly attenuated by administering nicotine 0.3 mg/kg either during or immediately before cessation of chronic alcohol administration (Lallemand et al., 2007). In other studies, intracerebellar infusions of nicotine or pre-treatment with nicotine also attenuated ethanol-induced motor incoordination in a dose-dependent manner (Dar et al., 1994).

It has also been demonstrated that pre-treating rats with nicotine, in a dose-dependent manner, prevents alcohol-induced impairment of both aerial righting reflex and performance as well as reference and working memory in an eight-arm radial
Fig. 6. Release of glutamate from the nucleus accumbens of adult male, M (a) and female, F (b) and adolescent male, YM (c) and female, YF (d) after first administration of ethanol, 2 g/kg or 3 g/kg or water during the first hour of microdialysis after 56 days or 49 days, respectively, of a ‘binge drinking’ regimen. Results are presented as mean ± standard error of the mean. For the statistical evaluation ∗P < 0.05, ∗∗P < 0.01.

maze (Tracy et al., 1999; Rezvani and Levin, 2002). In another study of male and female Lewis rats, which were administered nicotine over a 2-week period, a significantly higher spontaneous activity in both sexes was identified in comparison with saline-treated rats (Prus et al., 2008). In contrast, another study showed that a single nicotine dose, 0.2 mg/kg, significantly decreased locomotor activity in adolescent compared to adult rats (Rezvani and Levin, 2004). Co-administration of an acute dose of nicotine (0.2 mg/kg) and of alcohol 2.5 g/kg significantly decreased locomotor activity in adolescent rats compared to adult rats, thereby indicating a greater sensitivity to these two drugs of addiction in adolescent rats (Rezvani and Levin, 2004). In our present studies, nicotine reversed the decrease in locomotor activity after the first ‘binge drinking’ regimen in both male and female adolescents as well as adult female rats. The neurochemical basis for such results may be explained by the fact that both nicotine and ethanol actions are mediated, in part, via activation of central acetylcholine receptors located on brain dopamine neurons (Johnson et al., 1995); sub-chronic nicotine treatment will enhance the reinforcing and dopamine-activating properties of ethanol (Soderpalm et al., 2000).

Motor activity decreases during chronic alcohol administration (Lapin et al., 1987; Schaefer and Michael, 1992), but increases ~6–9 h after withdrawal (Dahchour and De Witte, 1999). Such alteration in motor activity has been associated with increases in glutamate release in specific brain regions, e.g. the hippocampus (Dahchour and De Witte, 1999), striatum (Rossetti and Carboni, 1995) and nucleus accumbens (Dahchour and De Witte, 1999). An increase in the NAc glutamate level has been reported in alcohol-prefering mice after repeated alcohol administration, 2 g/kg for 8 days, and was suggested to be an important determinant for regulating alcohol consumption, particularly high alcohol intake (Kapasova and Szumlinski, 2008). An increase in basal NAc glutamate levels after both ethanol doses was also evident in female adult rats in this present study. This could indicate that these rats had developed a tolerance to the increased NAc glutamate content that could be mediated via a variety of receptors including mGlu5 receptors that play a modulatory role on rodent locomotor behaviours (Kinney et al., 2003). It was also noteworthy that co-administration of nicotine to the adult female rats reduced the basal concentration of glutamate in both the 2 and 3 g/kg ‘binge drinking’ rats to that of the controls in the 2 g/kg, and to only a 2-fold increase in the 3 g/kg rats. However, there was no clear association between the glutamate extracellular NAc content and locomotor activity in our ‘binge drinking’ rat model thereby indicating that the phenotype and biochemical characteristics is totally different from that of chronic
alcohol abuse (Ward et al., 2008b). Previous studies have shown the importance of glutamate in inducing locomotor activity in the nucleus accumbens. For example, blocking glutamate uptake with L-pyrrolidine-2,4-dicarboxyl acid, in NAc of control rats, shows significant dose-dependent increases in both horizontal and vertical locomotor activity (Kim and Vezina, 1999) while increases in this brain region during ethanol withdrawal in chronically alcoholized rats (Dahchour and De Witte, 2003) similarly show enhanced locomotor activity. It could be concluded that acute increases in glutamate initiate change in locomotor activity while sustained elevation of glutamate, as observed in the female adult rats after a binge drinking regimen, do not elicit such locomotor activity changes.

Our studies have clearly identified significant differences in adult and adolescent rats, male and females, for one aspect of behavioural responses, locomotor activity after ethanol administration in a ‘binge type’ regimen. Chronic nicotine administration modulated some of the adverse effects of ethanol’s sedative effects, particularly in adolescent rats. Since there is a high rate of co-occurrence between smoking and alcohol, with between 70 and 90% of alcoholic subjects smoking, our results would indicate that nicotine might modulate some of the adverse effects of ethanol toxicity.

Fig. 7. Release of glutamate from the nucleus accumbens from adult male, M (a) and female, F (b) and adolescent male, YM (c) and female, YF (d) after first administration of ethanol, 2 g/kg or 3 g/kg, or water during the first hour of microdialysis after 56 days or 49 days, respectively, of a ‘binge drinking’ regimen after chronic supplementation with nicotine. Results are presented as mean ± standard error of the mean. For the statistical evaluation, *P < 0.05, **P < 0.01.

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