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Motor Stimulant Effects of Ethanol Injected into the Substantia Nigra Pars Reticulata: Importance of Catalase-Mediated Metabolism and the Role of Acetaldehyde

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A series of experiments was conducted to investigate the locomotor effects of local injections of ethanol and the ethanol metabolite, acetaldehyde, into substantia nigra pars reticulata (SNr). Infusions of ethanol into SNr resulted in a dose-related increase in locomotor activity, with maximal effects at a dose of 1.4 μmol. Ethanol injected into a control site dorsal to substantia nigra failed to stimulate locomotion, and another inactive site was identified in brainstem areas posterior to substantia nigra. The locomotor effects of intranigral ethanol (1.4 μmol) were reduced by coadministration of 10 mg/kg sodium azide, a catalase inhibitor that acts to reduce the metabolism of ethanol into acetaldehyde in the brain. SNr infusions of acetaldehyde, which is the first metabolite of ethanol, also increased locomotion. Taken together, these results indicate that SNr is one of the sites at which ethanol and acetaldehyde may be acting to induce locomotor activity. These results are consistent with the hypothesis that acetaldehyde is a centrally active metabolite of ethanol, and provide further support for the idea that catalase activity is a critical step in the regulation of ethanol-induced motor activity. These studies have implications for understanding the brain mechanisms involved in mediating the ascending limb of the biphasic dose–response curve for the effect of ethanol on locomotor activity.

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INTRODUCTION

Ethanol is a sedative-hypnotic drug that has motor stimulant effects at low to moderate doses, and sedative or ataxic effects at higher doses (Read *et al*, 1960; Pohorecky, 1977; Crabbe *et al*, 1982; Dudek and Phillips, 1990; Little, 2000; Phillips *et al*, 2002). Previous studies from our laboratory have demonstrated that central (ie, intraventricular) administration of low doses of ethanol in rats produce behavioral stimulant effects (Arizzi *et al*, 2003; Correa *et al*, 2003a, b). The neurochemistry and neuroanatomy of this effect are still unknown, but in view of research demonstrating that GABA is involved in some of the neurochemical and physiological effects of ethanol (Mereu and Gessa, 1985; Marrosu *et al*, 1989; Grobin *et al*, 1998; Criswell *et al*, 1993; Grant and Lovinger, 1995), brain areas at which GABA mechanisms modulate motor activity offer potential substrates for the motor actions of ethanol. One of the brain areas at which GABA mechanisms exert a powerful control over locomotion is the substantia nigra pars reticulata (SNr). SNr is a part of the basal ganglia circuitry that has been implicated in several types of motor activity, including muscle rigidity (Crocker, 1997), lever pressing (Trevitt *et al*, 2001; Correa *et al*, 2003c), tremor (Finn *et al*, 1997; Mayorga *et al*, 1999; Carlson *et al*, 2003a, b), catalepsy (Scheel-Kruger *et al*, 1977), circling (Scheel-Kruger *et al*, 1977), and locomotion (Scheel-Kruger *et al*, 1977, 1981; Abraini *et al*, 1999; Trevitt *et al*, 2002). Although the SNr was once viewed as a region that simply provided feedback regulation of dopamine neurons in the substantia nigra pars compacta, it has become evident in the last few years that the SNr is one of the two major output nuclei for the basal ganglia (Scheel-Kruger *et al*, 1981; Young and Penney, 1993; Bevan *et al*, 1996; Wichmann *et al*, 1999). SNr is a brain site at which several neurotransmitter systems interact to regulate motor activity. Interactions between dopamine D1 and $GABA_A$ receptors in SNr regulate various aspects of motor activity (Mayorga *et al*, 1999; Trevitt *et al*, 2002). GABAergic manipulation of the SNr produced profound effects on various aspects of motor activity (Scheel-Kruger *et al*, 1977, 1981). Moreover, SNr is a brain area at which $GABA_A$ agonists and antagonists exert very potent effects on locomotion (Trevitt *et al*, 2002). Drugs that act on $GABA_A$ receptors are very potent at modifying motor output when injected locally into SNr,

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with effects at doses as low as 6 ng for the $GABA_A$ agonist muscimol (Finn *et al*, 1997; Trevitt *et al*, 2002), and 12–18 ng for the $GABA_A$ antagonist bicuculline (Mayorga *et al*, 1999; Trevitt *et al*, 2002; Correa *et al*, 2003c).

Ethanol has several effects on the physiology and neurochemistry of SNr neurons. Peripherally administered ethanol was shown to inhibit the firing of neurons in the SNr through a GABAergic mechanism (Mereu and Gessa, 1985; Diana *et al*, 1993). Increased biochemical measures of GABA activity have been reported to occur in SNr after acute administration of ethanol, and decreased activity was observed after chronic ethanol exposure (Gonzalez and Czachura, 1989). Ethanol enhances GABA function within specific regions of the brain, including SNr, by affecting a $GABA_A$ receptor with specific structural components (Criswell *et al*, 1993). Ethanol in the SNr facilitates chloride channel opening, at least in part, through an interaction with the GABA–benzodiazepine receptor-gated chloride ionophore (Marrosu *et al*, 1989). In view of the findings indicating that SNr is a brain site that is involved in the regulation of locomotion, and that ethanol exerts modulatory effects over the physiology of SNr neurons, it is reasonable to investigate the possibility that local injections of ethanol into SNr will affect motor activity.

Another important question in this area is the role of ethanol metabolism in the motor effects of ethanol. Acetaldehyde is an active metabolite that contributes to several of the effects of ethanol (Myers and Veale, 1969; Amir *et al*, 1980; Hunt, 1996; Smith *et al*, 1997; Zimatkin and Deitrich, 1997; Quertemont and Tambour, 2004; Quertemont *et al*, 2005a). Acetaldehyde generally is more potent than ethanol when administered peripherally, both in terms of its behavioral (Quertemont *et al*, 2005b) and physiological (Foddai *et al*, 2004) effects. Peripherally administered acetaldehyde has been shown to decrease motor activity under several conditions (Correa *et al*, 2005). In contrast, acetaldehyde administered into the lateral ventricles was shown to increase locomotor activity (Correa *et al*, 2003b), and to increase lever pressing rate on a differential reinforcement of low rates 30 s schedule (DRL30), which is an operant paradigm that generates low rates of responding and therefore is sensitive to the stimulant or disinhibiting effects of drugs (Arizzi *et al*, 2003). Thus, it has been hypothesized that central acetaldehyde can be partially responsible for the motor-activating effects of ethanol.

The enzyme catalase is thought to be the major complex responsible for the conversion of ethanol to acetaldehyde in the brain, and considerable evidence indicates that brain catalase is involved in the modulation of ethanol-induced motor activity. Mice lacking catalase have lower levels of ethanol-induced locomotion than control animals (Aragon *et al*, 1992a; Aragon and Amit, 1993), and across several pharmacological manipulations there is a high positive correlation between ethanol-induced locomotor activity and brain catalase activity (Correa *et al*, 1999a, b, 2000, 2001, 2004a, b; Sanchis-Segura *et al*, 1999a–c; Pastor *et al*, 2002). Because catalase has been strongly implicated in ethanol metabolism in the brain (Aragon *et al*, 1991, 1992b; Gill *et al*, 1992), it is reasonable to suggest that this enzyme may regulate the motor effects of ethanol through a mechanism tied to the rate of cerebral acetaldehyde production (Aragon *et al*, 1989, 1991, 1992b). Previous research indicates that substantia nigra is one of the brain areas with the highest concentration of the ethanol-metabolizing enzyme catalase (McKenna *et al*, 1976; Brannan *et al*, 1981; Moreno *et al*, 1995; Zimatkin and Lindros, 1996), which suggests that SNr may be an important brain locus at which acetaldehyde would modulate locomotor activity.

For these reasons, the present experiments focused upon SNr as a possible substrate for the locomotor effects of ethanol, and the possible importance of ethanol metabolism in these effects. The first experiment studied the locomotor effects of direct local infusions of ethanol into SNr. The second experiment studied the effects of ethanol infused into control sites dorsal and posterior to the SNr. In view of the possible role of brain ethanol metabolism in mediating the locomotor effects of ethanol, the third experiment studied the effects of the catalase inhibitor, sodium azide, on the locomotor activity induced by SNr injections of ethanol. The fourth experiment studied the locomotor effects of acetaldehyde infused directly into SNr.

MATERIALS AND METHODS

Animals

A total of 177 male Sprague–Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were used in these experiments ($n = 63$ for the ethanol dose–response study, $n = 26$ for the dorsal control site study, $n = 10$ for the posterior control site study, $n = 37$ for the ethanol–sodium azide study, and $n = 41$ for the acetaldehyde study). Rats were housed in a colony maintained at 23°C with a 12 L : 12 D cycle (lights on at 0800). All rats weighed between 280 and 320 g at the beginning of the study. Water and food were available *ad lib* in the home cages. Animal protocols were approved by the institutional animal care and use committee, and the methods were in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press (1996).

Drugs and Selection of Doses

For surgery, xylazine and ketamine were purchased from Phoenix Pharmaceutical Inc. (St Joseph, MO). Ethanol (99.5%, USP grade, Pharmco Products) and acetaldehyde (Fisher Scientific) were dissolved in artificial cerebrospinal fluid (aCSF: 147.2 mM NaCl, 1.2 mM $CaCl_2$, and 4.0 mM KCl) to make up the final drug solutions, and aCSF also was the vehicle solution used for control treatments. All drug doses listed reflect the total amount of drug injected bilaterally (ie, both sides added together). Based on previous studies with intraventricular administration (Correa *et al*, 2003a, b), ethanol was injected in doses in the range of 0.175, 0.35, 0.7, 1.4, or 2.8 μmol (ethanol: 8.05, 16.1, 32.2, 64.4, or 128.8 μg). A previous paper from our laboratory reported that brain extract levels of ethanol following intraventricular infusion of 2.8 μmol were 9.6–23.7 mg/100 ml (Correa *et al*, 2003a, p 370), which were lower than reported values of peak brain ethanol concentrations obtained from AA and Wistar rats that self-administered ethanol (means approximately 65–71 mg/100 ml; Nurmi *et al*, 1999). Based upon the results of the

experiment involving ethanol injected into SNr, and previous studies showing that acetaldehyde and ethanol acted on locomotion and operant responding over a similar dose range following intraventricular injections (Correa *et al*, 2003a, b; Arizzi *et al*, 2003), acetaldehyde was injected in a dose range of 0.35, 0.7, 1.4, or 2.8 μmol (acetaldehyde: 15.41, 30.83, 61.67, or 123.34 μg). In experiment 3, sodium azide (Fisher Scientific) was dissolved in physiological saline for intraperitoneal (i.p.) injections. At 30 min prior to testing, i.p. injections of sodium azide (0 or 10 mg/kg) were given. The dose and time interval were selected based upon the literature (Sanchis-Segura *et al*, 1999a), and after pilot studies across a range of doses were used to determine doses that would maximize the effects on catalase inhibition, and minimize any effects the drug would have on locomotion when administered alone.

Surgical Procedure

Rats were anesthetized with a solution (1.0 ml/kg, i.p.) that contained ketamine (100 mg/ml) and xylazine (20 mg/ml). Bilateral guide cannulae (23 ga stainless steel tubing, Small Parts) were chronically implanted to be 1.0 mm dorsal to the target structure. The coordinates for the different placements were obtained from Pellegrino and Cushman's (1967) sterotaxic atlas: SNr: AP −3.0 mm (from bregma), ML + 1.9 mm lateral (from midline), and DV −7.8 mm ventral (from the surface of the skull); dorsal control: AP −3.0 mm (from bregma), ML + 1.9 mm lateral (from midline), and DV −5.2 mm ventral (from the surface of the skull); posterior brainstem control: AP −3.8 mm (from bregma), ML + 1.0 mm lateral (from midline), and DV −8.0 mm ventral (from the surface of the skull). The incisor bar on the stereotax was set to 5.0 mm above the interaural line. All animals were single-housed following surgery, and were allowed to recover for 10 days before behavioral testing. Stainless steel stylets were kept in the guide cannulae to maintain their integrity.

Intracranial Drug Injection Procedures

Intracranial drug injections were made via 30 ga stainless steel injectors extending 1.0 mm below the tip of the guide cannulae. The injectors were attached to 10.0 μl Hamilton syringes by PE-10 tubing. A syringe pump (Harvard Apparatus) drove the injections at a rate of 0.5 μl/min for a total volume of 0.5 μl per side. Following the infusion, the injectors remained in place for 1 min to allow for diffusion of the drug, after which the injectors were removed, stylets were replaced, and animals were immediately placed into the activity chamber for testing.

Behavioral Procedures

Rats were tested for locomotor activity in an automated Plexiglas stabilimeter chamber (28 cm × 28 cm × 28 cm) inside a sound-attenuating box. The floor of the chamber consisted of two moveable wire mesh panels (27 cm × 13 cm) mounted 6.0 cm above the box floor on a centered rod attached at either end to the sides of the chamber, allowing for slight vertical movement of the floor panels. Movement of the panels was detected by microswitches mounted outside the chamber at the ends of each panel. The depression of a given quadrant (quadrant = 1/2 of each panel) closed the circuit on the microswitch, and each closing of a microswitch was counted as a single activity count. All animals were habituated to the chamber and to the injection procedure in a total of two sessions of 30 min each conducted 2 days before testing to decrease activational effects of the mild restraint used during the injection process and the novelty-activating effect of the chamber. On the day of testing, animals were placed in the chamber immediately after intracranial drug injections and locomotor behavior was recorded for 10 min. In the sodium azide experiment (experiment 3c), animals were i.p. injected, placed back into their home cages for 30 min, and then injected intracranially and placed in the activity chamber.

Catalase Activity Determination

Brain catalase activity was determined in independent groups of male rats ($n = 6$ per group). Rats were perfused using heparinized (1000 U/l) isotonic saline. The whole brain was removed and homogenized in a phosphate buffer (50 mmol/l; pH 7.0) with digitonin (0.01%). Brain homogenates were centrifuged at 10 000 r.p.m. (8730 *g*) for 10 min in an Eppendorf microcentrifuge. Supernatant aliquots were used to determine brain catalase levels. Catalase activity was assayed spectrophotometrically in the supernatants by measuring the decrease in absorbance of H_2O_2 at 240 nm ($\varepsilon_{240} = 0.00394\ \text{mmol}^{-1} \times \text{mm}^{-1}$) (Aebi, 1974). Protein levels were determined from supernatants (Bradford, 1976).

Experiments

Four experiments were conducted.

Experiment 1: SNr injections of ethanol. The first experiment assessed the effects of local injections of vehicle or ethanol (0.175, 0.35, 0.7, 1.4, or 2.8 μmol) into the SNr on locomotor activity ($n = 63$). Based upon the results of this experiment, the 1.4 μmol dose of ethanol was the highest dose used in experiment 2a, and the only dose used in experiments 2b and 3.

Experiment 2: Ethanol injections into control sites. Experiment 2a studied the locomotor effects of injections of vehicle or ethanol (0.35, 0.7, and 1.4 μmol) into a control site dorsal to the SNr ($n = 26$). Experiment 2b examined the effects of vehicle and 1.4 μmol ethanol injected into a control site posterior to SNr ($n = 12$).

Experiment 3: Effect of catalase inhibition on ethanol-induced activity. There were three components to this experiment. Experiment 3a was conducted to determine if 10 mg/kg of the catalase inhibitor sodium azide had any effect on locomotion when administered alone ($n = 32$). Animals received i.p. injections of either saline or 10 mg/kg sodium azide, and were tested for locomotion as described above. Experiment 3b studied the effect of 10 mg/kg sodium azide on brain catalase activity ($n = 12$). Rats received i.p. injections of either saline or 10 mg/kg sodium azide, and were assessed for brain catalase activity as described above. Experiment 3c employed a two-factor design to study the effect of catalase blockade on the locomotion induced by intranigral injections of ethanol ($n = 37$). Rats received an

i.p. injection of either vehicle or 10 mg/kg of the catalase inhibitor sodium azide, and also received an injection of either vehicle or 1.4 μmol ethanol directly into the SNr.

Experiment 4: SNr injections of acetaldehyde. The final experiment assessed the locomotor effects of local injections of vehicle or acetaldehyde (0.35, 0.7, 1.4, or 2.8 μmol) into the SNr ($n = 41$).

Histology

After the experiments were completed, all animals were intracardially perfused with 0.9% saline. Brains were then stored refrigerated in 3.7% formaldehyde solution for at least 5 days prior to slicing. The placements of the injectors were verified histologically by collecting consecutive 50 μm sections through the relevant brain areas. Sections were mounted on slides and stained with cresyl violet solution to aid in detection of the injector tracts. Coverslipped slides were viewed microscopically to assess accuracy of implantation. Any animal with improper placement in either hemisphere (ie, not in the target structures, such as SNr), or significant damage around the injection site, was not included in the statistical analyses of behavioral data (22.7% of all implantations were rejected). See Figure 1 for a photomicrograph of a representative SNr cannula and injector, showing placement in the SNr and negligible amounts of gliosis.

Statistical Analysis

Total locomotor activity data across the 10 min sessions were analyzed using one-way analysis of variance (ANOVA) in experiments 1, 2a, 2b, and 4. Planned comparisons of differences between each drug dose and its respective vehicle control data were conducted using the LSD test. The chosen level of significance was set at $p \leqslant 0.05$. A *t*-test was used to analyze experiments 3a and 3b because they involved only two groups. Experiment 3c was analyzed using a 2×2 factorial ANOVA. These analyses were conducted using a computerized statistical program (Systat). For experiments 1 and 4, orthogonal analysis of trend was used to determine if the dose–response curves were characterized by linear or quadratic (ie, biphasic) functions (SPSS). A quadratic trend is one that displays a single bend either upward or downward; this is sometimes referred to as an inverted-U shape (Keppel, 1991).

RESULTS

Experiment 1: SNr Injections of Ethanol

A one-way ANOVA for the between factor dose of ethanol indicated that infusion of ethanol into the SNr led to a significant overall increase in locomotor activity ($F(5,57) = 3.52$, $p < 0.01$) (see Figure 2). The planned comparisons revealed that all doses of ethanol except the lowest (ie, 0.175 μmol) produced increases in activity that were significantly different from the effects of vehicle (1.4 μmol $p < 0.01$; and 0.35, 0.7, and 2.8 μmol, $p < 0.05$). Trend analysis revealed that the overall dose–response curve showed a significant linear trend ($F(1,57) = 5.0$, $p < 0.05$), and also showed a robust, statistically significant quadratic trend ($F(1,57) = 10.3$, $p < 0.005$). None of the other trends were significant. The significant quadratic trend indicates that the dose–response curve for ethanol had an inverted-U shape, with a peak at 1.4 μmol. Figure 3 shows injector placements for animals that received the 1.4 μmol dose of ethanol.

Experiment 2: Ethanol Injections into Control Sites

Ethanol (0.0, 0.35, 0.7, or 1.4 μmol) injected into the dorsal control site produced no significant change in behavior compared to the vehicle group, and there was also no effect of ethanol injections into the posterior control site (see Table 1; dorsal control: $F(3,22) = 0.486$, n.s.; posterior control: $F(3,8) = 0.53$, n.s.). Figures 4a and b show injector placements in the dorsal and posterior brainstem control sites, respectively.

Experiment 3: Effect of Catalase Inhibition on Ethanol-Induced Activity

Experiment 3a was conducted to determine if 10 mg/kg of the catalase inhibitor sodium azide had any effect on

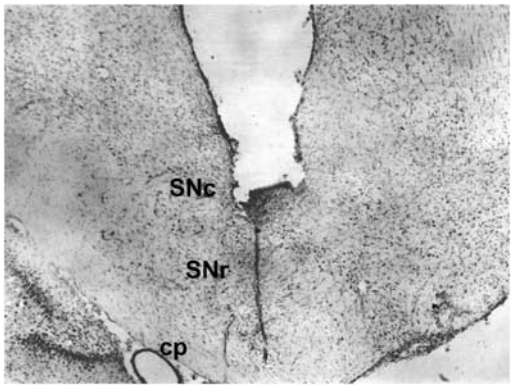

Figure 1 Photomicrograph of SNr with cannula and injector tracts. The animal received an injection of 2.8 μmol of ethanol. SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; cp: cerebral peduncle.

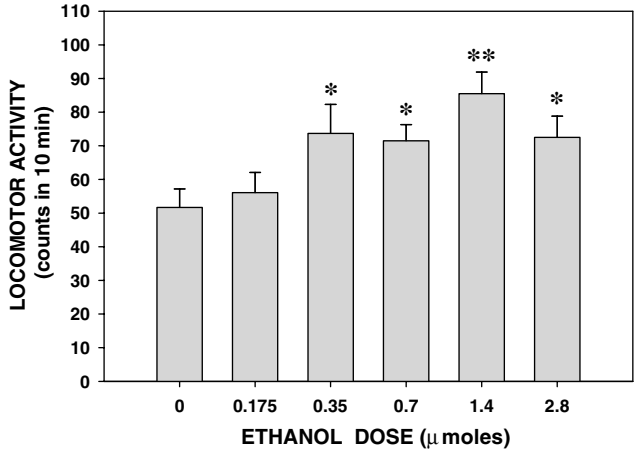

Figure 2 Effect of intranigral ethanol administration (0.0, 0.35, 0.7, 1.4, or 2.8 μmol) on locomotor activity in rats. Mean ± SEM represent counts in 10 min. $^{**}p < 0.01$, $^{*}p < 0.05$ significantly different from vehicle.

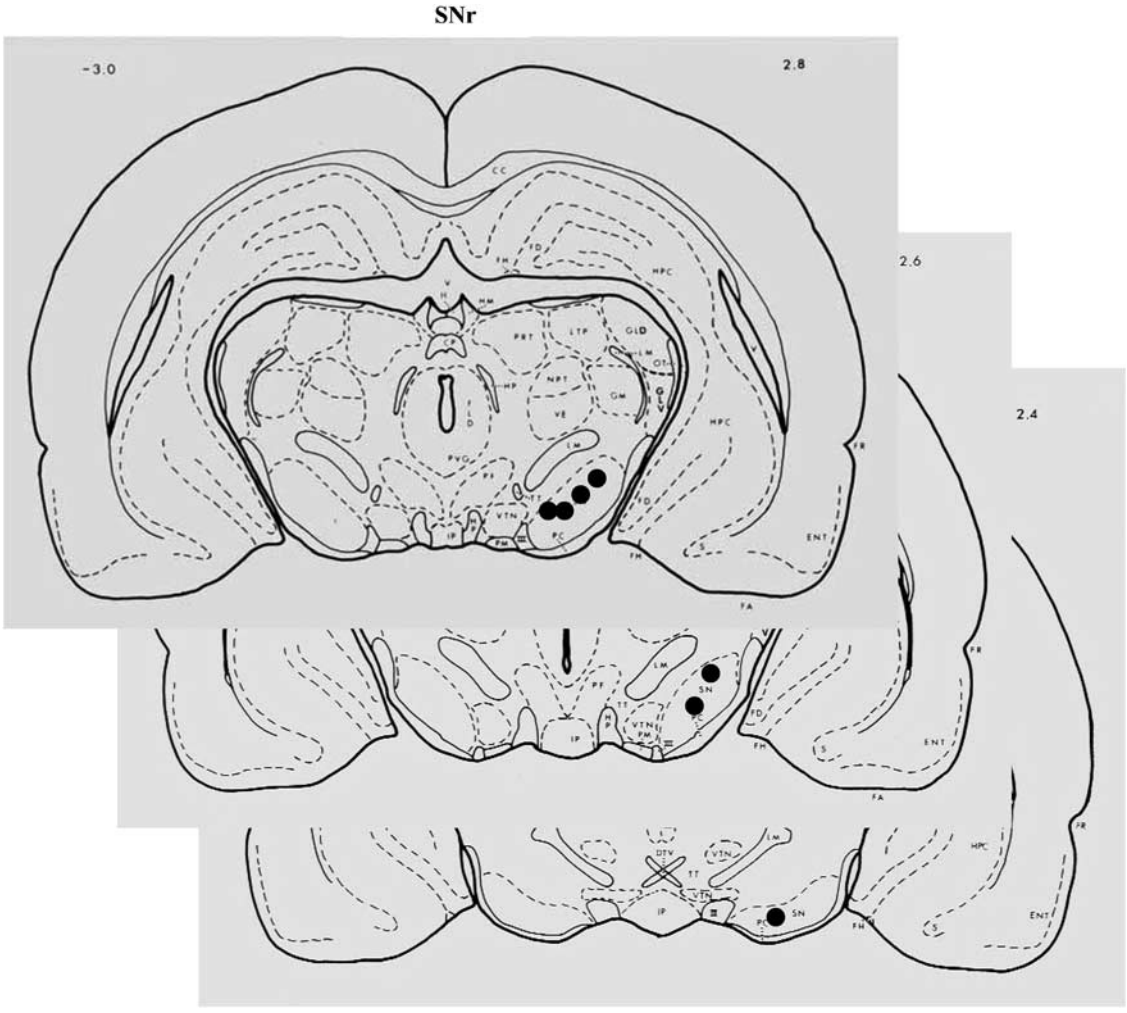

Figure 3 Cannula placements for animals that received the 1.4 μmol dose of ethanol into SNr (right side only; Pellegrino and Cushman, 1967, plates 47–49) and the dorsal control site (left side only; Pellegrino and Cushman, 1967, plates 47 and 48).

Table 1 Effect of Ethanol Injection into Dorsal and Brainstem Control Sites on Locomotor Activity in Rats

	Ethanol dose (μmol)			
	Vehicle	**0.35**	**0.7**	**1.4**
Dorsal control	61.4±7.3	56.6±8.7	47.6±5.2	55.3±6.9
Posterior control	71.0±15.7	84.0±10.0	71.5±10.5	60.3±12.6

Mean±SEM represent counts in 10 min.

locomotion when administered alone. Mean (±SEM) numbers of locomotor counts were as follows: saline vehicle, 46.7 (±4.6); 10 mg/kg sodium azide, 40.4 (±5.0). Statistical analysis showed no difference in locomotor activity between 10 mg/kg sodium azide and vehicle ($t=0.94$, df = 30, n.s.). Experiment 3b studied the effect of 10 mg/kg sodium azide on brain catalase activity. It was observed that 10 mg/kg sodium azide decreased brain catalase activity in rats by 40% 30 min after i.p. injection: vehicle ($n=6$) 1.067±1.11 mmol H_2O_2/min/mg protein; sodium azide ($n=6$) 0.636±0.44 mmol H_2O_2/min/mg protein ($t=3.58$, df = 10, $p<0.005$).

Figure 5 shows the effect of i.p. administration of the catalase inhibitor sodium azide (0 or 10 mg/kg) on the locomotion induced by intranigral ethanol (0.0 or 1.4 μmol). The two-way factorial ANOVA showed a significant overall effect of sodium azide treatment ($F(1,33)=19.015$, $p<0.01$), a significant effect of the ethanol factor ($F(1,33)=12.282$, $p<0.01$), and a significant sodium azide × ethanol interaction ($F(1,33)=10.099$, $p<0.01$), indicating that the effect of sodium azide on locomotion was different in animals treated with vehicle compared to those treated with ethanol. *Post hoc* comparisons with the Tukey test revealed that sodium azide alone did not suppress locomotion relative to the combined vehicle condition ($p<0.05$). Administration of ethanol significantly increased locomotion compared to vehicle ($p<0.05$), and sodium azide significantly reduced activity in ethanol-treated animals compared to ethanol alone ($p<0.05$). Rats treated with ethanol alone also significantly differed from rats receiving sodium azide alone ($p<0.05$).

Experiment 4: SNr Injections of Acetaldehyde

A one-way ANOVA with acetaldehyde dose as the main factor revealed that infusions of acetaldehyde directly into

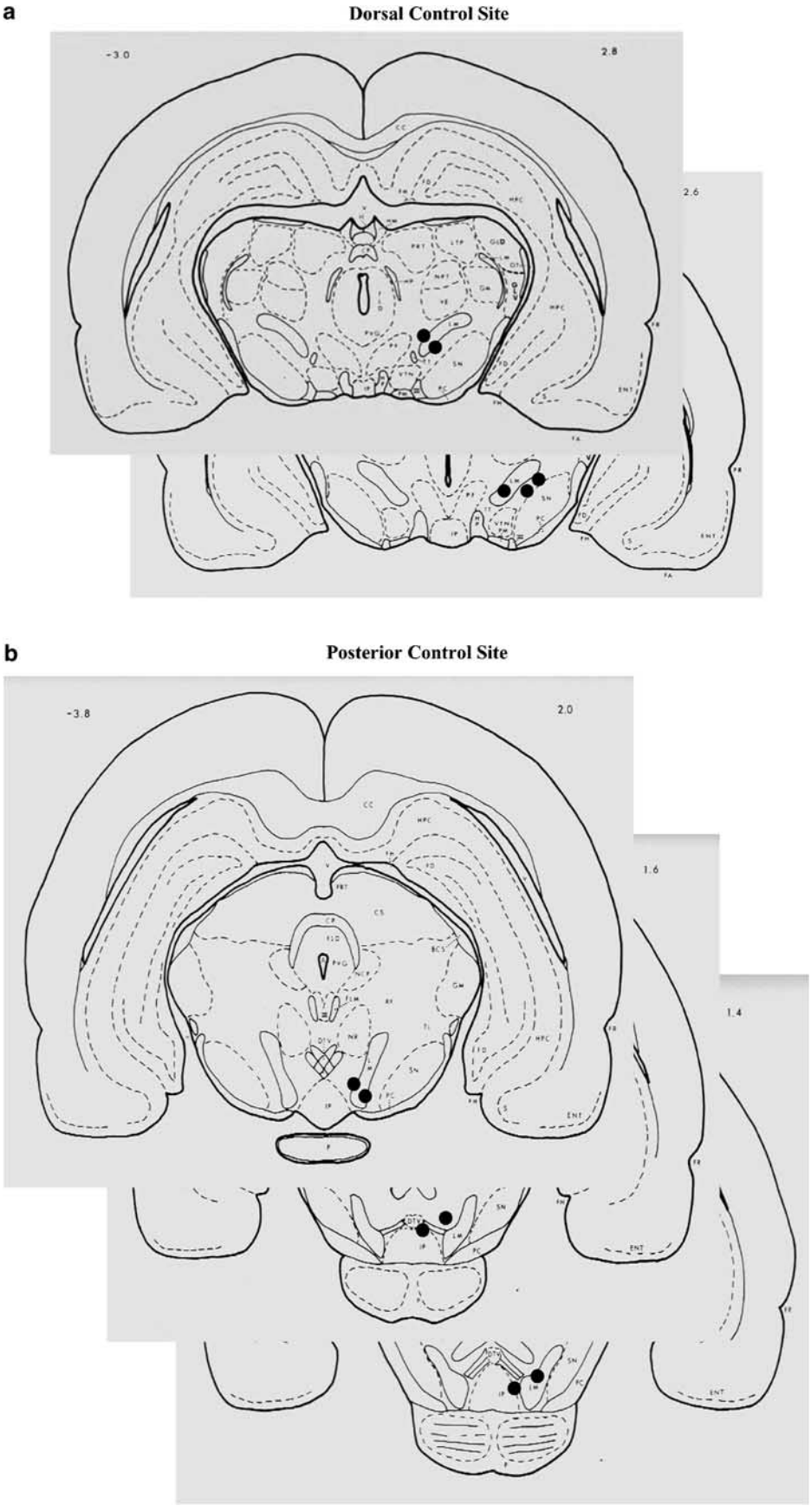

Figure 4 Placement of animals in the control site studies that received the 1.4 μmol dose of ethanol into the brainstem control site (Pellegrino and Cushman, 1967, plates 51, 53, and 54).

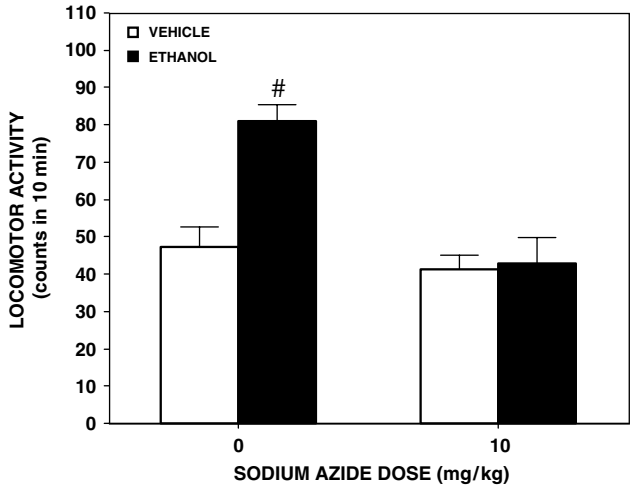

Figure 5 Effect of i.p. sodium azide injection (0 or 10 mg/kg, 30 min before test) on intranigral ethanol (0.0 or 1.4 μmol)-induced locomotion in rats. Mean ± SEM represent counts in 10 min. $^{*}p<0.05$ significantly different from 0 mg/kg sodium azide–1.4 μmol; $^{\#}p<0.05$ significantly different from all other groups.

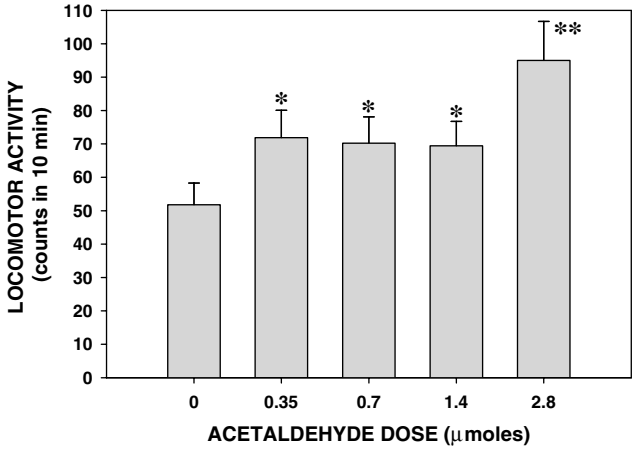

Figure 6 Effect of intranigral acetaldehyde administration (0.0, 0.35, 0.7, 1.4, or 2.8 μmol) on locomotor activity in rats. Mean ± SEM represent counts in 10 min. $^{**}p<0.01$, $^{*}p<0.05$ significantly different from vehicle.

SNr produced a significant increase in locomotor activity ($F(4,36)=3.36$, $p<0.05$). The planned comparisons indicated that all doses significantly differed from vehicle (2.8 μmol $p<0.01$; 0.35, 0.7, and 1.4 μmol $p<0.05$; see Figure 6). The overall dose–response curve for acetaldehyde was characterized by a significant linear dose-related trend ($F(1,36)=11.0$, $p<0.01$), but not a significant quadratic trend ($F(1,36)=0.05$, n.s.).

DISCUSSION

Summary of Results

As described above, a series of experiments was conducted to investigate the locomotor effects of local injections of ethanol, and the ethanol metabolite acetaldehyde, into SNr. The first experiment demonstrated that infusions of ethanol into SNr resulted in a dose-related increase in locomotor activity. Additional experiments demonstrated that infusions of ethanol into control sites dorsal and posterior to SNr did not increase locomotion. The locomotor effects of intranigral ethanol were reduced by administration of sodium azide, a catalase inhibitor that acts to reduce ethanol metabolism. Moreover, SNr infusions of the ethanol metabolite acetaldehyde also increased locomotion. Taken together, these results indicate that SNr is one of the sites at which ethanol and acetaldehyde may be acting to induce locomotor activity. These experiments support the hypothesis that intracranial infusions of low doses of ethanol in rats can induce locomotion, and also provide further support for the hypothesis that metabolites of ethanol can be centrally active.

Effects of SNr Infusions of Ethanol

The first experiment demonstrated that infusions of ethanol directly into SNr resulted in a significant induction of locomotor activity. Although previous studies have employed intraventricular injection procedures, this is the first study reporting that locomotor activity can be induced by local infusions of ethanol into a discrete brain locus. With SNr infusions, the overall dose–response curve for the effect of ethanol tended to have an inverted-U shape, with 1.4 μmol being the dose that produced the highest increase in locomotion. It is unlikely that these results occurred because of some general or nonspecific effect of infusion of ethanol somewhere into the brain, since additional experiments demonstrated that infusions of ethanol into control sites dorsal and posterior to SNr did not affect locomotion. In experiment 2, it was shown that injections of ethanol into a control site 2.6 mm dorsal to SNr failed to stimulate locomotor activity. In addition, placement sites in brainstem areas posterior to SNr also were shown to be inactive. Taken together, the results of these experiments indicate that SNr is a brain locus at which local infusions of ethanol can stimulate locomotor activity.

These studies have implications for understanding the brain mechanisms involved in mediating the ascending limb of the biphasic dose–response curve for the effect of ethanol on locomotor activity. The activating effects of systemic administration of ethanol have typically been shown in mice (Read *et al*, 1960; Frye and Breese, 1981; Crabbe *et al*, 1982; Masur *et al*, 1986; Aragon *et al*, 1992a; Phillips and Shen, 1996; Correa *et al*, 1999a, 2000, 2001, 2004b). Although some studies have shown that selectively bred alcohol-preferring rats can show ethanol-induced increases in locomotion (Paivarinta and Korpi, 1993; Colombo *et al*, 1998; Rodd *et al*, 2004), in most previous studies, peripheral administration of ethanol to other strains of rats failed to stimulate locomotor activity, and generally has resulted in a suppression of motor activity (Frye and Breese, 1981; Masur *et al*, 1986; Waller *et al*, 1986; Spivak *et al*, 1987; Aragon *et al*, 1989; Gingras and Cools, 1996; Correa *et al*, 2003a). More recently, through the use of intraventricular administration of ethanol directly into the brain, it has been demonstrated that central administration of ethanol in rats can induce locomotor activation (Correa *et al*, 2003a, b) and also increased operant responding on a DRL30 schedule, which is a sign of behavioral stimulant effects (Arizzi *et al*, 2003). These previous results are

important, because they demonstrated that after an acute challenge of ethanol rats are capable of showing increased locomotor activity. However, these previous studies involving intraventricular administration did not assess the effects of local injections of ethanol directly into discrete brain areas. The current study demonstrated that ethanol can produce locomotor activation after infusion directly into the SNr.

With studies involving intracranial injections of drugs, there often are questions about the doses used, and the possibility of brain damage. The doses employed in the present study were at or below the doses that were used in recently published articles involving intraventricular administration (Arizzi *et al*, 2003; Correa *et al*, 2003a, b; Crankshaw *et al*, 2003), and were lower than those used in several earlier papers (Brown *et al*, 1978; Smith *et al*, 1984). Also, as described in detail by Correa *et al* (2003a, Selection of Intraventricular Doses, pp 369–370), the highest dose of intraventricular ethanol that was used in that study was 650–5200 times lower than a typical systemic dose in rats such as 1.0 g/kg (eg, Quertemont *et al*, 2003). This ratio between intracranial and systemic doses of ethanol compares very favorably with the published literature involving intracranial injections of other drugs (Trevitt *et al*, 2001; Carlson *et al*, 2003b). For example, the obtained intracranial/systemic ratio for ethanol mentioned above is much lower than the intracranial/systemic ratio of the doses of the 5-HT 2 antagonist mianserin used in a recently published study (1 to 450 ratio; Carlson *et al*, 2003b). Thus, compared to other drugs that have been used both systemically and intracranially, the present doses of ethanol injected into the brain were generally lower when expressed as a fraction of the systemic dose range. Finally, it should be emphasized that in the present studies, as well as the previous papers involving intraventricular infusions (Arizzi *et al*, 2003; Correa *et al*, 2003a, b), none of the behavioral signs of administration of a high dose of ethanol (ie, ataxia, suppressed locomotion, suppressed lever pressing) were observed at any of the doses tested. Taken together, this evidence indicates that the doses of ethanol used in the present studies (0.175–2.8 μmol) were not excessively high.

The possibility of brain damage induced by intracranial injections of ethanol or acetaldehyde also is important to address. In the Correa *et al* (2003a) article, a microscopic examination of intraventricular injection sites was conducted, and there was no significant difference in the severity of brain damage between animals that received 15 injections of 2.8 μmol ethanol and those that received 15 injections of vehicle. In the present study, any animals that had signs of substantial damage in the vicinity of the injector relative to control rats were rejected from the study. Microscopic examination of the injection sites in the remaining animals indicated that the doses of ethanol used in the present study did not produce extensive damage (eg, Figure 1).

The specific neural mechanisms through which ethanol can induce locomotor activity are unknown, but several brain areas have been suggested to be involved in mediating the motor effects of ethanol, including the cerebellum (Dar, 2002), medial septal area (Givens and Breese, 1990), the central nucleus of the amygdala (Demarest *et al*, 1998), the arcuate nucleus of the hypothalamus (Sanchis-Segura *et al*, 2000, 2005), and basal ganglia (Dar, 2001). The present results demonstrate some degree of site specificity for the effects of SNr infusions of ethanol, because injections into control sites were ineffective. Nevertheless, it is not clear that SNr is the only active brain site for this effect. Preliminary data from our laboratory indicate that local injections of 1.4 μmol ethanol into the posterior ventral tegmental area also can increase locomotor activity (Correa *et al*, 2005). Additional research will be necessary to map the ventral midbrain sites leading to ethanol-induced motor activity. In addition, the basic neurochemical effects of ethanol leading to the induction of locomotion also remain uncertain. Ethanol has been shown to interact with several neurotransmitter systems (Imperato and Di Chiara, 1986; Givens and Breese, 1990; Dar, 2001; Phillips and Shen, 1996; Ericson *et al*, 2003; Rodd-Henricks *et al*, 2003; Sanchis-Segura *et al*, 2004). Based upon the data reviewed above, it is possible that ethanol facilitates GABAA receptor function on SNr output neurons (Diana *et al*, 1993; Criswell *et al*, 1993, 1999). These neurons in turn project to various brainstem motor areas, including reticular formation and the pedunculopontine nucleus, which are thought to be involved in locomotion (Fallon and Laughlin, 1995). Thus, it is possible that local infusion of ethanol into SNr is stimulating locomotion through a mechanism that is similar to the one that is thought to be involved in muscimol-induced locomotion (Trevitt *et al*, 2002). However, further research is necessary to study this hypothesis.

Role of Ethanol Metabolism

Consistent with previous studies involving peripheral administration of ethanol in mice (Aragon *et al*, 1992a; Aragon and Amit, 1993; Correa *et al*, 1999a, b, 2000, 2001, 2004a, b; Sanchis-Segura *et al*, 1999a–c; Pastor *et al*, 2002), the present studies suggest that the metabolism of ethanol into acetaldehyde is involved in the locomotor stimulant effects of ethanol. In experiment 3, the locomotor effects of intranigral ethanol were blocked by peripheral administration of sodium azide, a catalase inhibitor that acts to reduce brain ethanol metabolism (Sanchis-Segura *et al*, 1999a; Correa *et al*, 2004b). The 10 mg/kg dose of sodium azide used in the present study reduced brain catalase activity by 40%, but did not affect locomotion when administered on its own in two separate experiments. This is consistent with previous results indicating that sodium azide did not suppress the locomotion induced by *d*-amphetamine or tert-butanol (Sanchis-Segura *et al*, 1999a). These results suggest that catalase-induced metabolism of ethanol, and hence the production of acetaldehyde, is a plausible mechanism for some of the behavioral effects observed after ethanol administration. This statement is supported by the finding of increased locomotion after infusions of acetaldehyde into the SNr (experiment 4). Acetaldehyde infusions into the SNr in the same dose range as ethanol produced a monophasic induction of locomotion, with the maximum effect at 2.8 μmol of acetaldehyde. The present findings are consistent with previous studies showing that intraventricular injections of acetaldehyde can induce locomotor activity (Correa *et al*, 2003b) and increase

responding on a DRL30 operant schedule (Arizzi *et al*, 2003) over the same dose range as ethanol. It is not clear why acetaldehyde appears to have approximately the same potency as ethanol for inducing locomotion after intracranial injections. Based upon peripheral injection studies, one might think that acetaldehyde should be more potent than ethanol (eg, Quertemont *et al*, 2005b). However, the dose of intracranial acetaldehyde needed to induce locomotion could be influenced by many factors, and the rapid enzymatic elimination of acetaldehyde from the brain may be acting to reduce the apparent potency of this substance (Majchrowicz *et al*, 1967; Truitt and Walsh, 1971; Amit and Smith, 1985).

Although there is general acceptance of the concept that acetaldehyde is peripherally active as an ethanol metabolite (Holtzman and Schneider, 1974; Hillbom *et al*, 1983; Myers *et al*, 1984; Quertemont and Grant, 2002; Tampier and Quintanilla, 2002), there has been some controversy about the potential role of acetaldehyde in mediating some of the central effects of ethanol (Hunt, 1996). This controversy is due mainly to uncertainties about the presence of acetaldehyde in the brain after ethanol consumption, and the relatively low levels of this metabolite that have been detected (Hunt, 1996; Smith *et al*, 1997; Zimatkin and Deitrich, 1997). Nevertheless, increases in acetaldehyde-metabolizing enzymes in the brain have been reported after previous ethanol exposure in rats, suggesting that acetaldehyde is present in the brain after consumption of ethanol (Amit *et al*, 1977; Amir, 1978). Moreover, recent studies have demonstrated the consistent detection of acetaldehyde in the brain after peripheral ethanol administration (Ward *et al*, 1997; Jamal *et al*, 2003). Some of the acetaldehyde present in the brain can be the result of acetaldehyde molecules that are produced peripherally and then reach the brain, although it is difficult for acetaldehyde to cross the blood–brain barrier because of the metabolic barrier presented by large concentrations of ALDH (Hunt, 1996; Quertemont and Tambour, 2004). Nevertheless, some acetaldehyde can be directly formed in the brain through the actions of catalase (Aragon *et al*, 1992b; Reddy *et al*, 1995; Zimatkin and Lindros, 1996; Hamby-Mason *et al*, 1997; Eysseric *et al*, 1997; Zimatkin *et al*, 1998). The notion that ethanol metabolism in the brain is important for some of the behavioral effects of ethanol is supported by the reports that manipulations of catalase activity exert a powerful effect on ethanol-induced behavior (Aragon *et al*, 1992a; Aragon and Amit, 1993; Correa *et al*, 1999a, b, 2000, 2001, 2004a, b; Sanchis-Segura *et al*, 1999a–c; Pastor *et al*, 2002). Additional support is provided by studies showing behavioral effects of acetaldehyde after central administration (Myers and Veale, 1969; Brown *et al*, 1978, 1979, 1980; Smith *et al*, 1984; Arizzi *et al*, 2003; Correa *et al*, 2003b, c; Rodd-Henricks *et al*, 2002; Rodd *et al*, 2005). The central administration of acetaldehyde avoids the issue of brain penetrability, and therefore more directly assesses the effects of central acetaldehyde on distinct aspects of behavior.

According to the current model (eg, Aragon and Amit, 1985), because it is difficult for peripheral acetaldehyde to escape liver metabolism and to cross the blood–brain barrier (Sippel, 1974; Eriksson and Sippel, 1977; Quertemont and Tambour, 2004), it is more likely that ethanol escapes metabolism, crosses the blood–brain barrier, and is then converted into acetaldehyde in the brain via catalase (Cohen *et al*, 1980; Aragon and Amit, 1985; Aragon *et al*, 1992b). With *in vitro* studies, it has been demonstrated that brain acetaldehyde concentration could be decreased by the administration of catalase inhibitors, but not by cytochrome P-450 or ADH inhibitors, supporting the idea that the primary brain mechanism for the metabolism of ethanol into acetaldehyde after acute ethanol exposure is catalase (Aragon *et al*, 1992b). The present results, together with the published findings, indicate that ethanol-induced locomotion in rodents depends on brain catalase activity. In mice, there is a very high correlation across a broad range of conditions between brain catalase activity and the degree of locomotor response to systemic ethanol administration (Correa *et al*, 2001). The results of experiment 3 indicate that motor activity induced by SNr infusions of ethanol in rats also depends upon catalase activity.

Conclusions

Taken together, the results of the present experiments indicate that SNr is one of the brain sites at which ethanol and acetaldehyde may be acting to induce locomotor activity. Together with the data showing that acetaldehyde is a biologically active compound that stimulates locomotor activity, these results also indicate that the catalase-mediated conversion of ethanol into acetaldehyde in the brain is an important biochemical step involved in the locomotor stimulation induced by low doses of ethanol. In view of the hypothesis that central acetaldehyde is partially mediating the activating effects of ethanol, it is important to examine the biochemical pathways and neural mechanisms that could be involved in these effects. In addition, it is critical to determine how acetaldehyde is exerting its basic cellular actions. Previous research on the effects of acetaldehyde on GABA-related mechanisms has yielded inconsistent results (Kuriyama *et al*, 1987; Hashimoto *et al*, 1989; Ward *et al*, 1997; Mascia *et al*, 2001). In a recent review (Quertemont *et al*, 2005b), it was noted that there is no clear evidence that acetaldehyde significantly interacts with glutamate or GABA transmission. Clearly, additional studies are needed to characterize the cellular mechanisms through which ethanol and acetaldehyde are acting to influence the function of neurons involved in the regulation of locomotion. Recent studies have shown that peripherally administered acetaldehyde can activate ventral tegmental neurons in a manner similar to ethanol (Foddai *et al*, 2004). In terms of future behavioral studies, it will be important to determine if the effects of ethanol or acetaldehyde injected into the SNr are blocked by administration of benzodiazepine antagonists or inverse agonists, in order to assess the role of the $GABA_A$/benzodiazepine receptor complex in these effects.

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