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Agmatine reduces balance deficits in a rat model of third trimester binge-like ethanol exposure

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Abstract

This study examined the effects of binge-like ethanol (ETOH) exposure in neonatal rats on a cerebellar-mediated balance task, and the ability of agmatine, an *n*-methyl-*d*-aspartate receptor (NMDAR) modulator, to reverse such effects. Five neonatal treatments groups were used, including ETOH (6.0 g/kg/day), AG (20 mg/kg), ETOH plus AG (6.0 g/kg/day and 20 mg/kg), a maltose control, and a non-treated control. Ethanol was administered via oral intubation twice daily for eight days, (AG was administered with the last ETOH intubation only). Two exposure periods were used; PND 1-8 or PND 8-15. On PND 31-33, balance performance on a single dowel was tested. Treatment with AG during withdrawal in ETOH exposed animals improved performance relative to ETOH alone among the PND 1-8 exposure period. ETOH exposure during the 2nd postnatal week did not impair balance. These findings provide further support that exposure to ETOH during critical developmental periods can impair performance on a cerebellar-dependent balance task. Of perhaps greater significance, co-administration of agmatine reduced these deficits suggesting that NMDA modulation via polyamine blockade may provide a novel approach to attenuating damage associated with binge-like ETOH consumption.

Keywords: Agmatine; Alcohol; BAC; Balance; Glutamate; Ethanol; Ethanol withdrawal; Excitotoxicity; Fetal alcohol syndrome; FASD; NMDA; NR2B; Polyamines

1. Introduction

Ethanol abuse during pregnancy can cause permanent brain damage, resulting in behavioral, social, and cognitive dysfunctions (Hannigan and Armant, 2000). Such impairments can range from the more extreme Fetal Alcohol Syndrome (FAS) to more subtle fetal alcohol effects. All of these fit the umbrella term Fetal Alcohol Spectrum Disorder (FASD). The incidence of FASD has been reported to be approximately 9.1/1000 live births (Sampson et al., 1997) with an estimated annual cost of 3.6 billion dollars (Lupton et al., 2004).

Among the reports of FASD-associated impairments, deficiencies in motor skills are common, including delayed motor development, poor eye-hand coordination and fine motor dysfunction. Additionally, problems with balance in children exposed to ethanol prenatally have been noted (Kyllerman et al., 1985; Streissguth et al., 1980). Autopsy, MRI, and PET studies have associated these deficits with alterations in cerebellar volume and function (Riley et al., 2004; Riley and McGee, 2005; Roebuck et al., 1998).

Animal models have served as useful tools in the investigation of the effects of ethanol on the developing brain (Driscoll et al., 1990; Green, 2004; Tabakoff and Hoffman, 2000; Thomas and Riley, 1998; West et al., 1990; West and Goodlett 1990). In rat cerebellum, neonatal ethanol exposure produces a significant, dose-dependent loss of Purkinje cells. This neonatal exposure model is used to study a period of CNS development that overlaps the human 3rd trimester "brain growth spurt" (Dobbing and Sands, 1979). Reports of deficits in balance and other cerebellar type tasks resulting from neonatal ethanol exposure are common (Goodlett et al., 1991; Klintsova et al., 1998; Klintsova et al., 2000; Thomas et al., 1996). A particularly sensitive developmental period appears to span postnatal days (PND) 4-6 (Goodlett et al., 1998; Thomas et al., 1998). Behavioral tests of cerebellar function as well as stereological counts of cerebellar Purkinje cells have demonstrated that ethanol exposure on PND 4-5 results in more severe deficits than PND 8-9 exposure (Thomas et al.,

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1998), suggesting that the first neonatal week is a particularly sensitive period for ethanol's effects on the developing cerebellum.

A number of mechanisms have been proposed to explain how prenatal ethanol exposure affects the developing CNS (Goodlett et al., 2005; Riley et al., 2001). One mechanism that has received considerable attention and has shown potential as a clinical target for intervention involves glutamate receptor hyperactivity during withdrawal, specifically the *N*-methyl-D-aspartate receptor (NMDAR). Acute ethanol exposure inhibits NMDAR activity, and following chronic exposure a variety of compensatory responses can occur resulting in receptor function that may be up-regulated (Chandler et al., 1999; Hu and Ticku, 1995; Kalluri et al., 1998). During withdrawal, this up-regulation has been shown to result in glutamatergic hyperexcitability and cell death (Gibson et al., 2003; Hoffman et al., 1995; Iorio et al., 1993).

Thomas et al. (1997, 2002) have shown that administration of an NMDAR antagonist, MK-801, to neonatal pups during ethanol withdrawal reduced some of the deficits associated with prenatal ethanol exposure. The timing of MK-801 treatment, however, appeared critical (Thomas et al., 2001). If MK-801 was administered in the presence of ethanol, behavioral deficits were exacerbated. In contrast, when MK-801 was administered during ethanol withdrawal, behavioral deficits were reduced. NMDAR antagonists such as MK-801 have numerous limitations as potential treatments. MK-801 works via channel blockade and demonstrates little receptor subtype specificity. Thus, its wide-ranging actions can produce toxicity, may disrupt learning and memory, and can have abuse/psychotomimetic potential (Grant et al., 1991; Klein et al., 1999). Although the side effects of MK-801 preclude its use clinically, success in animal models of early ethanol exposure has generated interest in alternate NMDAR antagonists that may be more viable. At least three types of antagonists seem particularly promising; low-affinity, non-competitive NMDAR channel blockers such as memantine (Volbracht et al., 2006), which is currently used clinically for treating Alzheimer's disease, NR2B subunit antagonists such as ifenprodil or eliprodil (Nikam and Meltzer, 2002; Thomas et al., 2004), and agents that modulate rather than block NMDAR activity such as agmatine, which can act at the polyamine binding site, resulting in allosteric modulation of the receptor.

Polyamines play a variety of roles in CNS development (Slotkin and Bartolome, 1986) and can enhance NMDAR activity (Williams et al., 1991; Williams, 1994). Increased polyamine expression has also been reported in hippocampus, striatum, cortex, and cerebellum during periods of ethanol withdrawal (Davidson and Wilce 1998; Gibson et al., 2003). The concentration of polyamines is positively correlated with the severity of withdrawal-induced tremor and seizure in ethanol-dependant animals (Davidson and Wilce, 1998). Additionally, they have been shown to potentiate ethanol withdrawal-induced cell death *in vitro* (Prendergast et al., 2000; Gibson et al., 2003), and are implicated in the pathogenesis of FAS (Littleton et al., 2001; Sessa et al., 1987; Sessa and Perin, 1997). Taken together, these data suggest that inhibiting polyamine activity during ethanol withdrawal could reduce the severity of withdrawal-induced CNS damage (Littleton et al., 2001; Shibley et al., 1995), an effect that has been observed *in vitro* (Gibson et al., 2003), but not *in vivo*.

Agmatine, a polyamine precursor, is known to inhibit the NMDAR via binding at the polyamine site (Gibson et al., 2002). Exogenous administration of agmatine attenuates glutamate-induced neurotoxicity in cell cultures of rat cerebellum (Olmos et al., 1999), hippocampus (Wang et al., 2006), and cortex (Zhu et al., 2003). Additionally, agmatine reduces infarct and loss of cerebellar neurons following *in vivo* focal or global ischemia (Gilad et al., 1996; Kim et al., 2004) and brain weight loss following ischemia in neonates (Feng et al., 2002). Behaviorally, agmatine dose-dependently attenuates behaviors associated with ethanol withdrawal, including stereotypy, tremor, and wet-dog shakes, without affecting motor coordination in non-dependent animals (Uzbay et al., 2000).

In the current study, a third trimester model of chronic ethanol exposure was used to study the potential neuroprotective effects of agmatine on a cerebellar-mediated balance task. Additionally, two exposure periods (PND 1-8 and PND 8-15) were used to study temporal variables that could interact with ethanol and/or agmatine.

2. Materials and methods

2.1. Subjects

Offspring were Sprague-Dawley rats born at the University of Kentucky in the breeding colony maintained in the Psychology Department. Parent animals were obtained from Harlan Labs (Indianapolis, IN). Animals were mated nightly, and the presence of seminal plugs the following morning indicated copulation had occurred. Pregnant females were individually housed in plastic cages in a temperature controlled nursery (70°±2 F) on a 12 h light-dark cycle, with food and water provided *ad libitum*. On the day following birth (PND 1) litters were culled to 10 animals, maintaining a 1:1 ratio of males to females whenever possible.

2.2. Neonatal drug administration

Litters were randomly divided into five treatment conditions. These included 6 g/kg/day ethanol (ETOH), 20 mg/kg agmatine (AG), 6 g/kg/day ethanol plus 20 mg/kg agmatine (ETOH/AG), a maltose isocaloric control (MALT) and a non-treated control (NTC). No more than one male and one female were assigned to any treatment condition to avoid potential litter effects (Abbey and Howard, 1973). Drugs were administered via gastric intubation (.0278 ml/g bw) in a solution developed to nutritionally mimic rat milk (West et al., 1984). Animals not receiving ethanol (AG and MALT groups) instead received maltose, making the solutions for all intubated groups isocaloric. Agmatine was administered concurrently with the final ethanol exposure only, in order to have AG on board during the final withdrawal from ethanol.

Intubations were administered twice daily for eight days, at 1000 and 1400 h. Each intubation consisted of a 3 g/kg dose of

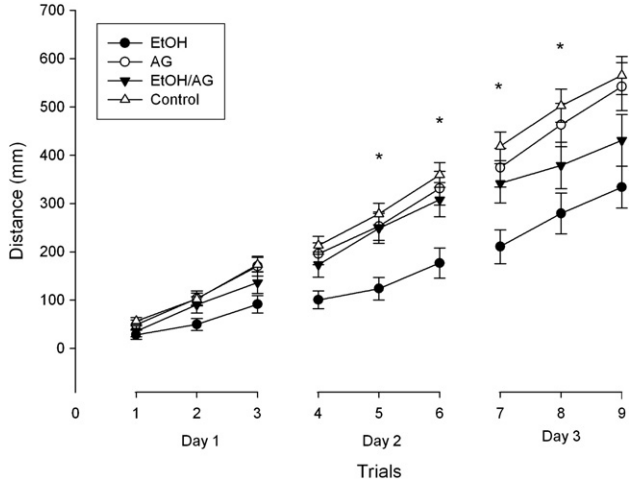

Fig. 1. Mean (±S.E.M). score on the balance task in 31–33 day old offspring treated on PND 1-8 as a function of neonatal treatment, day and trial. * indicates significant interactions ($p<0.05$) between the ETOH and the ETOH/AG offspring.

ethanol resulting in a dose of 6 g/kg/day ethanol. Litters were intubated on either PND 1-8 or PND 8-15. Animals were removed from the dam for approximately 20 min during each intubation session. Heating pads were used to help maintain pup body temperature during the intubation session. The solutions were intubated using a syringe connected to PE-50 and PE-10 polyethylene tubing (Clay Adams). Feeding tubes were coated with corn oil to ease esophageal passage.

On PND 21 animals were weaned and double housed with one same-sex conspecific until PND 30, when they were handled for 3 min and individually housed in preparation for PND 31 testing. All handling and testing was performed by an experimenter blind to treatment condition.

2.3. Balance testing

The balance apparatus consisted of a single elevated dowel rod (114 cm long, 1.85 cm diameter), raised (75 cm) above the ground, with a darkened escape box (21 × 10 × 17 cm) on one end. The floor beneath the rod was padded in case of falls. Each animal was habituated to the test room and escape box for 1 min each. During the first trial, the animal was placed on the rod, 10 cm from the escape box. Upon successfully reaching the escape box, the animal was allowed to remain in the box for 10 s, and then was removed to its home cage. If the animal did not successfully reach the escape box (either fell or swung from the rod), it was retrieved and placed in the escape box for 10 s, before being returned to the home cage for a 30 s intertrial interval. Subjects that were unsuccessful were retested at the same distance on the rod. Following successful completion of a trial, the distance on the next trial was increased by 13 cm. Hence, completion of the first three trials required traversing distances of 10, 23, and 36 cm, respectively. The dependant measure was the most recent successfully completed distance (if an animal fell on any trial, the last successfully completed distance was recorded for that trial). Each subject received three trials/day for three days as this schedule of testing in our laboratory has previously been shown to be sensitive to neonatal ETOH exposure. Following the final trial, body weights were recorded for analysis. The experimental protocols employed in this study were approved by the University of Kentucky Institutional Animal Care and Use Committee and are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4. Blood ethanol concentrations

Pups from 12 additional litters were intubated daily at 1000 and 1400 on either PND 1-8 or PND 8-15 for measurement of blood ethanol concentrations (BECs) to assess if there were age-dependent differences in blood ethanol concentrations and/or whether agmatine had any effects on ethanol metabolism. Blood was collected after the final ethanol intubation by making a 1 mm cut at the tip of the subject's tail on PND 8 or 15 and collecting 20 μl of blood. The BEC curve was established by collecting samples at 30, 60, 120, 240, 480 and 600 min following ethanol administration. In order to minimize stress, only three samples were collected per animal. Data were collected from four groups (ETOH 1-8, ETOH/AG 1-8, ETOH 8-15, ETOH/AG, 8-15), yielding 12 animals (6 males, 6 females) per group plus appropriate controls for standards. Plasma was separated and frozen at −70 °F. BECs were assayed using an Analox AM 1 Alcohol Analyzer (Analox Instruments).

3. Results

3.1. General statistical issues

A repeated measures ANOVA containing all variables was conducted (with trial nested within day); this 5 × 2 × 2 × 3[3] (Treatment × Exposure Period × Gender × Day[Trial]) analysis yielded main effects of all between subjects variables, including treatment $F(1,235)=3.548$, $p=.008$, exposure period $F(1,235)=10.404$, $p=.001$, and gender $F(1,118)=11.207$, $p=.001$. Animals in the earlier exposure period (PND 1-8) performed better than those exposed in the later period (PND 8-15). Additionally, female animals demonstrated enhanced performance. To better understand the data, each exposure period was analyzed separately. Of primary interest to these analyses was the interaction of agmatine and ethanol. Prior to investigating this

Table 1
PND 33 body weights (in g)±S.E.M. by group and exposure period

	Exposure period					
	PND 1-8			PND *8-15*		
Group	*n*	*M*	SEM	*n*	*M*	SEM
Ethanol (ETOH)	27	93.2*	±2.1	24	94.9*	±2.0
Agmatine (AG)	27	100.3	±2.5	28	102.0	±2.1
Ethanol+Agmatine (ETOH/AG)	20	94.0*	±2.4	29	97.3*	±1.6
Controls (MALT+NTC)	50	97.7	±1.6	22	97.9	±1.4

*ETOH exposed offspring differed from non ETOH exposed offspring p's <0.05.

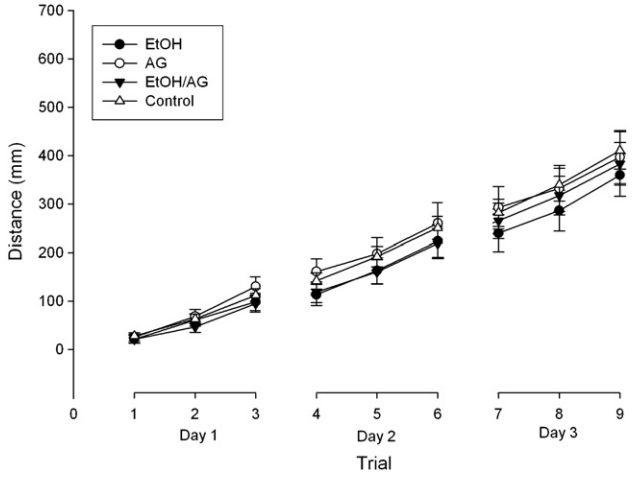

Fig. 2. Mean (±S.E.M.) score on the balance task in 31–33 day old offspring treated on PND 8-15 as a function of neonatal treatment, day and trial. No differences in balance performance were noted between groups.

interaction it was necessary to ensure that there were no differences between the MALT and NTC control groups. Post hoc analyses revealed no differences between controls for either exposure period. Therefore, in subsequent analyses, controls were collapsed into a single group and the data were examined using a (2×2×2×3[3]) factorial design with ETOH, AG and gender as between-group factors and day and trial as repeated measures (trial nested within day). A probability value of .05 was considered statistically significant.

3.2. PND 1-8 exposure balance performance

The repeated measures ANOVA revealed a significant ETOH×AG interaction, $F(1,118)=3.909$, $p=.05$. As shown in Fig. 1, treatment with agmatine during ethanol withdrawal improved performance relative to ethanol exposed animals that did not receive AG. There was also a significant ETOH×AG×Day [Trial] interaction, $F(4, 472)=3.393$, $p=.009$. The interaction was further broken down by conducting ANOVAs among individual trials, revealing significant interactions on trial 5, $F(1, 118)=7.285$, $p=.008$; trial 6, $F(1, 118)=5,707$, $p=.018$; and trial 7, $F(1, 118)=5.233$, $p=.024$ (after Bonferroni corrections). In all cases, these interactions reflected the poorer performance on the balance task of the ETOH group relative to the ETOH/AG group.

3.3. PND 33 body weights: PND 1-8 exposure

Body weights were recorded following the final trial of testing on PND 33 and analyzed using a 2×2×2 (ETOH×AG×Sex) ANOVA. Animals treated with ethanol weighed less than controls, as shown by a main effect of ETOH $F(1, 114)=9.641$, $p=.002$. These data are presented in Table 1, with group means collapsed across sex for ease of presentation, since sex did not interact with any other variables. Overall, male offspring weighed more than females $F(1,114)=54.181$, $p<.001$. In order to assess the potential contribution of body weight to balance performance, correlations of body weight with performance on the final trial collapsed across treatment condition, as well as correlations within each treatment, were examined. No significant correlations between weight and balance performance were observed.

3.4. PND 8-15 exposure balance performance

As shown in Fig. 2, no performance differences between neonatal treatment groups were detected for PND 8-15 exposed animals. Interestingly, female animals performed better than males $F(1, 123)=8.70$ $p=.004$.

3.5. PND 33 body weights: PND 8-15 exposure

A similar pattern of body weight results were observed in PND 33 offspring exposed to drug or control on PND 8-15 as described above for offspring exposed on PND 1-8. These data are also presented in Table 1. As with PND 1-8 exposure, the ETOH and ETOH/AG offspring weighed less than controls, as shown by a main effect of ethanol $F(1, 125)=5.043$, $p=.026$. Again, males weighed more than females $F(1,125)=35.818$, $p<.001$. Performance and body weight correlations were not investigated, due to the lack of performance differences within this exposure period.

3.6. Blood ethanol concentrations

Blood ethanol concentrations (BECs) on PND 8 and PND 15 are presented in Table 2. No differences in BECs were observed between the ETOH and ETOH/AG groups. The BEC was lower in the PND 1-8 animals 60 min after intubation $F(1,18)=14.127$, $p=.002$, marginally lower at 120 min $F(1,22)=3.752$,

Table 2
Blood ethanol concentration±SEM by group and exposure period*

	30 min	60 min**	120 min	240 min	480 min**	600 min
Group	M±SEM	M±SEM	M±SEM	M±SEM	M±SEM	M±SEM
PND 1-8 ETOH	192.8±6.3	220.6±8.1	219.3±9.6	180.7±11.2	38.2±8.7	3.8±1.4
PND 1-8 ETOH/AG	201.9±10.7	217.7±4.7	213.4±18.0	163.5±13.2	57.0±23.1	2.0±0.1
PND 8-15 ETOH	228.7±10.1	257.8±8.3	240.2±9.7	177.0±17.5	17.5±9.4	2.8±0.3
PND 8-15ETOH/AG	202.9±18.7	252.5±11.9	259.7±15.0	169.1±24.3	12.8±8.7	1.6±0.6

*n's ranged from 5–8 subjects per cell.
**1–8 and 8–15 exposed animals differed, p's<0.05.

$p = .072$, and higher at 480 min after intubation $F(1,22) = 5.239$, $p = .037$) compared with PND 8-15 exposed animals.

While area under the curve analyses (AUC) provide useful information regarding total ethanol exposure, this was not possible in the current study since individual animals were sampled at only three of the six time points, making the generation of complete curves for each animal (a requirement of AUC analyses) impossible. However, an estimate of the AUC values was calculated using the mean for each group. The mean values showed considerable overlap across treatment groups and exposure ages: 124.5 (PND 1-8 ETOH), 124.6 (PND 1-8 ETOH/AG), 125.3 (PND 8-15 ETOH) and 122.7 mg/dl/hr (PND 8-15 ETOH/AG).

4. Discussion

This study was designed to examine the effects of ethanol during a period of CNS development that overlaps the human 3rd trimester "brain growth spurt" (Dobbing and Sands, 1979). Chronic neonatal ethanol exposure during the first week of postnatal life (PND 1-8) was associated with deficits in a cerebellar-mediated balance task. Animals that received an identical course of exposure, but were administered agmatine with the final dose of ethanol, showed marked improvements on the balance task. Agmatine alone had no effects on this behavior. Although a difference in body weights between animals administered ethanol and controls was observed, body weight did not correlate with performance.

Neonatal ethanol exposure during the second week of postnatal life (PND 8-15) did not impair balance. Females, across treatment conditions, did perform better than males. Although this difference was significant only among the PND 8-15 exposure group, this may reflect a more general sex difference, as a similar trend was observed in the PND 1-8 exposure group (main effect of sex, $p = .071$). The most likely explanation for this gender difference in balance is due to body shape, length, or composition differences between genders.

Findings from the blood ethanol analysis showed no differences in BECs between ETOH and ETOH/AG animals, suggesting that the protective effects of AG were not due to any obvious effects on ethanol metabolism. Importantly, while the peak BECs for both groups would be considered clinically high, they are within the range displayed by human binge drinkers (Urso et al., 1981). Blood ethanol levels varied over time as a function of the age of exposure. The highest peak blood concentrations were noted in pups exposed to ethanol during the 2nd postnatal week, however ethanol remained on-board longer for pups exposed during the first postnatal week. The mean AUC values for each group suggested few, if any, differences between groups. Pierce and West (1986) have suggested that peak BEC is a critical factor in the observance of fetal ethanol effects, however this was not the pattern observed in the current study. Thus, the behavioral deficits observed in the current study were more likely due to the timing of ethanol exposure rather than modest differences in BECs between exposure periods, a conclusion supported by existing literature (Goodlett and Lundahl, 1996; Goodlett et al., 1998).

Our analyses demonstrated that ethanol was cleared between 480 and 600 min following the final ethanol intubation. In CNS, agmatine has a half-life of approximately 720 min (i.e., 12 h) (Roberts et al., 2005). Agmatine is readily absorbed through the stomach and intestinal walls (Molderings et al., 2002; for review see Molderings et al., 2003) and crosses the blood–brain barrier (Piletz et al., 2003). Taken together, these findings suggest that agmatine was biologically available during and following the onset of ethanol withdrawal.

While the balance paradigm used in this study clearly tests cerebellar function, there is also almost certainly a learning component. Since it is impossible to separate these components, the results from this study may reflect both balance and learning differences between groups. It is also possible that with continued exposure and practice, the ethanol-exposed offspring could improve to control levels. Motor rehabilitation has previously been shown to improve performance on balance related tasks following neonatal ethanol exposure (Klintsova et al 2000). While the ETOH/AG group clearly showed improved performance relative to the ETOH exposed offspring, this effect was not observed on the last trial in which the ETOH/AG group did not differ from either controls or the ETOH exposed group. This may be due to the increased task difficulty as the number of trials increased (since the distance required for successful completion of each trial increased accordingly). Only three days of testing were performed, based on the sensitivity of this paradigm to ethanol-associated deficits in previous work, however further work (i.e., extending the number of test days and/or trials) should be able to better address these issues.

The current work is consistent with previous studies showing cerebellar deficits among animals exposed neonatally to ethanol (Thomas et al., 1998). Additionally, the importance of the PND 1-8 period is supported by observations that exposure to ethanol during PND 4-6 predicts more severe damage than later periods (Thomas et al., 1998). It is unclear why behavioral deficits are so pronounced during this and not other postnatal exposure periods, however one hypothesis involves the altered subunit composition of the NMDA receptor during development. NMDARs are heteromeric tetramers (McBain and Mayer, 1994; Schorge and Colquhoun 2003), composed of both NR1 and NR2 subunits. NR1 subunits are ubiquitous in brain, and expressed as at least eight splice variants (Zukin and Bennett, 1995). Four transcripts for the NR2 subunit have been identified (NR2 A-D). These display a spatially distinct pattern of expression, and are implicated in the pharmacologic specificity of the receptor (for reviews see Cull-Candy et al., 2001; Monaghan et al., 1998; Scheetz and Constantine-Paton, 1994). Adding to this complexity, various subunit combinations can vary widely in their pharmacology (Wafford et al., 1993). A number of studies examining recombinant NMDARs suggest that those containing NR2A or NR2B subunits may display twice the sensitivity to ethanol as those including NR2C or NR2D (Kuner et al., 1993; Masood et al., 1994; Mirshahi and Woodward, 1995; for reviews see Allgaier, 2002; Sucher et al., 1996). NR2B-containing receptors also display the greatest sensitivity to polyamine stimulation, due to their increased number of polyamine binding sites (Williams, 1994; Williams et al., 1991). NR2B subunits

predominate early in development and are replaced by other NR2 subunits, depending on brain area. In the rat cerebellum, NR2B and NR2C transcript levels change in opposite directions during the first weeks of life (Monyer et al., 1994). Following the first week of life, the highly expressed NR2B decline to undetectable levels, whereas initially undetectable NR2C rapidly increase to adult levels (Zhong et al., 1995). Thus, developmental differences in subunit composition may account for the developmental differences in pharmacologic sensitivity to ethanol and to polyamine manipulation observed in this study.

The extent of excitotoxic damage following chronic early exposure to ethanol likely depends on a number of factors. Our research highlights the particular importance of subunit composition (by targeting an NR2B-associated modulatory site) and developmental age (by varying ethanol exposure between the first and second weeks of neonatal life) although further work is clearly needed to directly assess the contribution of these variables.

It should be noted that a main effect of exposure period was observed, such that animals treated during the first week of life showed better overall performance relative to those treated during the second. This difference is not generally seen in other behavioral paradigms within our lab, although to date we have not previously assessed differences in balance performance between these distinct developmental periods. Although no explanation for this difference is immediately obvious, our gavage procedure necessitates a period of brief maternal separation, an experience that has been shown to alter behavior and endocrine function in later development (for review see Champagne and Meaney, 2001). Such separation procedures are usually conducted across the first two weeks of life (Plotsky and Meaney, 1993). Thus, the recognition that separation during the first week may afford greater benefits than the second is novel, and requires further investigation.

One of the most interesting findings from the current study is that agmatine administered during ethanol withdrawal attenuated behavioral deficits. Agmatine's ability to reduce such damage is notable, however, agmatine does have additional actions in the CNS. It has been proposed that it may be a putative endogenous neurotransmitter. In addition, reports suggest that it may be a ligand of the imidazoline receptor (Zhong et al., 1995), may block neuronal nicotinic receptors (Li et al., 1994) and voltage-gated calcium channels (Loring, 1990; Weng et al., 2003), interact with alpha2-adrenoceptors (Molderings et al., 2000; Zheng et al., 2004) and some serotonin receptor subtypes, (Dias Elpo Zomkowski et al., 2004) and can inhibit nitric oxide synthase (Kim et al., 2004). Given the wide range of actions of agmatine throughout the CNS, it is possible that the protection observed in our study was partly based on some of these additional mechanisms, independent of NMDAR modulation. However, additional behavioral work from our laboratory and others (Thomas et al., 2004), using more specific NR2B or polyamine antagonists, as well as cell culture studies specifically examining the effects of agmatine and polyamines on glutamate-induced cell death (Gibson et al., 2003) favor an NMDAR/polyamine modulation hypothesis.

It should be noted that while agmatine was administered only on the last day of ethanol administration (and withdrawal), the animals must have experienced varying levels of withdrawal daily since ethanol was administered for seven days. Our rationale for using a single treatment of agmatine was due to the following: First, Thomas and colleagues have reported that ethanol-associated behavioral deficits are attenuated with MK-801 (an NMDAR channel blocker) when administration occurred 21 or 33 h following ethanol administration (Thomas et al., 2001). Since our treatment regiment involved a maximum of ~20 h between ethanol exposures, the withdrawal experienced on a daily basis may be relatively minor compared with the prolonged withdrawal following the final intubation. Furthermore, repeated treatment with agmatine could produce its own compensatory responses which would make interpretation of the findings difficult. Finally, an advantage of a single administration of agmatine is its ease for potential interventions in human neonates; pharmacotherapies may be provided postnatally, but are difficult to provide following prenatal binge-withdrawal cycles.

Newborns of alcoholic mothers, particularly those whose pattern of drinking may be characterized as binge-like in nature, may experience a critical period of withdrawal following birth. Although newborns may have been exposed to several binge-withdrawal cycles *in utero*, the withdrawal associated with birth may be unique, and perhaps uniquely damaging both due to its prolonged nature as well as the challenges associated with parturition. Excitotoxicity associated with such a withdrawal may be further complicated by hypoxic episodes during birth. Thus, the development of treatments addressing neonatal ethanol withdrawal is crucial. The current findings provide some intriguing insight into this problem and demonstrate the potential efficacy of agmatine to attenuate such damage.

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