Amphetamine Normalizes the Electrical Activity of Dopamine Neurons in the Ventral Tegmental Area following Prenatal Ethanol Exposure

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ABSTRACT
Prenatal ethanol exposure has been shown to produce a persistent reduction in the spontaneous activity of ventral tegmental area (VTA) dopamine (DA) neurons and in DA neurotransmission. Amphetamine-like stimulants are effective in treating attention deficit/hyperactivity disorder (ADHD), which is a major symptom in fetal alcohol syndrome. Because there is a link between reduced DA neurotransmission and ADHD, we investigated the possibility that amphetamine could restore the spontaneous activity of VTA DA neurons. Pregnant rats were administered 0 or 6 g/kg/day ethanol via intragastric intubation during gestation days 8 to 20. The spontaneous activity of VTA neurons was studied in 6- to 8-week-old male offspring using extracellular single-unit recording in unanesthetized (paralyzed, locally anesthetized) or chloral hydrate-anesthetized rats. Prenatal ethanol exposure reduced the number of spontaneously active DA neurons without changing the firing rate or firing pattern in both groups of animals. Acute amphetamine administration (2 mg/kg, i.v.) increased the number of spontaneously active DA neurons after prenatal ethanol exposure. Because amphetamine inhibited DA neuron firing rate in ethanol-exposed animals, it is possible that amphetamine restored the number of spontaneously active neurons by alleviating the depolarization block. These results show that the reduction in the number of spontaneously active DA neurons resulting from prenatal ethanol exposure is not confounded by using general anesthesia. Furthermore, acute amphetamine treatment can normalize the activity of DA neurons after prenatal ethanol exposure. This mechanism may contribute to the therapeutic effects of amphetamine-like stimulants in attention problems observed in children with fetal alcohol syndrome.

Results from previous studies have shown that prenatal ethanol exposure leads to profound changes in midbrain dopamine (DA) systems. Evidence from animal studies demonstrates that prenatal ethanol exposure causes reductions in DA uptake and receptor binding sites, DA content, and the DA metabolites homovanillic acid and 3,4-dihydroxyphenylacetic acid in the somatodendritic and terminal areas (Rathbun and Druse, 1985; Cooper and Rudeen, 1988; Druse et al., 1990). Prenatal ethanol exposure also results in morphological changes (e.g., smaller cell bodies and retarded dendritic growth) in midbrain DA neurons (Shetty et al., 1993). In addition, DA receptor-mediated behaviors, such as locomotion and catalepsy, are altered following prenatal ethanol exposure (Hannigan and Randall, 1996).

In the past few years, using the electrophysiological approach, we have observed a persistent reduction in the number of spontaneously active midbrain DA neurons without a loss of DA neurons after prenatal ethanol exposure (Shen et al., 1999). We have also seen changes in the functions of DA receptors after prenatal ethanol exposure (Shen et al., 1995). The DA receptor changes include a supersensitivity in the somatodendritic DA autoreceptors and a subsensitiveness in postsynaptic D-1 DA receptors in nucleus accumbens (Shen et al., 1999). Because the spontaneous activity of DA neurons plays an important role in controlling the synthesis and release of DA (Gonon and Buda, 1985; Suau-Chagny et al., 1992), the reduction in DA content and its metabolites produced by prenatal ethanol exposure could be caused by a reduction in the spontaneous activity of DA neurons. Interestingly, acute administration of the DA agonist apomorphine at a low dose can restore the number of spontaneously active DA neurons after prenatal ethanol exposure, suggesting that the depolarization block is the underlying mechanism for the reduced number of spontaneously active DA neurons after prenatal ethanol exposure (Shen et al., 1999).

Several lines of evidence suggest that decreased DA neurotransmission may contribute to the etiology of the attention/hyperactivity problem commonly observed in children with fetal alcohol syndrome/fetal alcohol effect (FAS/FAE;
Nanson and Hiscock, 1990; Streissguth et al., 1991). For example, in experimental animals, hyperactivity during early development can be induced by selectively depleting midbrain DA neurons with 6-hydroxydopamine (Shaywitz et al., 1976). A reduction in the DA metabolite homovanillic acid in the cerebrospinal fluid has been observed in children with attention deficit/hyperactivity disorder (Shaywitz et al., 1977). Although amphetamine-like stimulants are effective in treating attention deficit/hyperactivity disorder, including the attention problems in children with FAS/FAE (Morrow, 1991; Oesterheld et al., 1998), the underlying mechanism of their therapeutic effects is not clear. Because acute apomorphine and amphetamine exert similar inhibitory effects on DA neuron firing rate, it is possible that, similar to the effect of apomorphine, amphetamine can reverse the depolarization block and restore the number of spontaneously active DA neurons after prenatal ethanol exposure. The restored number of spontaneously active DA neurons in turn compensates for DA hypofunction and leads to the alleviation of attention problems. In the present study, we examine the possibility that acute amphetamine can restore the number of spontaneously active DA neurons.

The majority of the experiments investigating the spontaneous activity of DA neurons, including our previous studies, are conducted in anesthetized animals. Recently, a group of investigators have raised the possibility that the reduction in the number of spontaneously active DA neurons and the depolarization block seen after experimental treatment are “artifacts” due to the use of general anesthesia (Mereu et al., 1995). Therefore, to verify the hypothesis that the depolarization block is the underlying mechanism for the reduction in the number of spontaneously active DA neurons after prenatal ethanol and examine if amphetamine can restore this deficit by reversing the depolarization block, recordings in the present study are performed in unanesthetized (paralyzed, locally anesthetized) rats. Additional recordings are performed under chloral hydrate anesthesia for the purpose of comparison. We focus our studies on DA neurons in the ventral tegmental area (VTA) because these neurons project to the prefrontal cortex, which is important in mediating the attention process (Smith and Jonides, 1999). We believe the results from the present study can further our understanding of the cellular mechanisms leading to reduced DA neurotransmission after prenatal ethanol exposure, as well as help to clarify how amphetamine-like stimulants can ameliorate the behavioral symptoms in children with FAS/FAE.

Materials and Methods

Prenatal Ethanol Administration. Timed-pregnant Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were delivered on gestation day 6 to allow time for handling. To mimic the binge drinking behavior that produces high blood ethanol concentrations in humans at risk for FAS/FAE, rats were administered ethanol via intragastric intubation from gestation day 8 through gestation day 20. Animals were treated with a daily dose of 0 or 6 g/kg ethanol (20% w/v in 0.9% saline), except during weekends. Treatment was carried out by two intubations at 0 or 3 g/kg (5–6 h apart; between 10:00 AM and 5:00 P.M.) during weekdays. A single daily treatment was carried out by two intubations at 0 or 3 g/kg (5–6 h apart; except during weekends. Treatments in humans at risk for FAS/FAE, rats were administered ethanol (20% w/v in 0.9% saline), except during weekends. An additional control group, which was not intubated or pair-fed, was also included. Dams in the ethanol group also received thiamine injections (8 mg/kg; i.m. twice a week) to avoid thiamine deficiency induced by ethanol treatment.

Rearing and Cross-Fostering. We had observed a certain degree of negligence toward pups by ethanol treated dams in our current laboratory setting. Therefore, a cross-fostering procedure was used. On postnatal day 1, pups were individually weighed and examined for gross physical abnormalities, and the litters were culled randomly to 10 pups of 5 males and 5 females when possible. The litters were then transferred to surrogate dams that did not receive any treatment and had delivered two days earlier. The pups were mixed with bedding from the surrogate dams’ cage and then transferred to the surrogate dams. The surrogate dams’ litters were removed before the transfer. Litters were weaned and weighed on postnatal day 21. Only male offspring were used in the present study. To control litter effects, no more than three litters were used in the same experiments. The offspring used in the present study were acquired from 44 litters.

Surgical Procedures. The electrophysiological recordings of DA neurons were performed in unanesthetized or in chloral hydrate-anesthetized rats between 6 and 8 weeks old. When the unanesthetized preparation was used, all surgical procedures were performed under temporary halothane anesthesia. All incision sites and blunt pressure points were infiltrated with a long-acting local anesthetic, bupivacaine (0.25%, Abbott Laboratory, North Chicago, IL) before surgical procedures. Although anesthetized with halothane, each rat was tracheotomized, cannulated with a tracheal tube, mounted in a stereotaxic apparatus, and the skull and dura overlapping the VTA were removed. The tail vein was cannulated. Rats were then paralyzed with gallamine triethiodide [25 mg/kg intravenous (i.v.), Sigma-RBI, St. Louis, MO] injected through the i.v. cannula, and halothane was withdrawn. Each rat was respired immediately with a mixture of O2/N2/O (70%/30%) by connecting the tracheal cannula with a rodent ventilator (Edco Scientific, Inc., Chapel Hills, NC). Each rat was respired for minimum of 20 min before each electrophysiological recording. Expired CO2 levels were continuously monitored with a CO2 monitor (Biochem 9000 Capnograph-Oximeter, Biochem, Wankesha, WI) and maintained between 28% and 43%. Body temperature was monitored and maintained between 36°C and 37°C. Heart rate and blood oxygen saturation were monitored with an oximeter (Nonin 8600V, Plymouth, MN). Heart rate was maintained between 250 to 400/min. Blood oxygen was maintained above 90%. Gallamine triethiodide was administered every 30 min.

For comparison purposes, some recordings were performed under chloral hydrate anesthesia. Rats were anesthetized with chloral hydrate (400 mg/kg intraperitoneal [i.p.]) and underwent the same stereotaxic surgical procedures as used in the other groups (see below). A ventilator was not used, and only body temperature was monitored. Supplement of chloral hydrate (100 mg/kg i.p.) was administered every 30 to 60 min.

Electrophysiology. Electrophysiological recordings were carried out as previously described (Shen et al., 1999). Extracellular action potentials were recorded with single-barrel glass micropipettes (1.5 mm o.d.; Sutter Instrument Co., Novato, CA) filled with 2 M NaCl (in vitro impedance, 2–4 MΩ at 135 Hz) lowered into the VTA and monitored with a high input impedance amplifier (bandpass filter settings, 0.3–3 kHz). The output was sent to an analog oscilloscope, audiomonitor, window discriminator, and a 486 personal computer. To perform the cells-per-track technique, the recording electrode was passed systematically 12 times through a stereotaxically defined block in the VTA. The electrode tracks were separated by 200 μm.
The prenatal ethanol treatment and surgical procedures were consequently administered to all animals to reactivate the same neuron to antagonist, haloperidol (0.1 mg/kg i.v.; Sigma-RBI) was subsequently administered to all animals to reactivate the same neuron to ensure a true inhibition by amphetamine instead of a loss of signals. The prenatal ethanol treatment and surgical procedures were conducted in accordance with National Institutes of Health and American Association for Accreditation of Laboratory Animal Care animal care guidelines, and were approved by the Institutional Animal Care and Use Committee at University at Buffalo.

Data Analysis. The comparisons between groups for numbers of spontaneously active DA neurons and firing rates were made by one-way or two-way analysis of variance (ANOVA/multivariate ANOVA (MANOVA), followed by Tukey honest significant difference (HSD) post hoc comparison (Statistica Software, Tulsa, OK). Percent bursting cells were compared between groups with $\chi^2$ square tests.

Results

The Number of Spontaneous Active DA Neurons and Their Firing Rates after Prenatal Ethanol Exposure.

Prenatal ethanol exposure significantly decreased the number of spontaneously active DA neurons in the VTA in 6- to 8-week-old male rats in both unanesthetized and anesthetized preparations (two-way ANOVA; $F_{1,4o} = 71.66; P < 0.001$; Fig. 1A). Although there was a slight increase in the number of spontaneously active DA neurons in unanesthetized animals when compared with that in anesthetized animals, it did not reach statistical significance (two-way ANOVA; $P > 0.05$). The mean numbers of cells-per-track in the 0 g/kg control groups acquired from unanesthetized and anesthetized animals were 1.06 $\pm$ 0.10 (mean $\pm$ S.E.M.) and 1.02 $\pm$ 0.09, respectively. These two groups did not differ from each other. The mean number of cells-per-track in the untreated control group was 0.94 $\pm$ 0.05, which was not different from the two 0 g/kg control groups (one-way ANOVA; $P > 0.05$). In animals from the 6 g/kg prenatal ethanol-exposed groups, the numbers of spontaneously active DA neurons in unanesthetized and anesthetized animals were 0.48 $\pm$ 0.07 and 0.35 $\pm$ 0.04 cells-per-track, respectively. This represents a 55 to 66% decrease in the number of spontaneously active DA neurons in the VTA produced by prenatal ethanol exposure relative to the 0 g/kg control groups.

In some animals, the cells-per-track sampling procedure
was extended to the anterior VTA (24 tracks were used; see Materials and Methods). Results acquired from these animals were similar to that obtained when only the more posterior VTA was sampled. There was a significant reduction in the number of spontaneously active DA neurons after prenatal ethanol exposure regardless of the use of general anesthesia (two-way ANOVA; $F_{1,27} = 54.6; P < 0.005$). In unanesthetized animals, the mean number of cells-per-track was 0.77 ± 0.07 in the 0 g/kg control group and 0.28 ± 0.08 in the 6 g/kg prenatal ethanol exposed group, respectively. In anesthetized animals, the mean number of cells-per-track was 0.62 ± 0.02 in the 0 g/kg control group and 0.22 ± 0.04 in the 6 g/kg prenatal ethanol exposed group, respectively.

Despite the reduction in the number of spontaneously active DA neurons after prenatal ethanol exposure, there were no differences in firing rates of those neurons that were spontaneously active (two-way ANOVA; $P > 0.05$; Fig. 1B). The mean firing rates in the 0 g/kg control groups acquired from unanesthetized and anesthetized animals were 4.82 ± 0.21 and 4.98 ± 0.20 spikes/s, respectively. The mean firing rate of VTA DA neurons from the untreated group was 4.4 ± 0.37 spikes/s. After prenatal ethanol exposure, the mean firing rates in anesthetized and unanesthetized animals were 5.29 ± 0.20 and 5.33 ± 0.37 spikes/s, respectively. Although small increases in firing rates were observed in prenatal ethanol-exposed groups when compared with the 0 g/kg control groups in both unanesthetized and anesthetized animals, they did not reach statistical significance (two-way ANOVA; $P > 0.05$).

Because there were no significant differences between the untreated and 0 g/kg control groups in the number of spontaneously active DA neurons, or in their firing rates, there was no evidence that undernutrition associated with the pair-feeding procedure influenced the number of spontaneously active DA neurons in these offspring. Therefore, the reduction in the number of spontaneously active DA neurons was a specific effect of prenatal ethanol exposure.

**Burst Firing Pattern.** To determine the influence of prenatal ethanol exposure on the burst firing pattern of VTA DA neurons in the unanesthetized and anesthetized animals, interspike intervals of 500 consecutive action potentials from each individual spontaneously active DA neuron were analyzed. A two-way MANOVA was conducted to detect overall group differences in these parameters except in percent bursting cells (measured by percent bursting cells) in unanesthetized and anesthetized animals. Therefore, the reduction in the number of spontaneously active DA neurons was a specific effect of prenatal ethanol exposure.

Separate $\chi^2$ tests indicated that there were more bursting cells (measured by percent bursting cells) in unanesthetized animals compared with those in anesthetized animals (between the two 0 g/kg control groups: $\chi^2 = 5.98, df = 1, P < 0.05$; between the two 6 g/kg ethanol-treated groups: $\chi^2 = 8.82, df = 1, P < 0.01$). However, prenatal ethanol exposure did not influence percent bursting cells in unanesthetized or anesthetized animals ($\chi^2$ tests; $P > 0.05$).

**Effects of Systemically Administered Amphetamine on the Number of Spontaneously Active DA Neurons.** Systemically administered amphetamine at 2 mg/kg (i.v.) exerted opposite effects in the number of spontaneously active DA neurons in the 0 g/kg control and 6 g/kg prenatal ethanol-exposed groups. In the 0 g/kg control group, amphetamine decreased cells-per-track from 1.15 ± 0.06 to 0.45 ± 0.06 (61% reduction; $n = 10$). On the contrary, in animals exposed to 6 g/kg ethanol during gestation, amphetamine administration within the same animal increased the number of spontaneously active DA neurons by 49%; from 0.45 ± 0.03 to 0.89 ± 0.05 (Fig. 2A). The opposite effects of amphetamine in the 0 g/kg control and in the 6 g/kg groups were reflected in a significant interaction effect between ethanol treatment and acute amphetamine administration (two-way ANOVA; $F_{2,18} = 232.97, P < 0.001$).

Amphetamine seemed to slightly decrease the average firing rates of DA neurons in the VTA (Fig. 2B). A significant difference was detected as an amphetamine main effect when

### Table 1

Burst firing patterns of spontaneously active VTA DA neurons following prenatal ethanol exposure in unanesthetized and anesthetized animals

<table>
<thead>
<tr>
<th>No. of</th>
<th>% Coefficient of Variation</th>
<th>Burst No.</th>
<th>No. of Doublets</th>
<th>Interburst Interval (ms)</th>
<th>Within Burst Interval (ms)</th>
<th>Burst Length (s)</th>
<th>% Bursting Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 g/kg Unanesthetized</td>
<td>119</td>
<td>103.5 ± 4.4*</td>
<td>80.6 ± 4.6**</td>
<td>33.0 ± 2.2*</td>
<td>351.0 ± 15.3*</td>
<td>60.7 ± 1.0*</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>6 g/kg Unanesthetized</td>
<td>61</td>
<td>116.5 ± 8.1*</td>
<td>71.4 ± 4.7**</td>
<td>31.6 ± 3.1*</td>
<td>383.6 ± 27.9*</td>
<td>61.9 ± 1.5**</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>0 g/kg Anesthetized</td>
<td>81</td>
<td>89.1 ± 5.2</td>
<td>55.3 ± 3.6</td>
<td>25.5 ± 2.2</td>
<td>304.9 ± 19.8</td>
<td>68.8 ± 1.4</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>6 g/kg Anesthetized</td>
<td>26</td>
<td>85.9 ± 8.2</td>
<td>50.6 ± 6.3</td>
<td>22.8 ± 3.7</td>
<td>300.0 ± 25.7</td>
<td>74.7 ± 2.7</td>
<td>3.4 ± 0.2</td>
</tr>
</tbody>
</table>

* $P < 0.05$; ** $P < 0.01$. Differences between unanesthetized and chloral hydrate-anesthetized animals in either the 0 g/kg control groups or the 6 g/kg ethanol-treated groups. Two-way ANOVAs followed by Tukey HSD post hoc tests were used to test for group differences in all burst parameters except % bursting cells. $\chi^2$ test was used to test for group differences in % bursting cells.
Fig. 2. Numbers of spontaneously active DA neurons and their firing rates sampled before and after systemic intravenous amphetamine administration (2 mg/kg i.v.) in the VTA in the 0 g/kg control group and 6 g/kg prenatal ethanol-exposed group. A, the numbers of cells-per-track in each animal sampled before and after amphetamine administration are presented as individual data points connected by solid lines. The average number of spontaneously active DA neurons in the 0 g/kg control group is decreased by amphetamine, whereas an increase is observed in the 6 g/kg prenatal ethanol-exposed group. B, the average firing rates of spontaneously active DA sampled before and after systemic intravenous amphetamine administration in the 0 g/kg control and 6 g/kg prenatal ethanol-exposed rats. The number of cells recorded in each group is indicated within each bar.

All groups were analyzed (two-way ANOVA, $F_{1,347} = 5.01$, $P < 0.05$). However, amphetamine did not significantly change DA neuron firing rate in either the 0 g/kg control or the 6 g/kg prenatal ethanol-exposed group (Tukey HSD post hoc test). The firing rates in the 0 g/kg control groups were $4.61 \pm 0.11$ (n = 139) and $4.23 \pm 0.22$ (n = 54) spikes/s before and after amphetamine treatment, respectively. In the 6 g/kg ethanol group, the firing rates were $4.52 \pm 0.17$ (n = 52) and $4.18 \pm 0.14$ (n = 103) before and after amphetamine treatment, respectively.

Discussion

The results from the present study show that prenatal ethanol exposure produces a significant reduction in the number of spontaneously active VTA DA neurons in young adult rats (6–8 weeks old), similar to that observed in older rats (3–5 months old and 14–16 months old) previously (Shen et al., 1999). These observations suggest that, after prenatal ethanol exposure, the hypofunction in DA neurotransmission mediated by the reduction in the number of spontaneously active DA neurons occurs throughout adulthood.

We used both the unanesthetized (paralyzed, locally anesthetized, and anesthetized [chloral hydrate-anesthetized]) preparations to avoid possible confounding effects of general anesthetics on the spontaneous activity of DA neurons. The extracellular single-unit recording and cells-per-track techniques were initially developed to examine the mechanisms of antipsychotics (for review, see Chiodo, 1988; White, 1996). With these techniques, many investigators have observed a reduction in the number of spontaneously active VTA DA neurons after chronic antipsychotic treatment and suggest that this mechanism mediates the efficacy of antipsychotics. Recently, a group of investigators have argued that the reduction in the number of spontaneously active DA neurons after chronic antipsychotic treatment can be observed only in the anesthetized preparation (Mereu et al., 1995). In other words, a reduction in the number of spontaneously active DA neurons after chronic antipsychotic treatment is an “artifact” caused by the use of general anesthesia. To verify if this was what happened previously when we examined the spontaneous activity in DA neurons after prenatal ethanol exposure under chloral hydrate anesthesia in older animals, and to truly understand the impact of general anesthesia on the spontaneous activity of DA neurons in young adult animals after prenatal ethanol exposure, we have chosen to use both the unanesthetized and anesthetized preparations. Our results show that prenatal ethanol exposure causes similar reductions in the number of spontaneously active DA neurons whether or not general anesthesia is used. These results do not support the view that the reduction in the number of spontaneously active DA neurons is an artifact produced by general anesthesia. They also validate our previous observations performed under chloral hydrate anesthesia in which prenatal ethanol exposure reduces the number of spontaneously active DA neurons in older rats (3–5 months old and 14–16 months old).

Although general anesthesia does not appear to alter the number of spontaneously active DA neurons, it does significantly decrease their burst activity. Therefore, the unanesthetized preparation appears to be a better approach to ex-
amine burst activity in DA neurons. Interestingly, despite a significant reduction in the number of spontaneously active DA neurons, prenatal ethanol exposure does not alter either burst activity or firing rate of the remaining spontaneously active DA neurons, indicating that there are independent mechanisms underlying burst activity and the number of spontaneously active DA neurons. Increased burst activity can elevate DA release (Suau-d-Chagny et al., 1992) and has been proposed to play an important role in maintaining adequate DA release after a loss of DA neurons in animal models of Parkinson’s disease (Hollerman and Grace, 1990). The lack of changes in burst activity in DA neurons after prenatal ethanol exposure suggests that this type of compensatory mechanism does not occur after prenatal ethanol exposure.

The results from the present study also demonstrate that acute amphetamine administration can normalize the number of spontaneously active DA neurons after prenatal ethanol exposure. What could be the underlying mechanism for this phenomenon? Acute amphetamine administration typically exerts an inhibitory effect on DA neurons by activating somatodendritic DA autoreceptors due to amphetamine-induced increases in dendritic DA release (Mercuri et al., 1989; Pothis et al., 1998), as well as by activating a feed back input to DA neurons due to increased DA release in the forebrain (Bunney and Aghajanian, 1976). The inhibitory effect of amphetamine on DA neurons is similar to the effects of other inhibitory agents that can restore the number of spontaneously active DA neurons. For example, systemically administered apomorphine has been shown to restore the number of spontaneously active DA neurons following chronic prenatal or postnatal ethanol treatment, or chronic antipsychotic treatment (Bunney and Grace, 1978; Shen et al., 1993, 1999). Other manipulations that normally inhibit of DA neurons such as locally applied GABA or membrane hyperpolarization can also restore the number of spontaneously active DA neurons (Bunney and Grace, 1978; Grace and Bunney, 1986). Based on these observation, it is hypothesized that the reduction in the number of spontaneously active DA neurons is produced by excessive depolarization leading to an impairment in action potential generation. This is called the depolarization block hypothesis. Because acute amphetamine has the same effect on DA neuron firing rates as acute apomorphine, we believe that amphetamine may also restore the number of spontaneously active DA neurons after prenatal ethanol exposure by reversing the depolarization block.

Although the reversal of the depolarization block appears to be the most likely mechanism mediating the amphetamine-induced restoration of the number of spontaneously active DA neurons after prenatal ethanol exposure, other possibilities should also be considered. Amphetamine has been shown to increase excitatory amino acid neurotransmission to DA neurons by activating α1 adrenergic receptors after blocking inhibitory inputs (Shi et al., 2000). The unmasked excitatory effect of amphetamine may play a role in the restoration of the number of spontaneously active DA neurons because prenatal ethanol exposure-induced morphological changes in DA neurons (Shetty et al., 1993) could potentially alter the afferent regulation of DA neurons. If this is the case, amphetamine would have to exert a direct excitatory effect only in previously “silent” DA neurons because no such effects were observed in spontaneously active DA neurons in the present study. This possibility also does not seem to reconcile with the fact that apomorphine can also normalize the number of spontaneously active DA neurons. Future studies using specific receptor antagonists are required to clarify the roles of DA autoreceptors and α1 adrenergic receptors in the amphetamine-induced reversal of the number of spontaneously active DA neurons after prenatal ethanol exposure.

In the present study, prenatal ethanol exposure also produced a left shift in the dose-response curves for the inhibitory effect of systemically administered amphetamine on VTA DA neurons, indicating that DA neurons are more sensitive to the inhibitory effect of amphetamine after prenatal ethanol exposure. This result could be due to a supersensitivity in somatodendritic DA autoreceptors first observed in our previous study (Shen et al., 1995). We have speculated that DA autoreceptor supersensitivity may be mediated by understimulation of these receptors due to either insufficient dendritic DA release or decreased dendrodendritic contacts among DA neurons (Shetty et al., 1993) after prenatal ethanol exposure. The results from the present study indicate that insufficent dendritic DA release may indeed occur due to decreased number of spontaneously active DA neurons. Interestingly, the sensitivity of DA autoreceptors returns to normal after chronic amphetamine treatment (Shen et al., 1995). This effect is similar to the down-regulation of DA autoreceptors in the VTA in response to chronic amphetamine treatment in nonethanol-treated rats (White and Wang, 1984). It is also noteworthy that chronic amphetamine treatment in normal rats could increase the number of spontaneously active DA neurons and their firing rates presumably by decreased sensitivity in DA autoreceptors (White and Wang, 1984). These results suggest that, under chronic amphetamine treatment, the doses of amphetamine required to optimally restore the number of spontaneously active DA neurons after prenatal ethanol exposure may need to be changed as the sensitivity of somatodendritic DA is modified.

Recently, the efficacy of amphetamine-like stimulants in treating attention problems in FAS/FAE has been clearly demonstrated (Oesterheld et al., 1998). Based on the results from the present study, we believe that, other than causing a direct increase in DA level in DA terminal areas (Kalivas and Stewart, 1991), the therapeutic effect of amphetamine in FAS/FAE may be due to its ability to restore the number of spontaneously active DA neurons. The restored spontaneous activity in DA neurons may consequently increase DA levels in the forebrain via elevating the release and synthesis of DA (Gonon and Buda, 1985; Suaud-Chagny et al., 1992).

Several lines of evidence suggest that a reduction in the number of spontaneously active DA neurons is not a unique outcome after prenatal ethanol exposure. For example, early postnatal lead exposure, prenatal cocaine exposure, or chronic ethanol treatment prenatally or postnatally all decrease DA neurotransmission and the number of spontaneously active DA neurons (Shen and Chiodo, 1995; Wang and Pitts, 1994; Shen et al., 1999; personal communication with D. K. Pitts). Interestingly, attention problems are major symptoms in all these conditions (Winneke et al., 1983; Nixon et al., 1995). It appears that the reduced number of spontaneously active DA neurons contributes to DA dysfunction and attention problems. The efficacy of amphetamine-like stimulants in treating attention problems in these conditions could also be partially mediated by normal-
izing the spontaneous activity in DA neurons. We believe the current animal model is an appropriate approach to investigate the detailed mechanisms of amphetamine-like stimulants in treating attention problems caused by the above conditions.

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References


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