Methylphenidate Restores Ventral Tegmental Area Dopamine Neuron Activity in Prenatal Ethanol-Exposed Rats by Augmenting Dopamine Neurotransmission

Kar-Chan Choong and Roh-Yu Shen

Research Institute on Addictions, State University of New York at Buffalo, Buffalo, New York

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ABSTRACT

Altered neurotransmission in the mesolimbic dopamine (DA) system has been suggested to be the underlying cause of attention problems commonly observed in children with fetal alcohol spectrum disorder (FASD). Methylphenidate is effective in treating attention problems in children with FASD. However, the underlying mechanism is currently unknown. We have shown previously that reduced ventral tegmental area (VTA) DA neuron activity in prenatal ethanol-exposed animals can be normalized by DA agonist treatment. In the present study, we investigated the possibility that similar mechanism mediates the effect of methylphenidate using the in vivo extracellular single-unit recording technique in anesthetized animals. We observed that reduced VTA DA neuron activity in prenatal ethanol-exposed animals was normalized by methylphenidate. The effect of methylphenidate was mediated by increased extracellular levels of DA instead of norepinephrine because this effect was not altered by the coadministration of prazosin, an α1 receptor antagonist, and was mimicked by the application of DA transporter blockers, nomifensine and 1-2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenyl)piperazine dihydrochloride (GBR 12909). These observations support our hypothesis that depolarization inactivation is the cause of prenatal ethanol exposure-induced reduction in VTA DA neuron activity. We speculate that methylphenidate normalized the activity of VTA DA neurons by increasing extracellular DA levels in the VTA and the activation of somatodendritic DA autoreceptors. As a result, the depolarization inactivation was removed by hyperpolarization. The normalized VTA DA neuron activity in prenatal ethanol-exposed animals may contribute to a restoration of DA neurotransmission and the therapeutic effect of methylphenidate in attention problems in children with FASD.

Prenatal ethanol exposure leads to abnormalities in the function of the midbrain dopamine (DA) system. For example, there is a reduction in DA uptake and receptor binding sites, DA content, and the DA metabolites in both the somatodendritic and terminal areas (Rathbun and Druse, 1985; Cooper and Rudeen, 1988; Druse et al., 1990) in prenatal ethanol-exposed animals. Prenatal ethanol exposure also can lead to changes in DA neuron morphology [e.g., smaller cell bodies and retarded dendritic growth in DA neurons (Shetty et al., 1995), DA receptor function (Shen et al., 1995; Wang and Shen, 2002), and DA receptor-mediated behavior (Hannigan and Randall, 1996)]. Recently, impairment in sustained attention, which could be modulated by DA (Davids et al., 2003), was also reported in prenatal ethanol-exposed animals (Hausknecht et al., 2003).

In previous electrophysiological studies, we have demonstrated that prenatal ethanol exposure leads to a persistent reduction in the electrical activity of the midbrain DA neurons without a loss of DA neurons (Shen et al., 1999; Xu and Shen, 2001). Because the electrical activity controls the synthesis and release of DA (Gonon and Buda, 1985; Suaud-Chagny et al., 1992), prenatal ethanol exposure-induced reduction in the electrical activity in DA neurons could contribute to the decrease in DA content and metabolites observed in previous studies. Given the connection between abnormal DA neurotransmission and attention problems, prenatal ethanol exposure-induced reduction in the electrical activity of DA neurons could underlie the attention problems often observed in children with fetal alcohol spectrum disorder (FASD) (Streissguth et al., 1994; Coles et al., 1997).

We have previously demonstrated that prenatal ethanol exposure-induced reduction in the electrical activity of DA neurons in the ventral tegmental area (VTA) can be normalized by acute administration of direct or indirect DA agonists such as apomorphine and amphetamine (Shen et al., 1999;
Xu and Shen, 2001). Interestingly, attention problems in children with FASD can be effectively treated with an amphetamine-like stimulant, methylphenidate (Oesterheld et al., 1998). It is likely that methylphenidate exerts its therapeutic effect by normalizing DA neurotransmission via the restoration of the electrical activity of VTA DA neurons. In the present study, we investigate the possibility that methylphenidate can restore the activity of VTA DA neurons.

In addition, we conduct pharmacological experiments to study whether the effect of methylphenidate is mediated by changing the adrenergic or DA neurotransmission. The results from the present study will provide important insights into the understanding of prenatal ethanol-induced changes in DA neurotransmission and the therapeutic effects of methylphenidate in children with FASD.

**Materials and Methods**

**Prenatal Ethanol Treatment and Surgical Procedure.** Timed-pregnant Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were delivered on gestation day 6. Each rat was handled (held and restrained) for 10 min on gestation day 7, so the rat was adapted to the restraining method for intubation starting on gestation day 8. To mimic the binge drinking behavior that produces high blood ethanol concentrations in humans at risk for FASD, rats were administered ethanol via intragastric intubation from gestation day 8 through 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. The 0 g/kg control group received the same volume of sucrose (30% w/v in 0.9% saline) to substitute for ethanol isocalorically. The blood ethanol concentration was measured in a previous study 1.5 h after the second daily dose of ethanol was between 281 and 341 mg/dl on gestation day 20 (Shen et al., 1999). To control for the possible effect of under nutrition, rats in the 0 g/kg control group were pair-fed 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 PM) during weekdays. A single daily dose of 0 or 4 g/kg ethanol was given during weekends. The 0 g/kg control group received the same volume of sucrose (30% w/v in 0.9% saline) to substitute for ethanol isocalorically. The blood ethanol concentration was measured in a previous study 1.5 h after the second daily dose of ethanol was between 281 and 341 mg/dl on gestation day 20 (Shen et al., 1999). To control for the possible effect of under nutrition, rats in the 0 g/kg control group were pair-fed with ethanol-treated dams. 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 have observed that ethanol-treated dams displayed a certain degree of negligence toward pups in the 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 maximum number of males. The litters were then transferred to surrogate dams that did not receive any treatment and had delivered 2 days earlier. Litters of control dams were switched among each other. Litters were weaned and weighed on postnatal day 21. To control for litter effects, no more than three littermates were used in the same experiments. Only male offspring were used for the electrophysiology recordings, and 90 male offspring of the treated dams at age 8 to 12 weeks were used for the present study.

**Electrophysiology.** The electrophysiological recordings of DA neurons were performed in chloral hydrate-anesthetized (400 mg/g i.p.) rats. The animal was mounted in a stereotaxic apparatus, and the skull and dura overlapping the VTA were removed. The lateral tail vein was cannulated with a 23-gauge needle. Body temperature was monitored by a rectal thermometer and maintained between 36 and 37°C by a thermostatically controlled heating pad (Finntronic, Orange, CT). An oximeter was used to ensure heart rate was between 280 and 400/min and blood oxygen level above 90% (Nonin 8600V; Nonin, Plymouth, MN). The clip-on probe of the oximeter was placed on the paw of one of the hind limbs. Recordings were aborted when the heart rate dropped below 280/min or the blood oxygen level was below 90%. If the heart rate rose above 400/min, supplement of chloral hydrate (100 mg/kg) was given (approximately every 30–60 min). Extracellular single-unit recordings were carried out with single-barrel glass micropipettes (1.5-mm o.d.; World Precision Instruments Inc., New Haven, CT) filled with 2 M NaCl. Electrode resistance ranged from 2 to 4 MΩ at 135 Hz. To perform the cells-per-track technique, the recording electrode was passed systematically 12 times through a stereotaxically defined block in VTA. The coordinates were: 2.8 to 3.4 mm anterior to lambda, 6.0 to 9.0 mm below the brain surface, and 0.6 to 1.0 lateral to the midline ( Paxinos and Watson, 1986). Each track was separated by 200 μm. Spontaneously active VTA DA neurons were identified by their characteristic waveform and firing patterns (Chiodo, 1988; Fig. 1A) including positive-negative action potential waveforms, long duration of action potentials (2–4 ms), and irregular single-spike or burst-firing pattern.

The acute effects of methylphenidate on the number of spontaneously active DA neurons in prenatal ethanol-exposed animals were examined using the cells-per-track technique within the same animals. The cells-per-track procedure was performed in one side of the VTA, followed by methylphenidate administration (1 mg/kg i.p.). The cells-per-track technique was then performed in the other side of the

**Fig. 1.** Number of spontaneously active VTA DA neurons and their firing rates in prenatal ethanol-exposed animals. A, recording traces depicting a typical positive/negative VTA DA neuron action potential waveform (left trace) and irregular firing activity (right trace). B, prenatal ethanol exposure reduced the number of spontaneously active VTA DA neurons. The numbers of spontaneously active DA neurons are shown as cells-per-track (mean number of DA neurons encountered from each electrode penetration ± S.E.M). C, prenatal ethanol exposure slightly but significantly reduced the average firing rates of VTA DA neurons. The number of cells recorded in each group was indicated inside each bar. *, P < 0.05; and ***, P < 0.001, Fisher’s LSD post hoc comparison between the 0 g/kg control group and 6 g/kg prenatal ethanol-exposed group.
Results

The Number of Spontaneously Active VTA DA Neurons and Their Firing Rates in Prenatal Ethanol-Exposed Animals. The number of spontaneously active VTA DA neurons was significantly reduced in 8- to 12-week-old male animals in prenatal ethanol-exposed animals (one-way ANOVA, P < 0.001; Fig. 1A). The mean number of cells-per-track (12 tracks in each rat) in the 6 g/kg prenatal ethanol-exposed group was 0.61 ± 0.10 (mean ± S.E.M., n = 15 rats) and in the 0 g/kg control group was 1.10 ± 0.08 (mean ± S.E.M., n = 15 rats). This represents a 45% decrease in the number of spontaneously active VTA DA neurons by prenatal ethanol exposure.

The firing rate of spontaneously active VTA DA neurons was significantly reduced in prenatal ethanol-exposed animals (one-way ANOVA, P < 0.05; Fig. 1B). The mean firing rate in the 6 g/kg control groups was 4.32 ± 0.09 spikes/s (n = 428 cells), whereas the mean firing rate in the prenatal ethanol-exposed group was reduced to 3.97 ± 0.13 spikes/s (n = 239 cells). This result was different from a previous study (Xu and Shen, 2001), where no difference in the firing rate of VTA DA neurons between the control and prenatal ethanol-exposed groups was observed. This discrepancy is caused by increased statistical power due to extremely large sample sizes (more cells sampled) in the present study. When such large sample sizes are used, the effect should be consid-

The inhibitory effect of methylphenidate on individual VTA DA neuron firing rate was studied by examining the dose-response curve of systemically administered methylphenidate. Single neuron firing rate was recorded every 10 s. After 60 s of stable baseline, methylphenidate was administered at cumulative doses (i.v.) of 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16 mg/kg. The increment doses were administered at 2- to 3-min interval. The inhibitory effect was considered reaching plateau when no changes in firing rate were observed after two continuous increment doses. To ensure a true inhibition, rather than a loss of single-unit activity, a DA receptor antagonist, haloperidol (0.1 mg/kg i.v., Sigma-Aldrich, St. Louis, MO) was subsequently administered to all animals to reactivate the same neuron. 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 the University at Buffalo.

Data Analysis. The comparisons between groups for the number of spontaneously active DA neurons and firing rates were made by one- or two-way analysis of variance (ANOVA) with or without repeated measures, followed by Fisher’s least significant difference (LSD) post hoc comparison (Statistica Software, Tulsa, OK). The repeated-measure ANOVA was used to compare pre- and postdrug effects (e.g., methylphenidate, nomifensine, prazosin, GBR 12909) within the same animals. Analysis of covariance (ANCOVA) was also used to compare the dose-response curves of methylphenidate with the baseline firing rate as the covariate.

Effects of Systemically Administered Methylphenidate on the Number of Spontaneously Active VTA DA Neurons and Their Firing Rates. Systemically administered methylphenidate at 1 mg/kg i.p. exerted different effects on the number of spontaneously active VTA DA neurons in the 0 g/kg control and 6 g/kg prenatal ethanol-exposed animals. In the 0 g/kg control group, methylphenidate did not affect the number of spontaneously active VTA DA neurons.

The cells-per-tracks were 1.02 ± 0.06 (n = 15 rats) and 1.02 ± 0.09 (n = 15 rats) before and after methylphenidate treatment, respectively (Fisher’s LSD post hoc test, P = 0.51).

Contrary to the effect in control animals, methylphenidate administration within the same animal from the 6 g/kg ethanol-exposed group increased the number of spontaneously active VTA DA neurons 2-fold, from 0.61 ± 0.10 (n = 15 rats) to 3.97 ± 0.08 (n = 15 rats; Fig. 2A; Fisher’s LSD post hoc test, P < 0.001). The opposite effects of methylphenidate in these two groups were indicated by a significant interaction effect between prenatal ethanol treatment and acute methylphenidate administration (two-way ANOVA; P,1.754 = 21.32, P < 0.001).

When all groups were analyzed, there was a significant main effect of methylphenidate on the firing rate of VTA DA neurons (two-way ANOVA, F1,174 = 4.33, P < 0.05). Methylphenidate administration significantly reduced the mean firing rate of VTA DA neurons in the 0% control group by 23% (Fisher’s LSD post hoc test, P < 0.001). The firing rates were

![Fig. 2. Number of spontaneously active VTA DA neurons (cells-per-track) and their firing rates sampled before and after systemic methylphenidate (MPH) administration (1 mg/kg i.p.) in control and prenatal ethanol-exposed animals. A, methylphenidate restored the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals to the level of control animals. The number of spontaneously active VTA DA neurons in the 0 g/kg control group was not affected by methylphenidate treatment. B, methylphenidate administration significantly reduced the firing rate of VTA DA neurons in the 0 g/kg control group but had no effect on VTA DA neurons in the 6 g/kg prenatal ethanol-exposed group. C, the numbers of cells-per-track in each animal sampled before and after methylphenidate administration are presented as individual data points connected by solid lines. **, P < 0.05; and ***, P < 0.001, Fisher’s LSD post hoc comparison of acute methylphenidate effect in the control or prenatal ethanol-exposed group. #, P < 0.001 between the 0 g/kg control group and 6 g/kg prenatal ethanol-exposed group.](image-url)
reduced from $4.66 \pm 0.14$ (n = 200 cells) to $3.61 \pm 0.13$ (n = 186 cells) spikes/s after methylphenidate administration. In the 6 g/kg ethanol group, methylphenidate reduced the firing rate of the spontaneously active VTA DA neurons slightly—from $4.42 \pm 0.19$ (n = 133 cells) to $3.99 \pm 0.13$ (n = 239 cells) spikes/s, which did not reach statistical significance (Fisher’s LSD post hoc test, \( P = 0.05437 \)).

The Effect of Systemic Administration of Nomifensine, GBR 12909, Prazosin, or the Coadministration of Methylphenidate and Prazosin on the Number of Spontaneously Active DA Neurons and Their Firing Rates. The cells-per-track experiment was also performed before and after treatment of drugs that modify dopaminergic or noradrenergic transmission (nomifensine and prazosin, respectively) to verify that the effect of methylphenidate was mediated by increasing DA or norepinephrine levels in the VTA. Nomifensine (1 mg/kg i.p.) exerted similar effects on the number of spontaneously active VTA DA neurons as methylphenidate in animals with prenatal ethanol exposure. In the 6 g/kg prenatal ethanol-exposed group, the number of spontaneously active VTA DA neurons was increased by 2-fold, from $0.58 \pm 0.17$ to $1.22 \pm 0.14$ cells-per-track, after nomifensine treatment (n = 5 rats; Fig. 3A; Fisher’s LSD post hoc test, \( P < 0.001 \)). However, in the 0 g/kg control group, nomifensine decreased cells-per-track from $1.13 \pm 0.13$ to $0.60 \pm 0.14$ (47% reduction; n = 5 rats; Fig. 3A; Fisher’s LSD post hoc test, \( P < 0.05 \)). The opposite effects of nomifensine in control and prenatal ethanol-exposed animals were reflected by a significant interaction effect between prenatal ethanol treatment and acute nomifensine administration (two-way ANOVA, \( F_{1,17} = 29.43, P < 0.001 \)).

The changes in firing rate of VTA DA neurons sampled before and after systematic administration of nomifensine were significantly different for the 0 g/kg control group and prenatal ethanol-exposed group (two-way ANOVA interaction effect, \( F_{1,119} = 4.28, P < 0.05 \)). Nomifensine treatment reduced the mean firing rate of the spontaneously active VTA DA neurons from $4.11 \pm 0.21$ (n = 67 cells) to $2.80 \pm 0.21$ (n = 36 cells) spikes/s (Fisher’s LSD post hoc test, \( P < 0.001 \)). On the other hand, there was no effect of nomifensine on VTA DA neurons in the prenatal ethanol-exposed group (Fisher’s LSD post hoc test, \( P = 0.59 \)). The firing rates were $3.66 \pm 0.30$ (n = 36 cells) and $3.88 \pm 0.26$ (n = 64 cells), before and after nomifensine administration, respectively.

To further verify whether the effect of nomifensine is indeed mediated by increasing extracellular DA levels, we investigated the effect of GBR 12909, a specific DA transporter blocker. In the 6 g/kg prenatal ethanol-exposed group, the number of spontaneously active VTA DA neurons increased by more than 3-fold, from $0.44 \pm 0.03$ to $1.42 \pm 0.5$ cells-per-track, after GBR 12909 treatment (n = 3 rats). In the 0 g/kg control group, GBR 12909 decreased cells-per-track from $1.13 \pm 0.04$ to $0.96 \pm 0.04$ (n = 2 rats; Fig. 3A). A trend for the opposite effects in the control and prenatal ethanol-exposed groups was apparent, although it did not reach statistical significance (two-way ANOVA interaction effect, \( F_{1,6} = 3.06, P = 0.13 \)). The changes in firing rate of VTA DA neurons sampled before and after systematic administration of GBR 12909 were similar to the effect of nomifensine, i.e., there was a decrease trend in the control animals and no change in prenatal ethanol-exposed animals. The firing rates before and after GBR 12909 treatment were $3.57 \pm 0.46$ (n = 26 cells) and $2.52 \pm 0.48$ (n = 23 cells) spikes/s, respectively, in the control animals. In prenatal ethanol-treated animals, the firing rates before and after GBR 12909 treatment were $3.27 \pm 0.58$ (n = 16 cells) and $3.58 \pm 0.32$ (n = 52 cells), respectively.

Prazosin (1 mg/kg i.p.) did not change the number of spontaneously active VTA DA neurons (two-way ANOVA; Fig. 3A) or their firing rates (two-way ANOVA; Fig. 3B) in either the 0 g/kg control or the 6 g/kg prenatal-exposed group. Cells-per-track for the 0 g/kg control group were $1.02 \pm 0.09$ and $1.08 \pm 0.12$ (n = 5 rats) before and after prazosin treatment, respectively. The number of spontaneously active VTA DA neurons in the prenatal ethanol-exposed group was decreased by nomifensine, GBR administration, or the coadministration of MPH and prazosin.

Fig. 3. Number of spontaneously active VTA DA neurons (cells-per-track) and their firing rates sampled before and after nomifensine administration (1 mg/kg i.p.), prazosin administration (1 mg/kg i.p.), the coadministration of methylphenidate (MPH; 1 mg/kg i.p.) and prazosin (1 mg/kg i.p.), or GBR 12909 administration (GBR; 8 mg/kg i.v.). A, the reduction in the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals was normalized by nomifensine or GBR administration. The average number of spontaneously active VTA DA neurons in the 0 g/kg control group was decreased by nomifensine and GBR treatment. Prazosin treatment had no effect on both groups of animals. The coadministration of methylphenidate and prazosin did not change the ability of methylphenidate to normalize the reduction in the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals. B, the average firing rate of spontaneously active VTA DA neurons in the 0 g/kg control group was decreased by nomifensine, GBR administration, or the coadministration of MPH and prazosin. *, \( P < 0.05 \); **, \( P < 0.01 \); and ***, \( P < 0.001 \), Fisher’s LSD post hoc comparison of acute MPH effect in the control or prenatal ethanol-exposed group. #, \( P < 0.001 \) between the 0 g/kg control group and 6 g/kg prenatal ethanol-exposed group.
neurons recorded from the 6 g/kg prenatal ethanol-exposed group were 0.42 ± 0.05 before prazosin treatment and 0.40 ± 0.06 after prazosin injection (n = 5 rats). Mean firing rates for the 0 g/kg control group were 3.69 ± 0.20 spikes/s (n = 61 cells) and 3.97 ± 0.21 spikes/s (n = 64 cells) before and after prazosin treatment, respectively (Fisher’s LSD post hoc test, P = 0.35). The firing rates of VTA DA active neurons in the prenatal ethanol-exposed group were 3.09 ± 0.33 spikes/s (n = 25 cells) and 4.05 ± 0.37 spikes/s (n = 23 cells) before and after prazosin treatment, respectively (Fisher’s LSD post hoc test, P = 0.06).

The effect of coadministration of methylphenidate (1 mg/kg i.p.) and prazosin (1 mg/kg i.p.) on the number of spontaneously active VTA DA neurons was similar to that obtained from the administration of methylphenidate alone. The number of cells-per-track for the 0 g/kg control group were 1.22 ± 0.19 before treatment and 1.15 ± 0.15 after treatment. In contrast, in the prenatal ethanol-exposed group, the cells-per-tracks were increased from 0.38 ± 0.11 to 0.78 ± 0.09 (Fisher’s LSD post hoc test, P < 0.05). The different effects of acute drug treatment in these two groups were reflected in a significant interaction effect (two-way ANOVA, \( F_{1,17} = 4.61, P < 0.05 \)).

Mean firing rates were reduced for both groups of animals after the coadministration of methylphenidate and prazosin. This is reflected in a main effect of acute drug treatment (two-way ANOVA, \( F_{1,1218} = 13.36, P < 0.001 \)). For the 0 g/kg control group, the firing rate was reduced from 4.05 ± 0.19 (n = 71 cells) to 3.33 ± 0.21 (n = 69 cells) spikes/s (Fisher’s LSD post hoc test, P < 0.05). In the prenatal ethanol-exposed group, the firing rates were 4.03 ± 0.32 (n = 27) spikes/s and 3.02 ± 0.20 (n = 55 cells) spikes/s, before and after treatment, respectively (Fisher’s LSD post hoc test, P < 0.01).

**Inhibitory Effect of Methylphenidate on Spontaneously Active VTA DA Neurons.** To understand how methylphenidate could normalize the number of spontaneously active VTA DA neurons, we also examined the effect of acute methylphenidate on individual spontaneously active VTA DA neurons in both groups of animals. Acute systemic methylphenidate administration exerted inhibitory action on the firing rate on individual VTA DA neurons (Fig. 4, A and B). In Fig. 4A, the normalized dose-response curve for the inhibitory effect of methylphenidate in animals exposed to ethanol parentally was significantly shifted to the left (two-way ANOVA, \( F_{7,183} = 3.90, P < 0.001 \)). The maximum inhibition was around 80% in both groups of animals. This effect was not due to baseline firing rate differences, which may lead to biased estimate of normalized dose-response curves. The averaged baseline firing rates for the control and prenatal ethanol-exposed groups were 5.38 ± 0.60 and 5.79 ± 0.62 spikes/s, respectively (\( t_{26} = 0.48, P = 0.64 \)). The result using ANCOVA with repeated measures on the raw firing rate data with baseline firing rate as the covariate further confirmed the differential inhibitory effects of methylphenidate on the firing rate of VTA DA neurons recorded from the control and prenatal ethanol-exposed animals (Fig. 4 B). This effect was reflected in a significant interaction effect between group and dose (two-way ANCOVA, \( F_{7,175} = 4.07, P < 0.05 \) after the.

**Fig. 4.** The inhibitory effect of methylphenidate (MPH) on the firing rate of individual spontaneously active VTA DA neurons. A, normalized dose-response curves. The normalized dose-response curve obtained from the 6 g/kg prenatal ethanol-exposed group was significantly shifted to the left when compared with that obtained from the 0 g/kg control group. The relevant EC_{50} values are also presented. B, dose-response curves for the inhibitory effect of methylphenidate on the raw firing rate data of individual spontaneously active VTA DA neurons show that the left shift in normalized dose response curves was not due to baseline differences. C, rate histogram depicting the inhibitory effect of MPH on VTA DA neurons sampled from the 0 g/kg control group (upper trace) and 6 g/kg prenatal ethanol-exposed group (lower trace). MPH was administered at cumulative doses (i.v.) of 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/kg. To ensure a true inhibition by MPH, a DA receptor antagonist, haloperidol (HA), was administered at the end of each recording to reactivate the same neuron.
covariate (baseline firing rate) has been taken into consideration.

Discussion

Methylphenidate Restores the Electrical Activity of the VTA DA Neuron by Increasing DA Neurotransmission. In the present study, we have demonstrated that the reduction in the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals can be normalized by acute systemic methylphenidate treatment. This result is similar to previous results obtained in our laboratory, in which we demonstrate that acute administration of apomorphine, a DA agonist, restores the number of spontaneously active VTA DA neurons following prenatal ethanol exposure (Shen et al., 1999). The restoration in the number of spontaneously active VTA DA neurons with pharmacological agents that normally inhibit the firing rate of individual DA neurons has led us to suggest that the reduction in the number of spontaneously active VTA DA neurons is mediated by depolarization inactivation. Depolarization inactivation in DA neurons is postulated to occur by an overexcitation that leads to an impairment of action potential generation (for review, see Grace et al., 1997). We would like to point out that most investigators, including us, propose the depolarization inactivation hypothesis based on the unconventional action of DA agonists without direct measuring VTA DA neuron membrane potential. The verification of depolarization inactivation requires the employment of an in vivo intracellular recording technique that is rarely performed due to technical difficulties.

Systemic administration of apomorphine is commonly used to alleviate the depolarization inactivation and normalize the number of spontaneously active DA neurons. Recently, we have demonstrated that systemic administration of amphetamine is also effective in alleviating depolarization inactivation (Xu and Shen, 2001; Shen, 2003). Amphetamine is an indirect DA receptor agonist. Under normal conditions, it inhibits the firing rate of individual spontaneous active DA neurons by increasing extracellular DA concentration in the VTA, which leads to an activation of somatodendritic DA autoreceptors (Mercuri et al., 1989). This could result in hyperpolarization of VTA DA neurons and the removal of depolarization inactivation, which leads to the normalization of VTA DA neurons as observed in prenatal ethanol-exposed animals. Methylphenidate, like amphetamine, increases DA neurotransmission by increasing extracellular DA levels (Denney, 2001). Therefore, methylphenidate could also restore the electrical activity in DA neurons by the same mechanism; however, both amphetamine and methylphenidate are adrenergic uptake blockers (Ritz et al., 1987) and, therefore, could augment adrenergic neurotransmission in the VTA. For example, amphetamine-mediated excitation has been observed in VTA DA neurons via adrenergic α1 receptor stimulation (Shi et al., 2000). To understand how methylphenidate normalizes the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals, we have conducted a series of pharmacological experiments. The results show that nomifensine, which has a high degree of selectivity for the DA transporters at the dose administered (1 mg/kg i.p.; Hunt et al., 1979) can restore the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals. This effect is similar to methylphenidate effect and supports that the normalization in the electrical activity of VTA DA neurons in prenatal ethanol-exposed animals is mediated through its effects on DA neurotransmission. We also have administered another specific DA transporter blocker, GBR 12909. The result shows that, similar to nomifensine and methylphenidate, GBR 12909 has little effect on the number of spontaneously active VTA DA neurons in control animals but leads to normalization in the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals. Furthermore, systemic administration of an α1 receptor blocker, prazosin, did not change the number of spontaneously active VTA DA neurons, indicating that there is no tonic excitatory adrenergic input to VTA DA neurons. Finally, the coadministration of methylphenidate and prazosin does not affect the ability of methylphenidate to restore the number of spontaneously active VTA DA neurons in prenatal ethanol-exposed animals. Taken together, these results indicate that the restoration of the number of spontaneously active VTA DA neurons by methylphenidate in prenatal ethanol-exposed animals is mediated by increasing extracellular levels of DA, rather than norepinephrine, which leads to hyperpolarization of VTA DA neurons and the removal of depolarization inactivation.

Different Pharmacological Profiles between Amphetamine and Methylphenidate. Systemic methylphenidate administration-induced hyperpolarization is also confirmed by the inhibitory effect on the firing rate of individual spontaneously active VTA DA neurons in both the control and prenatal ethanol-exposed animals. The result also shows that the dose-response curve of methylphenidate obtained from prenatal ethanol-exposed animals is shifted to the left. Methylphenidate has greater effects on individual spontaneously active VTA DA neurons in prenatal ethanol-exposed animals. This effect should be distinguished from the effect of methylphenidate in the cells-per-track experiments in which the averaged firing rate was calculated from all spontaneously active VTA DA neurons that had been “normalized” and fire action potentials. Therefore, the average firing rate in cells-per-track experiments does not reflect the “greater inhibitory effect” of methylphenidate as observed on individual spontaneously active VTA DA neurons.

This shift of dose-response curves of methylphenidate in prenatal ethanol-exposed animals is similar to the observation obtained from systemically administered amphetamine and could be caused by the supersensitivity in the somatodendritic DA autoreceptors demonstrated in other studies (Shen et al., 1999; Xu and Shen, 2001; Wang and Shen, 2002). Interestingly, unlike amphetamine (Xu and Shen, 2001), methylphenidate does not completely suppress the firing activity in individual spontaneously active VTA DA neurons in either control or prenatal ethanol-exposed animals. The maximum inhibition by methylphenidate is around 80% in both groups of animals. Amphetamine administration (1 mg/kg i.v.) also decreases the number of spontaneously active VTA DA neurons (Xu and Shen, 2001) in control animals, whereas methylphenidate administration as shown in the present study has no such effect. The lack of total inhibition in individual neurons by methylphenidate may explain why the number of spontaneously active VTA DA neurons does not change in control animals after methylphenidate treatment.
The failure to reach a total inhibition in individual VTA DA neurons by methylphenidate does not appear to be caused by insufficient doses. We have observed that methylphenidate at a cumulative dose as high as 16 mg/kg i.p. fails to shut down the firing activity of individual spontaneously active VTA DA neurons. Similarly, the administration of methylphenidate at a dose up to 10 mg/kg i.p. induces no change in the number of spontaneously active VTA DA neurons in control animals (data not shown).

The difference in pharmacological profiles of methylphenidate and amphetamine could be due to pharmacological differences between these two drugs. Methylphenidate is a DA reuptake blocker (Ritz et al., 1987). Therefore, its effect on DA neurotransmission in the VTA depends on the existing rate of DA release, which may not increase extracellular DA concentration sufficiently to shut down the firing activity of VTA DA neurons. Amphetamine, on the other hand, can block DA reuptake, promotes DA release in the VTA area, and may be able to lead to stronger inhibition in DA neurons (Zetterström et al., 1988). This assumption, however, is not supported by the result from the nomifensine and GBR 12909 experiments, in which we find that the number of spontaneously active VTA DA neurons in control animals is reduced, similar to that obtained with amphetamine administration. Nomifensine and GBR 12909 are specific DA reuptake blockers and yet their effect is more similar to amphetamine than to methylphenidate. Therefore, the differences in the effect of amphetamine and methylphenidate on DA neuron activity could be due to other factors dictating the in vivo effects of these drugs (e.g., differences in bioavailability, brain pharmacokinetics, binding to different sites, potency to block the dopamine transporter; Katz et al., 2000). Interestingly, some investigators have reported that nomifensine has the properties of a DA releaser (Hurd and Ungerstéd, 1989) and weak DA receptor agonist (Gianutsos et al., 1982) in addition to pure transporter blocker (e.g., methylphenidate). These properties could lead to more activation of the somatodendritic DA autoreceptors and inhibition in VTA DA neurons, which results in more reduction in VTA DA neuron activity than methylphenidate.

One of the important questions that remains to be answered is whether the normalization in basal spontaneous electrical activity by methylphenidate or other DA transporter blockers in prenatal ethanol-exposed animals can lead to full functional recovery in DA neurotransmission in the mesolimbic DA system. A recent report indicates that the spontaneous electrical activity of DA neurons responds to input modulation in distinctive patterns from various brain regions and modulates in different patterns of DA release in the terminal area (Floresco et al., 2003). It would be interesting to investigate in the future the possibility that DA agonist and transporter blocker administration in prenatal ethanol animals can result in normalized input modulation of VTA DA neuron activity and release of DA in the terminal areas.

Clinical Implications. The results of the present study also show that in prenatal ethanol-exposed animals, the reduction in the electrical activity of VTA DA neurons can be normalized by a low dose of methylphenidate (1 mg/kg). The low-dose requirement is due to the reversal of depolarization inactivation that is mediated by hyperpolarization of VTA DA neurons with the activation somatodendritic DA autoreceptors, which are far more sensitive than the postsynaptic DA receptors in DA terminal areas (Chiody, 1988). In control animals, this dose of methylphenidate does not lead to behavioral activation (McNamara et al., 1993), perhaps due to a lack of effect on the activity of DA neurons and extracellular DA levels in the terminal areas to activate postsynaptic DA receptors (Kuczenski and Segal, 2002). These results are consistent with the low-dose requirement of stimulants in treating attention deficit/hyperactivity disorder (ADHD) and may explain why, at least in some ADHD cases (Sachdev and Trollor, 2000), doses of methylphenidate insufficient for behavioral activation can treat attention problems.

The findings from the present study may have important clinical implications. The synthesis and release of DA is controlled by electrical activity of DA neurons. Reduced electrical activity in VTA DA neurons in prenatal ethanol-exposed animals could result in altered DA neurotransmission. Since abnormal DA neurotransmission has long been suggested as the underlying cause of ADHD problems (Denney, 2001), the reduced activity may contribute to the attention problems often observed in children with FASD (Nanson and Hiscock, 1990; Streissguth et al., 1994). Based on the observation that attention problems in children with FASD are different in nature from typical ADHD, the effect of standard stimulant therapy in treating attention problems in children with FASD has been questioned (Coles et al., 1997; O’Malley and Nanson, 2002). Nonetheless, methylphenidate has been shown to be effective in alleviating the attention problems in children with FASD (Oesterheld et al., 1998). The efficacy of methylphenidate is supported by the results from the present study demonstrating that methylphenidate can normalize the electrical activity of VTA DA neurons in animals with prenatal ethanol exposure. Furthermore, the unique pharmacological profile of methylphenidate among stimulants may render it to be a more “flexible” stimulant to treat attention problems in children with FASD.

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References

Address correspondence to: Dr. Roh-Yu Shen, Research Institute on Addictions, 1021 Main Street, Buffalo, NY 14203. Email: shen@ria.buffalo.edu