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Peripubertal Diazepam Administration Prevents the Emergence of Dopamine System Hyperresponsivity in the MAM Developmental Disruption Model of Schizophrenia

Yijuan Du*[,1] and Anthony A Grace[1,2,3]

[1]Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA; [2]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA; [3]Department of Psychology, University of Pittsburgh, Pittsburgh, PA, USA

Schizophrenia is believed to arise from an interaction of genetic predisposition and adverse environmental factors, with stress being a primary variable. We propose that alleviating anxiety produced in response to stress during a sensitive developmental period may circumvent the dopamine (DA) system alterations that may correspond to psychosis in adults. This was tested in a developmental rat model of schizophrenia based on prenatal administration of the mitotoxin methyl azoxymethanol acetate (MAM). MAM administration leads to a hyperdopaminergic state consisting of an increase in the number of DA neurons firing spontaneously, which correlates with an increased behavioral response to amphetamine. MAM-treated rats exhibited a heightened level of anxiety during adolescence. Peripubertal administration of the antianxiety agent diazepam was found to prevent the increase in DA neuron activity and blunt the behavioral hyperresponsivity to amphetamine in these rats. These data suggest that the pathophysiological factors leading to the onset of psychosis in early adulthood may be circumvented by controlling the response to stress during the peripubertal period.
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Keywords: schizophrenia; MAM; diazepam; dopamine; prevention

INTRODUCTION

Schizophrenia is a neurodevelopmental disorder that afflicts approximately 1% of the population worldwide. Although there is a genetic linkage in the heritability of schizophrenia, it is clear that genetic predisposition alone is insufficient to account for the onset of this disorder. Instead, several investigators have proposed a two-hit model of schizophrenia, based on a genetic predisposition plus an early life risk factors that lead to the onset of psychosis in late adolescence or early adulthood (Maynard *et al*, 2001). Several risk factors have been identified as related to schizophrenia; a major one that has shown a strong association with schizophrenia is stress (Walker and Diforio, 1997; Corcoran *et al*, 2003; Thompson *et al*, 2004). Thus, a number of retrospective studies reported that individuals with psychotic symptoms experienced more major stressful life events preceding the onset of psychosis (for review, see Phillips *et al*, 2007). Furthermore, stress sensitivity is likely associated with transition to psychosis. Owens *et al* (2005) reported that, in adolescents at high genetic risk for schizophrenia, those who showed a higher premorbid anxiety response to stress tended to be the same individuals who eventually converted to schizophrenia later in life. The impaired stress tolerance is associated with a range of prodromal symptoms and poor function over time (DeVylder *et al*, 2013) and may be predictive of the development of psychosis (Yung *et al*, 2005).

Previous studies have shown that rats exposed during gestational day (GD) 17 to the mitotoxin methyl azoxymethanol acetate (MAM) exhibit behavioral, pharmacological, and anatomical characteristics consistent with an animal model of schizophrenia (Grace *et al*, 1998; Flagstad *et al*, 2004; Moore *et al*, 2006; for review, see Lodge and Grace, 2009). Moreover, these rats exhibit increases in dopamine (DA) neuron population activity (ie, the proportion of spontaneously active DA neurons) that correlates with the enhanced locomotor response to amphetamine (Lodge and Grace, 2007). In addition, consistent with the onset of psychosis in schizophrenia patients, the emergence of hyperresponsiveness to amphetamine develops after puberty.

Stress is known to damage the hippocampus (Mondelli *et al*, 2010, 2011), a region commonly reported to be altered in postmortem (Benes, 1999) and structural imaging studies (Nelson *et al*, 1998) of schizophrenia and which is proposed to underlie the DA system overdrive in the MAM model of schizophrenia (Lodge and Grace, 2007). We have proposed previously that deficits in prefrontal cortical function could limit the ability of this structure to attenuate stress

*Correspondence: Y Du, Department of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, USA, Tel: +1 412 624 7332, Fax: +1 412 624 9198, E-mail: yid8@pitt.edu
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responses (Rosenkranz and Grace, 2001), leaving the susceptible individual vulnerable to the deleterious effects of stress (Thompson *et al*, 2004).

Given the evidence that stress and stress intolerance early in life may be a factor in the transition to schizophrenia in humans, we therefore hypothesize that attenuating the response to stress in the premorbid, peripubertal period may circumvent the process leading to a hyperdopaminergic state in the adult. We tested this hypothesis by administering an antianxiety drug diazepam across puberty and evaluated, in adult rats, the electrophysiological activity of DA neurons as well as the behavioral response to amphetamine.

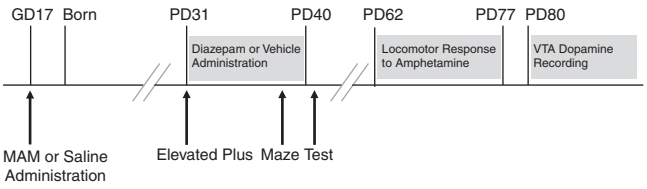

Figure 1 Experimental design. Pregnant rats were administered with MAM or saline on GD 17. The elevated plus maze test was performed on litters on either PD31 or PD38-41. Litters were further divided into four groups by 10 daily oral administration of diazepam or vehicle during PD31–PD40. These rats were used for electrophysiology or behavioral tests as adults.

MATERIALS AND METHODS

Animal

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* by the USPHS and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Pregnant Sprague-Dawley dams were obtained from Hilltop on GD 15 and administered the mitotoxin MAM (20 mg/kg, i.p. obtained from Midwest Research Institute, Kansas City, MO) or saline on GD 17. Litters were weaned on postnatal day 23 (P23) and housed in pairs. For the elevated plus maze test, 3 litters with 3–6 pups from each litter were used for each experimental group (Sal- *vs* MAM-treated rats), and 2litters with 2 pups from each litter were used for each experimental group (MAM:Veh *vs* MAM:DZ rats). For VTA DA neuron recordings, 5 litters with 1–3 pups from each litter were used for each experimental group. To assess locomotor responses to amphetamine, 6 litters with 1–4 pups from each litter were used for each experimental group.

Oral Administration of Diazepam

Diazepam (2 mg tablets, obtained from Watson Laboratories, Inc., Corona, CA) was ground to powder and mixed with sweetened condensed milk (Eagle Brand), sugar powder and ground mini Nile Wafers (Kraft Food). Approximately half of the pups from each litter were fed with this diazepam mixture across puberty, daily on 10 consecutive days (P31-40, 5 mg/kg); others were fed the same mixture without diazepam. The oral administration route was chosen because it is less stressful than i.p. injections and better mimics the preferred route of drug administration to patients. In addition, it allows paired housing that is beneficial for rats with minimal separation (time necessary to consume the wafers, usually <15 min) (Ferguson and Doctor, 2009). Male offspring were used for neurophysiology (P80-140) and locomotor tests (P62-77) as adults (Figure 1).

In vivo Recording from VTA DA Neurons

In vivo extracellular recordings were performed with investigators blinded to treatment. Rats were anesthetized with chloral hydrate and mounted on a stereotaxic frame (Kopf, Tujunga, CA). The body temperature was maintained at 37 °C using a thermostatically controlled feedback heating pad (Fintronics, New Haven, CT). A burr hole was drilled in the skull overlying the right VTA. Extracellular recording microelectrodes were pulled from Omegadot 2.0 mm glass tubing on a Narishige P-5 vertical electrode puller, the tip broken back under microscopic control, and filled with 2 M NaCl containing 2% Pontamine Sky Blue dye. The impedance of the electrodes *in situ* ranged from 6 to 15 MΩ. The stereotaxic coordinates for the VTA were 5.3 mm posterior from bregma, 0.8 mm lateral to the midline, and 6.0–9.5 mm ventral from the brain surface. Single-unit activity was filtered using a highpass filter at 30 Hz and lowpass at 10 kHz. All data analysis was performed using custom software (Neuroscope). Only neuronal activity with a signal-to-noise ratio greater than 3 : 1 and at least 3 min of stable spontaneous activity were used.

Six to nine vertical tracks, separated by 200 μm, were sampled in a predetermined pattern within the VTA of each rat. DA neurons were identified according to well-established electrophysiological features (Grace and Bunney, 1983; Ungless and Grace, 2012), which included the following criteria: (1) an action potential duration >2.2 ms; (2) slow firing rate (1–10 Hz); and (3) irregular and burst firing patterns (the start of burst characterized by inter-spike interval <80 ms, and the end of burst characterized by inter-spike interval >160 ms). The activity of each identified DA neuron was recorded for at least 3 min. Three parameters of the population activity were analyzed: (1) the number of spontaneously active DA neurons per electrode track, (2) average firing rate and (3) the percentage of spikes that occurred in bursts (%SIB).

At the end of recordings, the recording site was marked via electrophoretic ejection of Potamine Sky Blue dye from the tip of the electrode (20 μA constant negative current, 30 min). Rats were euthanized by an overdose of anesthetic; the brains were taken out, fixed for at least 48 h in 8% paraformaldehyde, cryoprotected in 25% sucrose, and sectioned for histological confirmation of the electrode sites.

Locomotor Response to Amphetamine

Adult rats were tested in an open-field chamber (Coulbourn Instruments, Allentown, PA) in which locomotor activity was determined by beam breaks and recorded with TruScan software (Coulbourn Instruments). All experiments were conducted at the same time of each day. Spontaneous activity was recorded for 30 min. After that, rats were

injected with D-amphetamine sulfate (0.5 mg/kg, i.p.) and their locomotor activity was recorded for another 90 min.

Elevated Plus Maze

Two groups of rats at PD31 and PD38–PD41 were run in the elevated plus maze. On PD38-41, diazepam (5 mg/kg) was given to rats orally 90 min before the test. The apparatus had four elevated arms (50 cm above the floor), 50 cm long and 10 cm wide, arranged in a cross-like pattern, with two opposite arms enclosed by 40 cm high opaque walls, two open with a lip (1 mm thick and 5 mm high) and a central platform at their intersection ($10 \times 10\,cm^2$) that permitted access to any of the four arms. Rats were handled for 3 consecutive days and habituated to the testing room 1 day before the test. Each rat was placed on the central platform facing an open arm, and the behavior was recorded for 5 min. The floor of the apparatus was cleaned between rats. The time spent in the open arms relative to that in the closed arms was used as an index of anxiety-like behavior.

Statistics and Analysis

Electrophysiological analysis of DA neuron activity was performed by custom-designed software (Neuroexplorer). Two-way ANOVA (MAM × diazepam) followed by Bonferroni *post hoc* test was used for comparison of DA neuron population activity. Locomotor activity was analyzed by TruScan software and compared using repeated measures three-way ANOVA (time as a within-subject factor, MAM and diazepam treatment as between-subject factors), followed by Bonferroni *post hoc* test. Activity of MAM and saline rats on elevated plus maze was compared by Mann–Whitney rank sum test. All statistics were calculated using SigmaPlot (Systat Software, San Jose, CA) or SPSS statistics (IBM Corporation, Armonk, NY). All data are represented as the mean ± SEM.

RESULTS

MAM Rats Exhibited a Higher Anxiety Level During Adolescence that was Reversed by Diazepam Treatment

The elevated plus maze test was performed on a group of rats at PD31 before the administration of diazepam. Compared with rats with prenatal saline treatment, MAM-treated rats spent significantly less time on open arms relative to closed arms (Figure 2a; $p<0.05$), indicating a higher level of anxiety. This is consistent with the higher level of anxiety reported for human adolescents at high risk for schizophrenia (Owens *et al*, 2005; Yung *et al*, 2005). Administration of diazepam acutely to MAM-treated rats at PD38-41, at a time point approximating the end of the diazepam treatment phase, effectively reversed the increased anxiety level in the elevated plus maze. Thus, MAM rats with diazepam administration spent significantly more time on open arms relative to closed arms (Figure 2b), and made significantly more open arm entries (0.75 ± 0.11) relative to closed arms compared with vehicle-treated MAM rats (0.28 ± 0.08; MAM:Veh *vs* MAM:DZ, $p<0.05$, *t*-test).

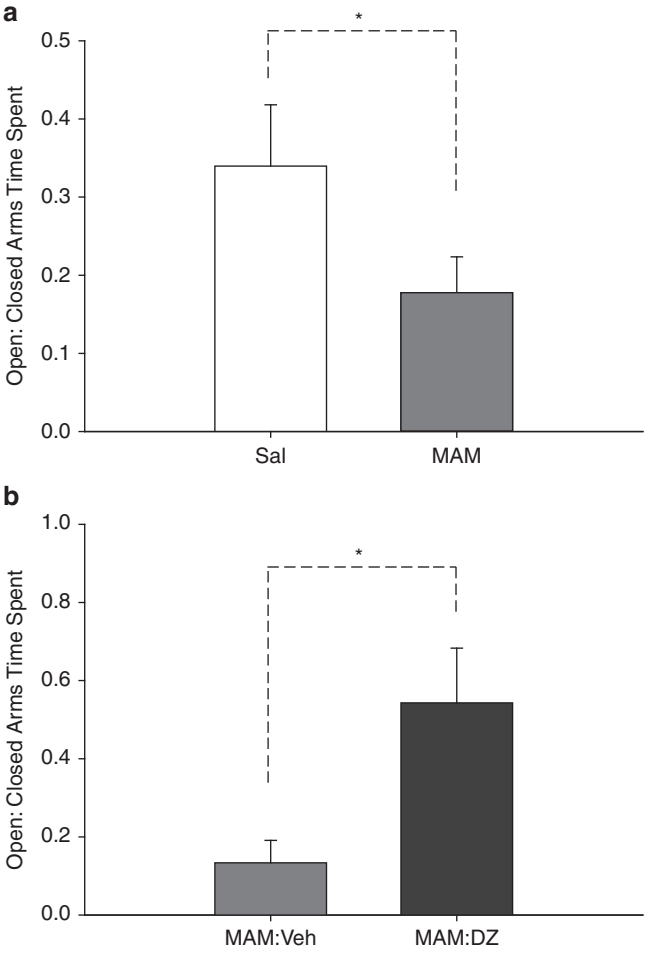

Figure 2 MAM rats exhibited a significantly higher level of anxiety compared with saline rats during adolescence, which was reversed by diazepam administration. (a) Adolescent (PD31) MAM rats ($n=14$) spent significantly less time on open arms relative to closed arms in the elevated plus maze compared with saline rats ($n=12$) (Mann–Whitney rank sum test, $p<0.05$). (b) Adolescent (PD38-41) MAM rats that were given diazepam acutely 90 min before testing (MAM:DZ, $n=4$) spent significantly more time on open arms relative to closed arms, compared with those treated with vehicle (MAM:Veh, $n=4$).

Diazepam Administration During Adolescence Did Not Affect Normal Weight Gain

The rats, measured on PD31 (first day of diazepam administration), PD35, and PD40 (last day of administration), showed a normal increase in body weight with age, independent of prenatal treatment (MAM or saline) or peripubertal drug administration (diazepam or vehicle) (Table 1). Only age produced a main effect on body weight ($F_{2,70}=2327.4$, $p<0.001$; repeated measures three-way ANOVA, age as a within-subject factor, MAM and diazepam treatment as between-subject factors).

Peripubertal Diazepam Administration Prevented VTA DA Hyperactivity in MAM-Treated Rats

Consistent with what has been reported previously (Lodge and Grace, 2007; Gill *et al*, 2011), Sal:Veh ($n=7$ rats, 63

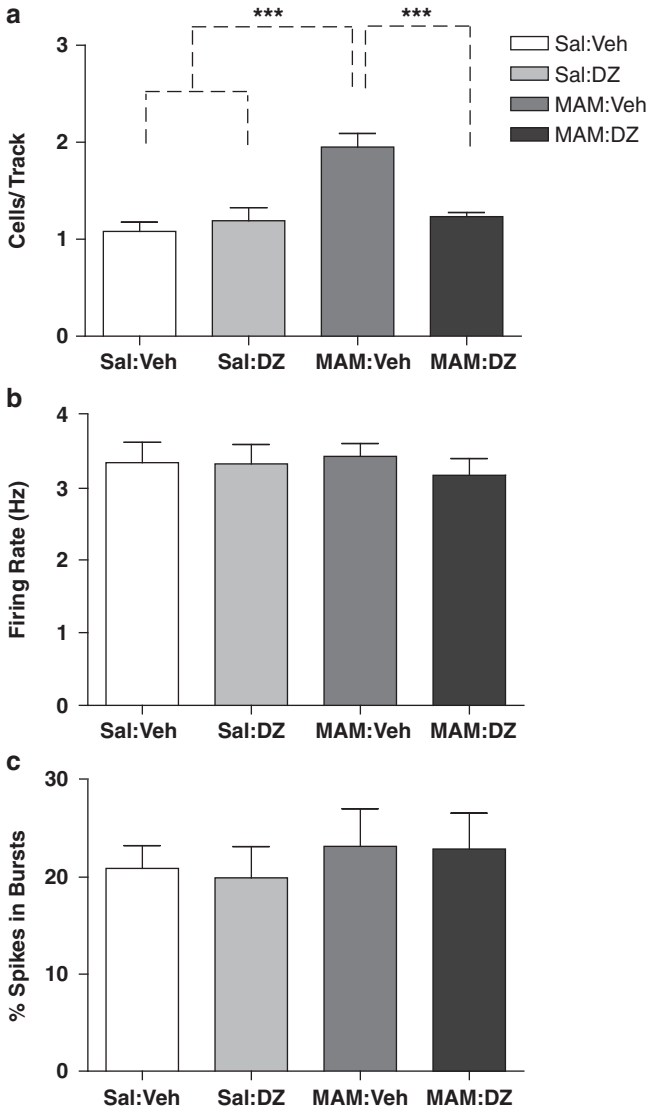

Figure 3 Peripubertal diazepam treatment (5 mg/kg, oral; daily, PD31–PD40) prevented the pathological increase in the number of spontaneously active dopamine (DA) neurons (presented as cells/track) in MAM-treated animals. (a) MAM:Veh ($n=7$) rats had a significantly higher number of DA neurons firing per electrode track compared with MAM:DZ ($n=7$), Sal:Veh ($n=7$), and Sal:DZ rats ($n=7$; Bonferroni *post hot* test). In contrast, diazepam treatment did not significantly alter the number of DA neurons firing in saline rats (Sal:Veh *vs* Sal:DZ rats, $p>0.05$). (b) Average firing rate and (c) percentage of spikes fired in bursts were not significantly different. ***$p<0.001$.

neurons) rats demonstrated an average of 1.1 ± 0.1 spontaneously active DA neurons per electrode track, an average firing rate of 3.3 ± 0.3 Hz, and $20.9 \pm 2.3\%$ of spikes fired in bursts (Figure 3a–c). Compared with Sal:Veh rats, recordings from MAM:Veh rats ($n=7$ rats, 120 neurons) showed a significantly greater number of spontaneously active DA neurons per electrode track (1.9 ± 0.1 cells/track, $p<0.001$), with no significant difference in average firing rate (3.4 ± 0.2 Hz) or %SIB ($23.0 \pm 3.9\%$).

The number of spontaneously active DA neurons were significantly affected by prenatal MAM ($F_{1,24}=16.3$, $p<0.001$), peripubertal diazepam administration ($F_{1,24}=7.2$, $p<0.05$), and their interaction ($F_{1,24}=13.4$, $p<0.01$; two-way ANOVA MAM × diazepam). Compared with MAM:Veh rats, MAM:DZ rats ($n=7$ rats, 72 neurons) showed significantly fewer spontaneously active DA neurons (1.2 ± 0.04 cells/track; Bonferroni *post hoc* test). Furthermore, the numbers of DA neurons in the MAM:DZ *vs* the Sal:Veh rats were not significantly different ($p>0.05$). In contrast, peripubertal diazepam treatment did not have a significant effect in saline-pretreated animals. Sal:DZ rats ($n=7$ rats, 64 neurons) showed an average of 1.2 ± 0.1 cells/track, which was not significantly different from Sal:Veh rats ($t=0.7$, $p>0.05$). The firing rate (3.2 ± 0.2 Hz in MAM:DZ and 3.3 ± 0.3 Hz in Sal:DZ rats) and %SIB ($22.9 \pm 3.7\%$ in MAM:DZ and $20.0 \pm 3.2\%$ in Sal:DZ rats) did not differ significantly across all four groups ($p>0.05$; two-way ANOVA).

Peripubertal Diazepam Administration Prevented the Enhanced Response to Amphetamine in MAM-Treated Rats

Previous studies have shown that rats treated with MAM on GD 17 exhibited an enhanced locomotor response to amphetamine (Flagstad *et al*, 2004; Moore *et al*, 2006; Lodge and Grace, 2007). Consistent with those studies, MAM:Veh ($n=10$) rats showed significantly higher levels of locomotor activity in response to amphetamine administration (0.5 mg/kg i.p.) compared with Sal:Veh rats (Figure 4a; $p<0.001$, Bonferroni *post hoc* test followed by repeated measures two-way ANOVA, $F_{1.36}=11.8$, $p<0.001$). In contrast, MAM:DZ rats ($n=10$) showed a significantly lower level of amphetamine-stimulated locomotion compared with MAM:Veh rats ($p<0.05$), and were not significantly different from Sal:Veh rats ($p>0.05$). Furthermore, peripubertal diazepam treatment in saline rats did not produce a significant alteration in amphetamine-stimulated locomotion (Sal:Veh *vs* Sal:DZ rats, $n=9$, $p>0.05$). The total movement distance after amphetamine administration revealed a similar result (Figure 4b), which was significantly affected by MAM ($F_{1,36}=21.5$, $p<0.001$) and the interaction of MAM and diazepam treatment ($F_{1,36}=10.9$, $p<0.01$). MAM:Veh rats showed a significantly higher total movement distance compared with MAM:DZ ($p<0.05$), Sal:Veh ($p<0.001$), and Sal:DZ rats ($p<0.01$). The spontaneous activity in a novel environment did not differ significantly among all four groups.

DISCUSSION

Although antipsychotic drugs have revolutionized the treatment of schizophrenia, in actuality this mode of treatment suffers from a low efficacy, the potential for producing untoward side effects, and the psychosis-induced alterations in brain function that may not be readily reversible pharmacologically (Lieberman *et al*, 2005). Moreover, studies have shown that the duration of untreated schizophrenia correlates with a worsened prognosis in patients (Hill *et al*, 2012). Therefore, a more effective approach to schizophrenia may be one of prevention. In this study, we examined an approach that we propose may be an effective method to prevent the transition to psychosis in susceptible individuals.

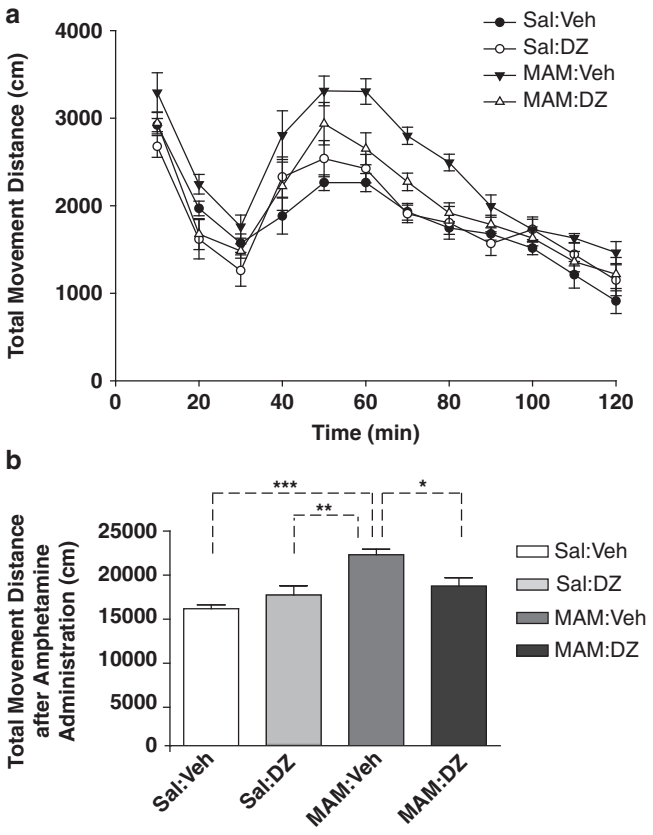

Figure 4 Rats treated peripubertally with diazepam (5 mg/kg, oral; daily PD31–PD40) showed an attenuation of the aberrant enhancement of the locomotor response to D-amphetamine (0.5 mg/kg, i.p.) that was observed in MAM-treated rats. (a) MAM:Veh ($n=10$) rats showed significantly higher amphetamine-induced locomotion compared with MAM:DZ ($n=10$), Sal:Veh ($n=11$), and Sal:DZ ($n=9$) rats. In contrast, MAM:DZ, Sal:Veh, and Sal:DZ rats were not significantly different. Repeated measures of three-way ANOVA revealed a significant effect of time ($F_{8,288}=67.8$, $p<0.001$), MAM treatment ($F_{1,36}=22.0$, $p<0.001$), and the interaction of MAM and diazepam treatment ($F_{1,36}=10.6$, $p<0.01$). Locomotor activity was calculated within each bin (bin width = 10 min). D-amphetamine injection is indicated by the dashed line. (b) MAM:Veh rats showed significantly higher locomotor activity than MAM:DZ, Sal:Veh, and Sal:DZ rats. *$p<0.05$; **$p<0.01$; ***$p<0.001$. Spontaneous activity before amphetamine injection did not show significant difference among all four groups.

Stress Sensitivity in Adolescence is Associated with Schizophrenia in Humans

Stress is known to be a risk factor in schizophrenia (Walker and Diforio, 1997; Corcoran *et al*, 2003; Phillips *et al*, 2007) and is correlated with the propensity of at-risk individuals who undergo transition to psychosis (Holtzman *et al*, 2012). Adolescents that convert to psychosis in later life showed higher sensitivity and intolerance to stress, a heightened anxiety level and a higher cortisol level (Owens *et al*, 2005; Yung *et al*, 2005; Walker *et al*, 2010; Corcoran *et al*, 2012; Devylder *et al*, 2013).

As for the biological response to stress, some studies (Walker *et al*, 2010) showed that baseline cortisol levels in adolescents with schizotypal symptoms predict severity of their schizotypal symptoms later in life. In addition, there were reports of an enlarged hypothalamus (Goldstein *et al*, 2007) and pituitary gland (Garner *et al*, 2005; Habets *et al*, 2012) in schizophrenia patients and nonpsychotic relatives, which is consistent with sustained stress. Moreover, a larger baseline pituitary volume was a significant predictor of future transition to psychosis within the ultra-high-risk group (Garner *et al*, 2005).

Stress Sensitivity in MAM Rats

Previous studies from our group showed that adult MAM rats are more vulnerable to stress exposure (Goto and Grace, 2006). In the current study, rats were not exposed to additional external stressors during the peripubertal period. Nonetheless, the fact that MAM rats showed high baseline anxiety levels peripubertally as assessed by the elevated plus maze suggests that even normal levels of stress (eg, use of wire-bottom caging, cage cleaning, transport and handling, etc) may have been sufficient to result in increased measures of anxiety as assessed by the elevated plus maze test. Whether the anxiety was endogenous or was due to a decreased tolerance to low-level stressors, administration of diazepam reversed the increased anxiety level observed in the MAM rats. This heightened anxiety during adolescence is proposed to contribute to the dopaminergic hyperresponsivity that occurs in the adult.

Stress Sensitivity as a Potential Target for Early Intervention

There is substantial evidence to suggest that many of the biological and social changes underpinning the development of schizophrenia may already be active in the pre-psychotic or prodromal phase. Indeed, the pre-psychotic phase may be the most sensitive part of the 'critical period' for preventive efforts (Phillips *et al*, 2002). To circumvent the effects of stress, we chose to use a potent antianxiety agent, diazepam. When administered at adolescence, diazepam significantly decreased the elevated anxiety of adolescent MAM rats as assessed by the elevated plus maze. By relieving anxiety and stress during the pre-psychotic period, we propose that diazepam may prevent the conversion to psychosis. Thus, in MAM rats peripubertal administration of diazepam was found to prevent the pathological increase in dopaminergic activity that is proposed to underlie psychosis in schizophrenia (Laruelle *et al*, 1999). In this manuscript, we focused on the antianxiety effects of diazepam; however, given the known disruption of the GABA system in MAM rats (Lodge *et al*, 2009) and in schizophrenia (Zhang and Reynolds, 2002; Lewis *et al*, 2012), part of the actions may be a restoration of GABA balance that may underlie schizophrenia. Indeed, administration of a selective $GABA_A$ alpha 5 benzodiazepine-like drug was found to reverse the hyperdopaminergic state in adult MAM rats (Gill *et al*, 2011).

Although the peripubertal administration of diazepam was found to be effective in circumventing the markers of hyperdopaminergic function in rodents, it is likely that other stress-relieving interventions will also be effective. Thus, administration of antipsychotic drugs to adolescent rats in the maternal immune activation model of schizophrenia has been shown to circumvent the emergence

Table 1 Body Weight (g) on PD31 (Before Diazepam Administration), PD35, and PD40 (end of administration) of MAM- or Saline-Treated Offspring with Peripubertal Diazepam (5 mg/kg, oral; daily, PD31–40) or Vehicle Administration ($n = 9$–11)

Groups	Prenatal treatment	Peripubertal treatment	PD31 (first diazepam administration)	PD35	PD40 (end of administration)
			Mean ± SEM body weight (g)		
Sal:Veh	Saline	Vehicle	92.5 ± 3.2	124.9 ± 4.3	170.6 ± 4.9
Sal:DZ	Saline	Diazepam	92.2 ± 7.1	121.1 ± 9.0	165.7 ± 10.7
MAM:Veh	MAM	Vehicle	87.8 ± 5.3	116.3 ± 7.9	163.6 ± 7.3
MAM:DZ	MAM	Diazepam	85.0 ± 4.6	115.8 ± 5.7	157.9 ± 7.5

Repeated measures three-way ANOVA (MAM and diazepam as between-subject factors, and age as a within-subject factor) yielded only significant effects of age ($F_{2,70} = 2327.4$, $p < 0.001$), but not of MAM ($F_{1,35} = 1.0$, $p > 0.05$), diazepam treatment ($F_{1,35} = 0.2$, $p > 0.05$), or their interaction ($F_{1,35} = 0.0$).

of several correlates of schizophrenia in adult rats (Pointkewitz *et al*, 2011, 2012). While it is unclear which of several potential mechanisms may be involved, it is well known that antipsychotic drugs do attenuate the cortisol response in schizophrenia patients (Walker *et al*, 2008), which would be consistent with our studies of diazepam. In addition, antidepressants, which also have anxiolytic properties, have been reported to be beneficial to treatment of prodromal schizophrenia in adolescents (Cornblatt *et al*, 2007). We propose that controlling the effects of stress in at-risk individuals during the prodromal period may be an effective means to prevent the transition to schizophrenia later in life.

One issue that this raises is who should be given the treatment? Individuals that are at risk for schizophrenia can be identified based on genetic background and family history (Sullivan *et al*, 2003; Straub and Weinberger, 2006). Structured interviews for evaluating psychosis risk such as the Structured Interview for Psychosis Risk Syndromes (Miller *et al*, 2003) and the Comprehensive Assessment of At-Risk Mental States (Yung *et al*, 2005) also contribute substantially toward creating a reliable and valid system for identifying risk before psychosis onset. Moreover, studies show that at-risk individuals who undergo transition to psychosis often show increased stress responsivity in childhood and adolescence (Owens *et al*, 2005; Yung *et al*, 2005; Corcoran *et al*, 2012; Devylder *et al*, 2013). Given these data, we propose that individuals that are at risk for schizophrenia could be tested for their response to stress, and simply treat the stress hyperreactivity. Both pharmacological and non-pharmacological interventions that are effective at reducing stress, drawing from our data, may be effective means to prevent the eventual transition to psychosis later in life.

Mechanisms Underlying the Effects of Stress on DA Hyperactivity

Our previous studies showed that the increase in DA neuron activity was due to hyperactivity in the ventral hippocampus, because pharmacological inactivation of this structure (Lodge and Grace, 2007) or administration of a hippocampal-selective $GABA_A$ alpha 5-positive allosteric modulator (Gill *et al*, 2011) could reverse this increase in DA neuron population activity as well as the augmented increase in locomotor response to amphetamine. These data are also consistent with clinical results showing hyperactivity in the limbic hippocampus that correlated with psychosis (Malaspina *et al*, 1999; Medoff *et al*, 2001; Molina *et al*, 2003), an increase in amphetamine-induced DA release in schizophrenia that correlated with exacerbation of psychosis (Laruelle *et al*, 1999), and in ultra-high-risk individual alterations in hippocampal glutamate levels that occurred in concert with increased presynaptic indices of dopaminergic function (ie, fluorodopa uptake; Schobel *et al*, 2009; Stone *et al*, 2010; Howes *et al*, 2011). Taken together, these data are consistent with a model in which hippocampal hyperactivity leads to increases in DA neuron population activity, rendering the system hyperresponsive to stimuli (Lodge and Grace, 2007). The hippocampal hyperactivity is proposed to be due to a loss of parvalbumin interneurons in the MAM model (Penschuck *et al*, 2006; Lodge *et al*, 2009), which is consistent with postmortem observations in schizophrenia brains (Zhang and Reynolds, 2002).

We propose that the loss of parvalbumin interneurons occurs secondary to stress-induced damage of the hippocampus. Stress has been shown to lead to hippocampal atrophy (Magarinos and McEwen, 1995; Lupien *et al*, 1998; Conrad *et al*, 1999). Moreover, studies have shown that pharmacological activation of the amygdala, a region known to be involved in stress and anxiety, will lead to decreases in hippocampal parvalbumin neuron number (Barretta *et al*, 2001).

Altogether, our findings indicate that treating the deleterious effects of stress, which may be magnified in at-risk individuals, during the peripubertal period may circumvent the cascade of events that leads to the emergence of psychosis in the adult (Table 1).

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DISCLOSURE

Over the past 3 years, AAG has received compensation from Johnson & Johnson, Lundbeck, Pfizer, GSK, Puretech Ventures, Merck, Takeda, Dainippon Sumitomo, Otsuka, Lilly, Roche, and Asubio. YD declares no conflict of interest.

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