Published in final edited form as:

Behav Brain Res. 2019 April 19; 362: 258–265. doi:10.1016/j.bbr.2019.01.031.

Effect of estrous cycle on schizophrenia-like behaviors in MAM exposed rats

Stephanie M. Perez^{#a}, Jennifer J. Donegan^{#a}, and Danie J. Lodge^a

^aUT Health San Antonio, Department of Pharmacology, Center for Biomedical Neuroscience, 7703 Floyd Curl Drive, MC7764, San Antonio, TX, 78229, USA

[#] These authors contributed equally to this work.

Abstract

Although there are clear sex differences in individuals with schizophrenia, preclinical research has historically favored the use of male rats for behavioral studies. The methylazoxymethanol acetate (MAM) model is a gestational disruption model of schizophrenia and has been reported to produce robust behavioral, neurophysiological and anatomical alterations in male rats; however, whether similar effects are observed in female rats is less well known. In this study, we characterize the behavioral, electrophysiological and molecular alterations induced by prenatal MAM administration in female rats while also examining the potential effects of the estrous cycle on schizophrenia-like behaviors. Specifically, MAM-treated female offspring demonstrated deficits in sensorimotor gating, latent inhibition, and social interaction, consistent with those observed in male animals. Interestingly, amphetamine-induced locomotor activity, latent inhibition, and social interaction were also affected by the estrous cycle. To examine the potential cellular mechanisms associated with these behavioral alterations, we analyzed hippocampal parvalbumin (PV) interneurons. Deficits in PV interneuron number and high-frequency gamma oscillations were disrupted in female MAM-treated rats regardless of the stage of the estrous cycle; however, alterations in PV protein expression were more prominent during metestrus/diestrus. Taken together, these data suggest that prenatal MAM exposure in female rats produces robust behavioral, molecular, and physiological deficits consistent with those observed in the male MAM model of schizophrenia. Moreover, our results also suggest that specific schizophrenia-like symptoms can also be influenced by the estrous cycle, and further emphasize the importance of sex as a biological variable when using preclinical models.

Keywords

Schizophrenia; MAM; hippocampus; estrous cycle; parvalbumin

Corresponding Author: Jennifer Donegan, 7703 Floyd Curl Drive, MC7764, San Antonio, TX, 78229, USA, Phone: +1 210-567-4273, donegan@uthscsa.edu.

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1. Introduction

Schizophrenia is a psychiatric disorder that affects both male and female populations; however, sex differences have been observed and reported. A robust and consistent difference between sex is the age of onset, as men develop the disease at an earlier age when compared to women, early 20's versus mid-to-late 20's respectively [1–3]. Interestingly, a subset of women experience illness onset during their mid-to-late 40's, an effect not typically observed in men [2, 4, 5]. Additionally, clinical and pre-clinical studies provide evidence of a hormonal component to schizophrenia [2, 6], thus suggesting that the second peak in disease onset observed in female patients may be due to the drop in circulating hormones experienced during menopause [2]. Other reported sex differences include a greater degree of premorbid deficits in men [7] and distinct differences in symptom severity [i.e. female patients experience a higher severity of positive symptoms, while males exhibit enhanced negative and cognitive dysfunction [2, 8, 9]. Because of these sex differences, and a potential hormonal component to the disease, a better understanding of the neurobiology underlying these sex variations is essential to developing more targeted therapies specific for both sexes.

In order to study sex related differences, we used a gestational disruption model, whereby methylazoxymethanol acetate (MAM) is administered to pregnant dams on gestational day (GD) 17. Male MAM-treated rats have been extensively studied and display a multitude of deficits consistent with those observed in individuals with schizophrenia [10, 11]. Subsequently, MAM-treated female rats have been studied to a lesser extent and display sensorimotor gating deficits [11–13], an enhanced locomotor response to amphetamine (a correlate of positive symptoms) [14, 15], and deficits in social interaction (a correlate of negative symptoms) [12, 13, 16]. In contrast, MAM-induced alterations in cognition and latent inhibition have only been observed in male rats [11, 17, 18]. It is important to note that the studies detailed above did not account for changes in circulating hormone levels that occur during the rodent (and human) reproductive cycle, and are known to influence neuronal activity and behavior.

We have previously demonstrated that female MAM-treated rats display an augmented dopamine system function that is dependent on the stage of the estrous cycle [6]. Specifically, female MAM-treated rats exhibit an increase in ventral tegmental area (VTA) dopamine neuron population activity during estrus, and this is driven by progesterone signaling in the ventral hippocampus (vHipp) [6]. This aberrant VTA dopamine neuron population activity, has been consistently shown to be driven by hyperactivity in the vHipp in male rats [15, 16, 19] and attributable to a decrease in inhibitory interneuron function [16, 19, 20]. Decreases in interneurons containing the calcium binding protein parvalbumin (PV), in the vHipp, are observed in both the MAM model [10] and in individuals with schizophrenia [21–23]. Further, restoring vHipp interneuron function can normalize dopamine neuron function and ameliorate behavioral deficits in male MAM-treated rats [16, 19]. Moreover, gamma oscillations (30–120 Hz), which are dependent on the activity of PV interneurons, have been shown to fluctuate depending on the stage of the estrous cycle, such that there is an increase in vHipp gamma during estrus [24]. Taken together, it is likely that the effects of MAM treatment in female rats may be altered by hormonal fluctuations across

the estrous cycle. Here, we evaluate specific behavioral, electrophysiological and molecular alterations induced by MAM in female rats throughout their reproductive cycle.

2. Materials and Methods

All experiments were performed in accordance with the guidelines outlined in the USPH Guide for the Care and Use of Laboratory Animals and were approached by the Institutional Animal Care and the Use Committee of UT Health San Antonio.

2.1 Animals

MAM treatments were performed as previously described [11, 25]. In brief, timed pregnant female Sprague Dawley rats (Envigo; Indianapolis, IN, USA) were obtained on gestational day (GD) 16. Methylazoxymethanol acetate (MAM; 22 mg/kg, *i.p.*) administration was performed on GD17. Injections of saline (1 ml/kg, *i.p.*) were administered to control dams. Female pups (MAM and saline controls) were weaned on postnatal day 21 and housed with littermates in groups of 2-3 until adulthood (>postnatal day 60). All experiments were performed on multiple litters of female MAM- and saline-treated rats. For approximately 2 weeks prior to behavioral testing and electrophysiological recordings, the stage of the estrous cycle was determined daily by vaginal cytology (as previously reported [6, 26]) and rats were separated into two groups: metestrus/diestrus (met/di) and proestrus/estrus (pro/est).

2.2 Amphetamine-Induced Locomotor Response

Female MAM- and saline-treated rats were placed in an open field arena (Med Associates, VT, USA) and spontaneous locomotor activity in the *x-y* plane was determined for 30 minutes by beam breaks and recorded with Open Field Activity software (Med Associates). Following a 30 minute baseline recording, all rats were injected with increasing doses of D-amphetamine sulfate (0.5mg/kg and 2.0mg/kg, *i.p.*). Locomotor activity was recorded for an additional 30 minutes immediately following each dose. Locomotor data were analyzed by three separate three-way ANOVA's (prenatal treatment $\times$ estrous cycle stage $\times$ time), one for each of the relevant time periods (baseline, 0.5mg/kg and 2.0mg/kg) followed by a Holm-Sidak post-hoc test.

2.3 Pre-Pulse Inhibition of Startle (PPI)

Female MAM- and saline-treated rats were placed in a sound attenuated chamber (SD Instruments, San Diego, CA, USA) and allowed to acclimate to 65dB background noise for 5 minutes. Rats were then exposed to ten startle-only trials [40ms, 120dB, 15s average inter-trial intervals (ITI)]. Next, rats were exposed to 24 trials where a pre-pulse (20ms at 69dB, 73dB and 81dB) was presented 100ms before the startle pulse. Each pre-pulse + startle pulse and startle pulse only was presented 6 times in a pseudorandom order (15s average ITI). The startle response was measured from 10-80ms after the onset of the startle only pulse and recorded using SR-LAB Analysis Software (SD Instruments).

2.4 Latent Inhibition (LI)

Female MAM- and saline-treated rats were placed into a $30.5 \times 25.4 \times 30.5 \text{ cm}^3$ square conditioning chamber with metal walls and a stainless steel grid shock floor (Coulbourn Instruments, Whitehall, PA, USA). Rats were randomly assigned into two groups: tone pre-exposed and non-exposed. Pre-exposed rats were presented with a 20 second tone over 16 trials with a pseudo-random ITI averaging 2 minutes. Immediately following tone exposure, rats underwent an established fear conditioning procedure in which a 20 second tone co-terminated with a mild (0.8 mA, 0.5 sec) foot shock. The tone-shock pairing was presented 4 times with a pseudo-random ITI averaging 2 minutes. Twenty-four hours after conditioning, rats were returned to the conditioning chamber and re-exposed to the 20 second tone over 4 trials. Behavior was video recorded at each stage and freezing behavior was analyzed off-line using FreezeFrame and FreezeView software (Coulbourn Instruments). Freezing was defined as behavior below a motion index threshold of 10 lasting at least 1 second.

2.5 Social Interaction (SI)

SI was performed as described previously [27]. Briefly, rats were placed individually in a testing arena ($100 \times 100 \times 40 \text{ cm}$) for 5 minutes per day for 2 days prior to testing. On the test day, experimental rats were placed in the arena with a weight-matched “stimulus” rat. The 5 minute test was recorded by video camera for offline analysis by a blind experimenter. The dependent measure was time the test animal spent actively engaged in social interaction (i.e. sniffing, climbing on, following, grooming, or wrestling) with the stimulus rat.

2.6 Elevated Plus Maze (EPM)

The EPM procedure was adapted from [28]. The apparatus (AccuScan Instruments Inc., Columbus, OH, USA) was made of four plastic arms ($10 \times 50 \text{ cm}$), in the shape of a plus sign with a $10 \times 10 \text{ cm}$ central platform elevated 75cm from the floor. The two opposing “closed arms” were enclosed by 48cm tall walls. The two “open arms” had no walls but were fitted with a 0.5cm plastic rim to prevent animals from falling off. To begin the 5 minute test, rats were placed on the center platform facing the junction between an open and closed arm. The test was video recorded and analyzed by ANY-maze software (Stoelting, Kiel, WI, USA). The following data was collected from each trial: number of entries and time spent in each the central platform, open arms, and closed arms. From this data, the open / total ratios (OTR) for both time and entries, defined as the proportion of open arm exploration relative to total exploration in all arms (open / open + closed), were calculated.

2.7 Western Blot

PV expression in the vHipp was determined by western blot. Rats were anesthetized with 8% chloral hydrate (400mg/kg, *i.p.*) and killed by rapid decapitation. The vHipp was dissected on ice and homogenized in ice-cold homogenization buffer (50mM Tris-HCl, 150mM NaCl, 0.1% Triton X-100 and pH 7.4) containing a protease inhibitor cocktail (Sigma-Aldrich). Homogenates were centrifuged at 14,000 r.p.m. for 10 minutes and supernatant containing protein fractions was analyzed for PV and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression levels. Proteins (20µg per well) were separated (30-45 minutes at 200V) on Mini-Protean TGX Any kD gels (Bio-Rad) in Tris/

glycine/SDS buffer (Bio-Rad) and subsequently transferred to nitrocellulose/filter paper sandwiches, 0.2µm (Bio-Rad) with transfer buffer (25mM Tris-HCl, 192mM glycine, 20% w/v ethanol, pH 8.3) for one hour at 100V. Membranes were then blocked for 30 minutes (PV: 2% milk in TBST; GAPDH: 5% BSA in TBST; 15mM Tris-HCl, 137mM NaCl and 0.1% Tween 20) before incubation with primary antibody (anti-PV 1:5000; anti-GAPDH 1:1000 for 1 hour at 4°C). Next, membranes were washed three times in TBST (10 minutes each), followed by incubation in secondary antibodies (1:5000) at room temperature (1 hour; PV: goat anti-rabbit conjugated to HRP; GAPDH: goat anti-mouse conjugated to HRP). Lastly, membranes were treated with Pierce ECL Western Blotting substrate (1 minute) and exposed to high-performance chemiluminescence film (Amersham Hyperfilm ECL). Membranes were then washed three times in TBST (10 minutes) and stripped with Restore Western Blot Stripping Buffer (15 minutes; Thermo Fisher Scientific) following probing with PV to allow for re-probing with GAPDH. Western blot films were scanned on an Epson Perfection V700 and optical density measured using ImageJ.

2.8 Immunohistochemistry

The number of PV positive interneurons was determined by immunohistochemistry. Rats were transcardially perfused with saline (100mL) followed by 4% paraformaldehyde (150mL). Rats were decapitated and brains extracted, post-fixed for at least 24 hours, and cryoprotected in 10% sucrose in phosphate-buffered saline (PBS) until saturated (~24 hours). Free-floating coronal sections from the vHipp (50µm) were washed in PBS (3 times for 10 minutes each), blocked (30 minutes; 2% normal goat serum and 0.3% Triton X-100), then incubated with a primary antibody [rabbit anti-PV (1:2000)] at 4°C overnight. Sections were then washed in PBS (3 times for 10 minutes each), incubated with Alexa Fluor® 488 goat anti-rabbit IgG secondary antibody (1:1000; 1 hour) and mounted on slides and cover slipped with ProLong® Gold antifade reagent with DAPI (150µL). Sections were imaged using an AxioCam ICc 1 (Zeiss) camera attached to an Axio Lab.A1 microscope (Zeiss). The number PV-positive cells were counted on four sections per animal. Representative images were collected using an Olympus 1X81 Motorized Inverted microscope. Digital images were acquired with FV10-ASW software and enhanced using ImageJ.

2.9 Electrophysiology: Local Field Potentials (LFP)

Survival surgeries were performed in a semi-sterile environment under general anesthesia. Female rats (~250-300 g) were anesthetized with Fluriso™ (2-5% Isoflurane, USP with oxygen flow at 1L/min) and placed in a stereotaxic apparatus using blunt atraumatic ear bars. Custom length polyimide-insulated stainless steel electrodes (MS303/1-AIU/SPC; PlasticsOne, Roanoke, VA) were unilaterally implanted into the vHipp (A/P +4.8 mm from bregma; M/L +/-4.8 mm from midline; D/V -7.5 mm ventral of skull surface) and non-insulated stainless steel ground wires were wrapped around an anchor screw. The recording electrodes were fixed in place with dental cement and anchor screws. Rats recovered for two weeks prior to any electrophysiological recordings. Spontaneous LFP activity in the vHipp was recorded, using LabChart software (version 7.1; ADInstruments, Chalgrove, Oxfordshire, UK). Rats were placed in a standard housing cage and allowed 20-30 minutes to acclimate. LFP oscillatory activity was then recorded (sampling frequency: 1kHz; gain: 5K; low pass filter: 300Hz; high pass filter: 0.3Hz) for a period of 10 minutes daily until a

complete, consecutive estrous cycle was observed. Oscillations in the gamma (30-100Hz; excluding 60Hz noise) frequencies were quantified with commercially available computer software (LabChart version 7.1) and delta analysis was performed using Prism software (GraphPad Software, San Diego, CA, USA). At the cessation of all LFP recordings, brains were extracted and electrode placements were histologically verified.

2.11 Analysis

Data are represented as the mean $\pm$ s.e.m. with n values representing the number of animals per experimental group unless otherwise stated. Statistics were calculated using SigmaPlot (Systat Software Inc., Chicago, IL, USA). Data was analyzed by two-way ANOVA or three-way ANOVA and the Holm-Sidak post-hoc test, with significance determined at $p < 0.5$.

2.12 Materials

MAM was purchased from Midwestern Research Institute (Kansas City, MO, USA). Fluriso™ (Isoflurane, USP) was purchased from MWI Animal Health (Boise, ID, USA). Chloral hydrate, protease inhibitor cocktail (P8340) and anti-mouse IgG-HRP (A4416) was sourced from Sigma-Aldrich (St. Louis, MO, USA). Alexa Fluor® 594 goat anti-rabbit IgG (A11012) and ProLong® Gold antifade reagent with DAPI (P36931) were purchased from Life Technologies (USA). Anti-Parvalbumin antibody (Rb pAb to PV; ab11427), Anti-GAPDH antibody (Ms mAb to GAPDH; ab9484) and goat pAb to Rb IgG-HRP (ab6721) were purchased from Abcam (Cambridge, MA, USA). Laemmli sample buffer (#161-0737), 10× Tris/glycine/SDS buffer (#161-0732), Mini-Protein TGX Any kD gels (#456-9035) and nitrocellulose/filter paper sandwiches, 0.2µm (#162-0213) were purchased from Bio-Rad (California, USA). Pierce ECL western blotting substrate (#32106) and Restore western blot stripping buffer (#21059) were purchased from Thermo Fisher Scientific (USA). High-performance chemiluminescence film (Amersham hyperfilm ECL; 28906839) was purchased from GE Healthcare. All other chemicals and reagents were of either analytical or laboratory grade, and purchased from standard suppliers.

3. Results

3.1 Amphetamine-Induced Locomotor Response

Individuals with schizophrenia demonstrate a hyper-responsivity to psychomotor stimulants that can be modeled in rats by examining the locomotor response to amphetamine. We have shown previously that male MAM-treated rats display an increased locomotor response to amphetamine compared to saline-treated controls [15, 19, 29]. In contrast, in female rats, we did not observe a main effect of MAM treatment on locomotor activity. However, both saline- (Figure 1A; $n = 6-7$) and MAM-treated (Figure 1B; $n = 10$) female rats display an increased sensitivity to amphetamine during pro/est compared to rats in the met/di stages of the estrous cycle (main effect of estrous cycle at both 0.5mg/kg & 2.0mg/kg doses; Three-way ANOVA; factors: prenatal treatment $\times$ cycle $\times$ time; 0.5mg/kg: $F_{(1, 197)} = 7.638$; $t = 2.764$; $P = 0.006$; 2.0 mg/kg: $F_{(1, 197)} = 4.811$; $t = 2.193$; $P = 0.030$). These results suggest that hormonal increases enhance the locomotor-inducing effects of amphetamine.

3.2 Pre-Pulse Inhibition of Startle

Schizophrenia patients display deficits in sensorimotor gating [30], which can be evaluated using PPI. Consistent with previous literature, we found a main effect of pre-pulse intensity, evidenced by an increase in inhibition with increasing pre-pulse magnitude (Figure 2; $P < 0.001$; $n = 7-10$). In addition, a main effect of pre-natal treatment (MAM/saline) was observed (three-way ANOVA; $F_{(1, 98)} = 5.150$; $t = 2.269$; $P = 0.026$), with MAM-treated animals displaying a decrease in PPI compared to saline-treated animals. There were no differences in PPI between met/di and pro/est groups, suggesting that PPI deficits are driven primarily by prenatal treatment rather than hormonal changes.

3.3 Latent Inhibition

Although it did not reach statistical significance, there was strong evidence for latent inhibition in the Saline met/di group that was not seen in the other groups. This is consistent with our previous findings [16]. There was a main effect of estrous cycle on freezing behavior whereby deficits in latent inhibition were observed in both, saline- and MAM-treated rats during pro/est, and are largely driven by decreases in freezing observed in the no-tone group (Figure 3; Two-way ANOVA (no tone): $F_{(1, 38)} = 4.87$; $P < 0.05$). Post-hoc analysis determined that there was significantly less freezing in the no-tone groups that received saline and were in pro/est (Holm-Sidak: $t = 2.37$, $p < 0.05$; $n = 8$, $29.53 \pm 9.20\%$ freezing) and in MAM-treated rats in met/di (Holm-Sidak: $t = 2.06$, $p < 0.05$; $n = 11$, $35.32 \pm 7.80\%$ freezing) compared to saline-treated rats in met/di ($n = 9$, $59.46 \pm 8.67\%$ freezing). There was no difference between saline- or MAM-treated rats in pro/est (Holm-Sidak: $t = 0.09$, $p = 0.93$; $n = 12$, $28.48 \pm 7.51\%$ freezing). Further, there were no differences in freezing between any of the groups that received the tone (saline-met/di: $n = 11$, $35.88 \pm 7.98\%$ freezing; saline-pro/est: $n = 9$, $30.56 \pm 8.82\%$ freezing; MAM-met/di: $n = 29.89 \pm 7.98\%$ freezing; MAM-pro/est: $n = 11$, $27.61 \pm 7.98\%$ freezing).

3.4 Social Interaction

Schizophrenia patients experience reductions in social interaction (SI), a negative symptom that can be modeled in rats. In line with our previous observations [16], we observed a main effect of prenatal treatment (Saline/MAM) on SI time (Figure 4A; Two-way ANOVA; $F_{(1, 32)} = 8.024$; $P < 0.05$). Post-hoc analysis demonstrated that there was a significant decrease in social interaction time in the MAM-treated animals compared to saline controls specifically during the met/di phase of the estrous cycle (Holm-Sidak: $t = 2.263$; $p < 0.05$; Saline: $n = 7$; met/di = 117.14 ± 9.13 seconds; MAM: $n = 10$; met/di = 90.2 ± 7.64 seconds). Further, we also observed a main effect of estrous cycle on SI time (Two-way ANOVA; $F_{(1, 32)} = 4.99 \pm 0.03$), with a reduction in social interaction time during pro/est compared with met/di in both saline and MAM-treated animals. Post-hoc analysis indicated that there was not a significant decrease in social interaction time in the MAM-treated rats compared to saline controls during the pro/est phase of the cycle (Holm-Sidak: $t = 1.75$; $p = 0.09$; Saline: $n = 7$; pro/est = 95.29 ± 9.13 seconds; MAM: $n = 9$; pro/est = 74.00 ± 8.05 seconds). These results suggest that both prenatal treatment and hormonal fluctuations can influence social interaction time.

3.5 Elevated Plus Maze

In order to determine if the social interaction deficits were due to increased anxiety-like behavior, we performed the EPM. We found no significant effects of prenatal treatment (Saline/MAM) or estrous cycle stage on the OTR for time (Figure 4B; Two-way ANOVA; Prenatal treatment $F_{(1,29)} = 0.03$, Estrous cycle $F_{(1,29)} < 0.01$; Saline: $n = 7$, met/di = 0.50 ± 0.06 OTR; pro/estrus = 0.48 ± 0.06 OTR; MAM: $n = 8$, met/di = 0.47 ± 0.05 OTR; pro/est = 0.49 ± 0.05 OTR). Additionally, we found no significant effects of prenatal treatment or estrous cycle on the OTR for entries (Figure 4C; Two-way ANOVA; Prenatal treatment $F_{(1,29)} = 3.078$; Estrous cycle $F_{(1,29)} < 0.01$; Saline: met/di = 0.43 ± 0.04 OTR; pro/est = 0.46 ± 0.04 OTR; MAM: met/di = 0.53 ± 0.04 OTR; pro/est = 0.49 ± 0.04 OTR). These results suggest that neither prenatal treatment nor estrous cycle influence anxiety-like behavior.

3.6 Western Blot

A decrease in PV expression has been observed in schizophrenia patients [31], therefore we used western blots to measure PV protein expression the vHipp. We found a main effect of prenatal treatment on PV expression (Figure 5A; Saline/MAM; two-way ANOVA; $F(1, 26) = 7.478$; $t = 2.735$; $P = 0.011$). MAM-treated animals had a decrease in PV expression compared to saline-treated controls (Saline: $n = 6-8$; met/di = 1.56 ± 0.19 OD/GAPDH; pro/est = 1.59 ± 0.29 OD/GAPDH; MAM: $n = 8$; met/di = 0.88 ± 0.11 OD/GAPDH; pro/est = 1.30 ± 0.16 OD/GAPDH). This effect was largely driven by a significant decrease in the expression of PV in MAM-treated rats during the met/di phase of the cycle (Holm-Sidak; $t = 2.822$; $P = 0.009$). These results suggest that both prenatal treatment and hormonal changes can influence PV expression.

3.7 Immunohistochemistry

Although previous work has demonstrated a decrease in PV expression in schizophrenia patients, it is unclear whether there is a reduction in PV protein expression or actual loss of PV-positive cells. Therefore, we used immunohistochemistry to label and count PV-positive neurons in the vHipp (Figure 5C). We found a main effect of prenatal treatment (Saline/MAM) on the number of PV-positive cells in the vHipp (Figure 5B; Two-way ANOVA; $F_{(1,19)} = 12.85$). MAM-treated females had significantly fewer PV-positive cells in the vHipp compared to saline-treated controls across all stages of the estrous cycle (Saline: $n = 4-5$; met/di = 11.6 ± 0.72 cells per $250\mu\text{m}^2$; pro/est = 11.33 ± 0.80 cells; MAM: $n = 5-6$; met/di = 8.75 ± 0.67 cells; pro/est = 8.98 ± 0.72 cells; prenatal treatment: met/di; Holm-Sidak; $t = 2.94$; $P = 0.01$; prenatal treatment: pro/est; Holm-Sidak; $t = 2.18$; $P = 0.45$). As expected, the 4-day estrous cycle did not affect the number of PV-positive cells in the vHipp (Two-way ANOVA; $F_{(1,19)} < 0.01$; $P = 0.976$), suggesting that prenatal treatment can influence cell number while hormonal fluctuations produce a more dynamic regulation of protein expression.

3.8 Electrophysiology: Local Field Potentials

PV-positive interneurons are thought to drive oscillatory activity in the gamma frequency range (30-100Hz) and deficits in gamma oscillations have been observed in schizophrenia patients. Therefore, we measured spontaneous LFP activity in the vHipp to determine if the

changes in PV expression and cell number were accompanied by a change in gamma oscillations. We observed a main effect of prenatal treatment on gamma oscillations in the vHipp (Figure 6B; Saline/MAM; two-way ANOVA; $F_{(1,36)} = 12.41$; $t = 3.522$; $P = 0.001$). Gamma oscillations were significantly decreased across all stages of the estrous cycle in MAM-treated rats ($n = 5$; met = $0.0101 \pm 0.01 V^2$; di = $0.0119 \pm 0.01 V^2$; pro = $0.0118 \pm 0.01 V^2$; est = $0.0102 \pm 0.01 V^2$) when compared to saline-treated control rats ($n = 6$; met = $0.0354 \pm 0.01 V^2$; di = $0.0401 \pm 0.01 V^2$; pro = $0.0352 \pm 0.01 V^2$; est = $0.0267 \pm 0.01 V^2$). These results suggest that the changes in coordinated network activity are driven by prenatal treatment rather than hormonal fluctuations.

4. Discussion

Schizophrenia is a devastating psychiatric disorder characterized by positive, negative and cognitive symptoms. Although the lifetime prevalence of schizophrenia does not vary between males and females, differences have been observed in the age of onset and symptom profiles [32], suggesting that there may be a hormonal component to this disorder. To date, the majority of pre-clinical research on schizophrenia has been performed in male animals. Indeed, our lab has demonstrated that MAM administration to pregnant dams on GD 17 produces physiological, anatomical and behavioral deficits in male offspring that model schizophrenia [16, 18]. In the current experiments, we aimed to characterize the MAM model of schizophrenia in naturally cycling female rodents.

While human females undergo a 28-day menstrual cycle, rodents experience a four day estrous cycle. The stages of the rodent estrous cycle are: diestrus (di), metestrus (met), proestrus (pro), and estrus (est). In the current experiments, we used vaginal cytology [6, 26] to divide rats into two groups: (1) met/di and (2) pro/est. These groups were based on levels of circulating hormones as peak levels of the hormones, estrogen and progesterone, are observed during pro/est [33], while levels remain lower during met/di. Furthermore, we have previously demonstrated that these groups are sufficient to isolate differences in dopamine system function in MAM-treated female rats [6].

Various symptoms of schizophrenia correlate with phases of low hormone levels in humans, which led to the estrogen protection hypothesis of schizophrenia [8]. Evidence for this hypothesis comes from the observation that women are more susceptible to first episode or relapse when there is a large drop in estrogen, such as during the post-partum period [34, 35] or during menopause [36]. Conversely, pregnancy, which is accompanied by high levels of estrogen, confers a protective effect [37]. Further, it has been shown that psychosis is exacerbated during low estrogen phases of the menstrual cycle, while symptom improvements are observed during higher estrogen phases [38, 39]. These results are not surprising as estrogen has been shown to protect cells against excitotoxicity, oxidative stress, inflammation and apoptosis [40–42]. Further, estrogens can enhance neurogenesis and synaptic plasticity [43, 44]. Recent work has demonstrated that estrogen receptor modulators, such as raloxifene, may have a beneficial effect on schizophrenia symptoms in both males and females [45, 46].

One way in which hormonal alterations may influence schizophrenia-like symptoms is via their effects on the dopamine system, as estrogens have been shown to modify dopamine signaling in rats [47]. For example, changes in dopamine release have been observed across the estrous cycle [48]. Further, ovariectomy reduced the number of dopamine transporters in the striatum, which would increase the time and/or amount of dopamine in the synapse, an effect that was reversed by estrogen replacement therapy [49]. Pre-clinical studies have demonstrated that the number of spontaneously active dopamine cells [6], a measure that is correlated with dopamine release [50, 51], varies across the estrous cycle, with the highest levels during pro/est [6]. Interestingly, this effect was due to progesterone rather than estrogen, as the increase in dopamine population activity was blocked by intra-vHipp injections of mifepristone, a progesterone receptor antagonist, but not the estrogen receptor antagonist, fulvestrant [6].

In the current study, we examined multiple behaviors associated with schizophrenia thought to depend, at least in part, on dopamine signaling, including amphetamine-induced locomotor activity [52], LI [53], and PPI [54]. In the amphetamine-induced locomotor response assay, MAM- and saline-treated females displayed an increase in locomotor activity during pro/est when compared to met/di. This result is consistent with our previous data demonstrating aberrant dopamine system function during pro/est in female MAM-treated rats as aberrant VTA dopamine neuron activity is correlated with an enhanced sensitivity in the amphetamine-induced locomotor response assay [15, 19].

Deficits in PPI were observed in female MAM-treated rats across the estrous cycle, consistent with what is observed in male MAM-treated rats. In the current experiments, we did not observe a main effect of estrous cycle on PPI; however, there may be trend for female MAM-treated rats in met/di to have greater PPI deficits. These results are not surprising as ovariectomized rats display PPI deficits in response to a D2 agonist and can be reversed by estrogen, but not progesterone, replacement [55]. MAM-induced deficits in LI were also observed in all stages of the estrous cycle. Interestingly, deficits in latent inhibition were also observed in saline-treated controls during pro/est. This result is somewhat surprising as estrogen treatment has been shown to improve LI deficits caused by amphetamine [56]. However, our results are in line with others demonstrating that LI is impaired during pro/est [57].

Negative symptoms associated with schizophrenia were modeled using SI [58]. Male patients with schizophrenia experience a greater number and more severe negative symptoms than women [32]. We have previously demonstrated that male MAM-treated rats display deficits in SI, as evidenced by a decrease in the time spent interacting [16]. In the current study, we found that female MAM-treated rats also spend less time engaged in social activity. Interestingly, this effect was even more pronounced during pro/est in both saline- and MAM-treated animals, suggesting that higher levels of estrogen or progesterone may exacerbate negative symptoms associated with schizophrenia. This is consistent with previous evidence suggesting that progesterone can influence social behavior [59]. Specifically, a systemic injection of progesterone in ovariectomized mice caused a decrease in SI time, consistent with our data demonstrating decreased SI time in pro/est, when progesterone levels are high. Importantly, in female rats, there was no effect of MAM

treatment or estrous cycle on anxiety levels, as measured by the EPM. These results suggest that the SI effect is not due to changes in anxiety and confirm that anxiety observed in patients with schizophrenia may be secondary to the illness rather than a symptom of the disease.

Individuals with schizophrenia display hyperactivity in the anterior hippocampus [60–63]. Similarly, male MAM-treated rats also display increased activity in the analogous region, the vHipp [15, 19]. The vHipp has been shown to modulate both VTA dopamine neuron activity, associated with positive symptoms, and can directly regulate activity in the prefrontal cortex [64], a region implicated in the negative symptoms and cognitive deficits observed in schizophrenia. Furthermore, we have previously demonstrated that normalizing vHipp activity can reverse aberrant dopamine neuron activity and behavioral deficits in the MAM model [16, 19]. Hyperactivity in the vHipp is thought to be caused by a loss of inhibitory interneurons in this brain region. Human studies have shown a loss in PV-positive interneurons in hippocampal sub-regions [23, 65, 66]. Indeed, we have also demonstrated that male MAM-treated rats have reduced PV cell numbers in the vHipp [67] and that restoring interneuron function in the vHipp using cell-based therapies can improve positive, negative and cognitive symptoms of schizophrenia [16, 19]. Here we demonstrate that female MAM-treated animals also display a decrease in vHipp PV expression. Interestingly, this was more prominent during met/di, suggesting that PV expression levels are dynamically regulated by estrous cycle in MAM rats. To examine this further, we performed immunohistochemistry to evaluate the number of PV neurons in the vHipp. Consistent with previous data in rodents and patients, a ~20% decrease in the number of PV positive interneurons was observed in MAM-treated rats regardless of estrous cycle stage. This suggests that while MAM-induces PV cell loss in female rats, hormonal factors can regulate protein expression within the remaining neurons.

To determine the potential physiological effects of the observed changes in vHipp PV, we examined gamma oscillations in freely behaving rats using *in vivo* electrophysiology. PV-positive interneurons are both necessary and sufficient to evoke gamma oscillations, which correlate with interneuron activity [68, 69]. Individuals with schizophrenia exhibit abnormalities in synchronized cortical activity, specifically in the gamma frequency range (30-100Hz) [70]. Consistent with these data, robust decreases in gamma oscillations were observed in the vHipp of female MAM-treated rats that did not vary across the estrous cycle. These data suggest that it is the decreases in PV cell number, rather than alterations in PV expression, that lead to deficits in synchronous vHipp activity. It should be noted that Barth and colleagues have demonstrated increases in gamma oscillations in female mice during estrus [24]. However, the discrepancy may be due to the fact that these measures were taken during REM sleep, while our recordings were performed in awake, freely moving animals.

Deficits in parvalbumin cell function and alterations in coordinated neuron activity have been associated with schizophrenia-like deficits, including decreased social interaction and cognitive dysfunction. For example, decreasing gamma oscillations in the mPFC of rodents has been shown to decrease social interaction [71]. Therefore, it is not surprising that the MAM-induced decrease in social interaction time corresponded to a decrease in a gamma oscillations in the MAM-treated animals. Further, coordinated network activity is also

thought to underlie cognitive function [72]. For example, inhibiting coordinated activity in PFC interneurons has been shown to disrupt cognitive flexibility and stimulating interneurons can reverse cognitive inflexibility in rodent models [73]. We have shown previously that male MAM-treated animals have deficits in two forms of cognitive flexibility, reversal learning and extra-dimensional set-shifting [16, 18] and that restoring interneuron function can attenuate these deficits [16]. Therefore, we expect that the decrease in gamma oscillations that we observed in the female MAM animals would also coincide with deficits in cognitive flexibility. Future experiments will explore this possibility.

5. Conclusion

Taken together, we now demonstrate that female MAM-treated rats, like their male counterparts, display behavioral, physiological and pathological deficits analogous to those observed in schizophrenia. However, it is important to note that specific deficits can be altered by estrous cycle stage, and are likely regulated by circulating hormones. These data suggest that some schizophrenia-like behaviors may be modulated by the hormones estrogen and progesterone. These findings have important implications for the treatment of schizophrenia in female patients, as fluctuating hormone levels may affect the efficacy of pharmacological therapies in female patients with schizophrenia.

Acknowledgements

We thank Katie Rodgers for valuable assistance with electrophysiological experiments.

Funding and Disclosures

This work was supported by the National Institutes of Mental Health [DJL, MH090067]; individual postdoctoral fellowship from the San Antonio Life Sciences Institute [SMP]. The authors report no disclosures, nor conflicts of interest.

References

- [1]. Goldstein JM, Tsuang MT, Faraone SV, Gender and schizophrenia: implications for understanding the heterogeneity of the illness, *Psychiatry research* 28(3) (1989) 243–53. [PubMed: 2762431]
- [2]. Salem JE, Kring AM, The role of gender differences in the reduction of etiologic heterogeneity in schizophrenia, *Clin Psychol Rev* 18(7) (1998) 795–819. [PubMed: 9827322]
- [3]. Lieberman J, Jody D, Geisler S, Alvir J, Loebel A, Szymanski S, Woerner M, Borenstein M, Time course and biologic correlates of treatment response in first-episode schizophrenia, *Archives of general psychiatry* 50(5) (1993) 369–76. [PubMed: 8098203]
- [4]. Castle DJ, Wessely S, Murray RM, Sex and schizophrenia: effects of diagnostic stringency, and associations with and premorbid variables, *The British journal of psychiatry : the journal of mental science* 162 (1993) 658–64. [PubMed: 8149118]
- [5]. Castle DJ, Abel K, Takei N, Murray RM, Gender differences in schizophrenia: hormonal effect or subtypes?, *Schizophrenia bulletin* 21(1) (1995) 1–12. [PubMed: 7770731]
- [6]. Perez SM, Chen L, Lodge DJ, Alterations in dopamine system function across the estrous cycle of the MAM rodent model of schizophrenia, *Psychoneuroendocrinology* 47 (2014) 88–97. [PubMed: 25001958]
- [7]. Larsen TK, McGlashan TH, Johannessen JO, Vibe-Hansen L, First-episode Schizophrenia: II. Premorbid Patterns by Gender, *Schizophrenia bulletin* 22(2) (1996) 257–269. [PubMed: 8782285]
- [8]. Leung A, Chue P, Sex differences in schizophrenia, a review of the literature, *Acta psychiatrica Scandinavica* 101(401) (2000) 3–38.

- [9]. Goldstein JM, Seidman LJ, Goodman JM, Koren D, Lee H, Weintraub S, Tsuang MT, Are there sex differences in neuropsychological functions among patients with schizophrenia?, *The American journal of psychiatry* 155(10) (1998) 1358–64. [PubMed: 9766767]
- [10]. Lodge DJ, Grace AA, Gestational methylazoxymethanol acetate administration: A developmental disruption model of schizophrenia, *Behavioural brain research* 7(204(2)) (2009) 306–12.
- [11]. Moore H, Jentsch JD, Ghajarnia M, Geyer MA, Grace AA, A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia, *Biological psychiatry* 60(3) (2006) 253–64. [PubMed: 16581031]
- [12]. Le Pen G, Gourevitch R, Hazane F, Hoareau C, Jay TM, Krebs MO, Peri-pubertal maturation after developmental disturbance: a model for psychosis onset in the rat, *Neuroscience* 143(2) (2006) 395–405. [PubMed: 16973297]
- [13]. Hazane F, Krebs MO, Jay TM, Le Pen G, Behavioral perturbations after prenatal neurogenesis disturbance in female rat, *Neurotox Res* 15(4) (2009) 311–20. [PubMed: 19384565]
- [14]. Chen L, Perez SM, Lodge DJ, An augmented dopamine system function is present prior to puberty in the methylazoxymethanol acetate rodent model of schizophrenia, *Dev Neurobiol* 74(9) (2014) 907–17. [PubMed: 24554310]
- [15]. Lodge DJ, Grace AA, Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia, *The Journal of neuroscience : the official journal of the Society for Neuroscience* 27 (2007) 11424–11430.
- [16]. Donegan JJ, Tyson JA, Branch SY, Beckstead MJ, Anderson SA, Lodge DJ, Stem cell-derived interneuron transplants as a treatment for schizophrenia: preclinical validation in a rodent model, *Molecular psychiatry* 22(10) (2017) 1492–1501. [PubMed: 27480492]
- [17]. Gastambide F, Cotel MC, Gilmour G, O'Neill MJ, Robbins TW, Tricklebank MD, Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator, *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 37(4) (2012) 1057–66. [PubMed: 22129780]
- [18]. Perez SM, Shah A, Asher A, Lodge DJ, Hippocampal deep brain stimulation reverses physiological and behavioural deficits in a rodent model of schizophrenia, *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 16(6) (2013) 1331–9.
- [19]. Perez SM, Lodge DJ, Hippocampal interneuron transplants reverse aberrant dopamine system function and behavior in a rodent model of schizophrenia, *Molecular psychiatry* 18(11) (2013) 1193–8. [PubMed: 23979606]
- [20]. Boley AM, Perez SM, Lodge DJ, A fundamental role for hippocampal parvalbumin in the dopamine hyperfunction associated with schizophrenia, *Schizophrenia research* 157(1-3) (2014) 238–43. [PubMed: 24888524]
- [21]. Lewis DA, Hashimoto T, Volk DW, Cortical inhibitory neurons and schizophrenia, *Nat Rev Neurosci* 6(4) (2005) 312–24. [PubMed: 15803162]
- [22]. Heckers S, The hippocampus in schizophrenia, *American Journal of Psychiatry* 161(11) (2004) 2138–2139.
- [23]. Zhang Z, Sun J, Reynolds GP, A selective reduction in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia patients, *Chinese medical journal* 115(6) (2002) 819–23. [PubMed: 12123544]
- [24]. Barth AM, Ferando I, Mody I, Ovarian cycle-linked plasticity of delta-GABAA receptor subunits in hippocampal interneurons affects gamma oscillations in vivo, *Front Cell Neurosci* 8 (2014) 222. [PubMed: 25157218]
- [25]. Lodge DJ, The MAM rodent model of schizophrenia, *Current protocols in neuroscience / editorial board, Crawley Jacqueline N. [et al.] Chapter 9* (2013) Unit9.43.
- [26]. Marcondes FK, Bianchi FJ, Tanno AP, Determination of the estrous cycle phases of rats: some helpful considerations, *Brazilian journal of biology = Revista brasileira de biologia* 62(4A) (2002) 609–14. [PubMed: 12659010]

- [27]. Cecchi C, Emx2: a gene responsible for cortical development, regionalization and area specification, *Gene* 291(1-2) (2002) 1–9. [PubMed: 12095673]
- [28]. Lapiz-Bluhm MD, Bondi CO, Doyen J, Rodriguez GA, Bedard-Arana T, Morilak DA, Behavioural assays to model cognitive and affective dimensions of depression and anxiety in rats, *Journal of neuroendocrinology* 20(10) (2008) 1115–37. [PubMed: 18673411]
- [29]. Perez SM, Carreno FR, Frazer A, Lodge DJ, Vagal nerve stimulation reverses aberrant dopamine system function in the methylazoxymethanol acetate rodent model of schizophrenia, *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34(28) (2014) 9261–7. [PubMed: 25009259]
- [30]. Braff DL, Geyer MA, Sensorimotor gating and schizophrenia. Human and animal model studies, *Archives of general psychiatry* 47(2) (1990) 181–8. [PubMed: 2405807]
- [31]. Zhang ZJ, Reynolds GP, A selective decrease in the relative density of parvalbuminimmunoreactive neurons in the hippocampus in schizophrenia, *Schizophrenia research* 55(1-2) (2002) 1–10. [PubMed: 11955958]
- [32]. Mendrek A, Mancini-Marie A, Sex/gender differences in the brain and cognition in schizophrenia, *Neurosci Biobehav Rev* 67 (2016) 57–78. [PubMed: 26743859]
- [33]. Lebron-Milad K, Milad MR, Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders, *Biol Mood Anxiety Disord* 2 (2012) 3. [PubMed: 22738383]
- [34]. McNeil TF, A prospective study of postpartum psychoses in a high-risk group. 1. Clinical characteristics of the current postpartum episodes, *Acta psychiatrica Scandinavica* 74(2) (1986) 205–16. [PubMed: 3776667]
- [35]. Kendell RE, Chalmers JC, Platz C, Epidemiology of puerperal psychoses, *The British journal of psychiatry : the journal of mental science* 150 (1987) 662–73. [PubMed: 3651704]
- [36]. Hafner H, an der Heiden W, Epidemiology of schizophrenia, *Canadian journal of psychiatry. Revue canadienne de psychiatrie* 42(2) (1997) 139–51. [PubMed: 9067063]
- [37]. Munk-Olsen T, Laursen TM, Pedersen CB, Mors O, Mortensen PB, New parents and mental disorders: a population-based register study, *JAMA* 296(21) (2006) 2582–9. [PubMed: 17148723]
- [38]. Luggin R, Bernsted L, Petersson B, Jacobsen AT, Acute psychiatric admission related to the menstrual cycle, *Acta psychiatrica Scandinavica* 69(6) (1984) 461–5. [PubMed: 6540037]
- [39]. Hallonquist JD, Seeman MV, Lang M, Rector NA, Variation in symptom severity over the menstrual cycle of schizophrenics, *Biological psychiatry* 33(3) (1993) 207–9. [PubMed: 8448269]
- [40]. Arevalo MA, Santos-Galindo M, Lagunas N, Azcoitia I, Garcia-Segura LM, Selective estrogen receptor modulators as brain therapeutic agents, *Journal of molecular endocrinology* 46(1) (2011) R1–9. [PubMed: 21071476]
- [41]. Bryant DN, Dorsa DM, Roles of estrogen receptors alpha and beta in sexually dimorphic neuroprotection against glutamate toxicity, *Neuroscience* 170(4) (2010) 1261–9. [PubMed: 20732393]
- [42]. Yang LC, Zhang QG, Zhou CF, Yang F, Zhang YD, Wang RM, Brann DW, Extranuclear estrogen receptors mediate the neuroprotective effects of estrogen in the rat hippocampus, *PloS one* 5(5) (2010) e9851. [PubMed: 20479872]
- [43]. Li J, Siegel M, Yuan M, Zeng Z, Finnucan L, Persky R, Hurn PD, McCullough LD, Estrogen enhances neurogenesis and behavioral recovery after stroke, *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 31(2) (2011) 413–25.
- [44]. Woolley CS, McEwen BS, Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat, *The Journal of neuroscience : the official journal of the Society for Neuroscience* 12(7) (1992) 2549–54. [PubMed: 1613547]
- [45]. Heringa SM, Begemann MJ, Goverde AJ, Sommer IE, Sex hormones and oxytocin augmentation strategies in schizophrenia: A quantitative review, *Schizophrenia research* 168(3) (2015) 603–13. [PubMed: 25914107]
- [46]. Bratek A, Krysta K, Drzyzga K, Baranska J, Kucia K, The role of selective estrogen receptor modulators in the treatment of schizophrenia, *Psychiatr Danub* 28(Suppl-1) (2016) 45–48.

- [47]. Silverman JL, Koenig JJ, Evidence for the involvement of ERbeta and RGS9-2 in 17-beta estradiol enhancement of amphetamine-induced place preference behavior, *Horm Behav* 52(2) (2007) 146–55. [PubMed: 17493623]
- [48]. Castner SA, Xiao L, Becker JB, Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies, *Brain research* 610(1) (1993) 127–34. [PubMed: 8518920]
- [49]. Le Saux M, Di Paolo T, Influence of oestrogenic compounds on monoamine transporters in rat striatum, *Journal of neuroendocrinology* 18(1) (2006) 25–32. [PubMed: 16451217]
- [50]. Legault M, Wise RA, Injections of N-methyl-D-aspartate into the ventral hippocampus increase extracellular dopamine in the ventral tegmental area and nucleus accumbens, *Synapse* 31(4) (1999) 241–9. [PubMed: 10051104]
- [51]. Blaha CD, Yang CR, Floresco SB, Barr AM, Phillips AG, Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens, *European Journal of Neuroscience* 9(5) (1997) 902–11. [PubMed: 9182943]
- [52]. Young JW, Minassian A, Geyer MA, Locomotor Profiling from Rodents to the Clinic and Back Again, *Current topics in behavioral neurosciences* 28 (2016) 287–303. [PubMed: 27418071]
- [53]. Weiner I, Arad M, Using the pharmacology of latent inhibition to model domains of pathology in schizophrenia and their treatment, *Behavioural brain research* 204(2) (2009) 369–86. [PubMed: 19433114]
- [54]. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR, Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review, *Psychopharmacology* 156(2-3) (2001) 117–54. [PubMed: 11549216]
- [55]. Gogos A, Van den Buuse M, Estrogen and progesterone prevent disruption of prepulse inhibition by the serotonin-1A receptor agonist 8-hydroxy-2-dipropylaminotetralin, *The Journal of pharmacology and experimental therapeutics* 309(1) (2004) 267–74. [PubMed: 14722325]
- [56]. Arad M, Weiner I, Disruption of latent inhibition induced by ovariectomy can be reversed by estradiol and clozapine as well as by co-administration of haloperidol with estradiol but not by haloperidol alone, *Psychopharmacology* 206(4) (2009) 731–40. [PubMed: 19169876]
- [57]. Arad M, Weiner I, Fluctuation of latent inhibition along the estrous cycle in the rat: modeling the cyclicity of symptoms in schizophrenic women?, *Psychoneuroendocrinology* 33(10) (2008) 1401–10. [PubMed: 18819755]
- [58]. Wilson CA, Koenig JJ, Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia, *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* 24(5) (2014) 759–73. [PubMed: 24342774]
- [59]. Koonce CJ, Frye CA, Progesterone facilitates exploration, affective and social behaviors among wildtype, but not 5alpha-reductase Type 1 mutant, mice, *Behavioural brain research* 253 (2013) 232–9. [PubMed: 23886595]
- [60]. Heckers S, Rauch SL, Goff D, Savage CR, Schacter DL, Fischman AJ, Alpert NM, Impaired recruitment of the hippocampus during conscious recollection in schizophrenia, *Nature neuroscience* 1(4) (1998) 318–23. [PubMed: 10195166]
- [61]. Schobel SA, Lewandowski NM, Corcoran CM, Moore H, Brown T, Malaspina D, Small SA, Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders, *Archives of general psychiatry* 66(9) (2009) 938–46. [PubMed: 19736350]
- [62]. Malaspina D, Storer S, Furman V, Esser P, Printz D, Berman A, Lignelli A, Gorman J, Van Heertum R, SPECT study of visual fixation in schizophrenia and comparison subjects, *Biological psychiatry* 46(1) (1999) 89–93. [PubMed: 10394477]
- [63]. Medoff DR, Holcomb HH, Lahti AC, Tamminga CA, Probing the human hippocampus using rCBF: contrasts in schizophrenia, *Hippocampus* 11(5) (2001) 543–50. [PubMed: 11732707]
- [64]. Jay TM, Witter MP, Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin, *The Journal of comparative neurology* 313(4) (1991) 574–86. [PubMed: 1783682]
- [65]. Heckers S, Konradi C, Hippocampal neurons in schizophrenia, *Journal of neural transmission* 109(5) (2002) 891–905. [PubMed: 12111476]

- [66]. Konradi C, Yang CK, Zimmerman EI, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, Heckers S, Hippocampal interneurons are abnormal in schizophrenia, *Schizophrenia research* 131(1-3) (2011) 165–173. [PubMed: 21745723]
- [67]. Lodge DJ, Behrens MM, Grace AA, A loss of parvalbumin-containing interneurons is associated with diminished oscillatory activity in an animal model of schizophrenia, *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29(8) (2009) 2344–54. [PubMed: 19244511]
- [68]. Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI, Driving fast-spiking cells induces gamma rhythm and controls sensory responses, *Nature* 459(7247) (2009) 663–7. [PubMed: 19396156]
- [69]. Sohal VS, Zhang F, Yizhar O, Deisseroth K, Parvalbumin neurons and gamma rhythms enhance cortical circuit performance, *Nature* 459(7247) (2009) 698–702. [PubMed: 19396159]
- [70]. McNally JM, McCarley RW, Gamma band oscillations: a key to understanding schizophrenia symptoms and neural circuit abnormalities, *Curr Opin Psychiatry* 29(3) (2016) 202–10. [PubMed: 26900672]
- [71]. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K, Neocortical excitation/inhibition balance in information processing and social dysfunction, *Nature* 477(7363) (2011) 171–8. [PubMed: 21796121]
- [72]. Uhlhaas PJ, Singer W, Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology, *Neuron* 52(1) (2006) 155–68. [PubMed: 17015233]
- [73]. Cho KK, Hoch R, Lee AT, Patel T, Rubenstein JL, Sohal VS, Gamma rhythms link prefrontal interneuron dysfunction with cognitive inflexibility in *Dlx5/6*(+/-) mice, *Neuron* 85(6) (2015) 1332–43. [PubMed: 25754826]

Highlights

- Female rats treated with MAM on gestational day 17 exhibit behavioral deficits that model positive and negative symptoms of schizophrenia.
- The behavioral deficits observed in female MAM rats vary across the estrous cycle.
- Female MAM rats also display deficits in GABAergic interneurons in the ventral hippocampus.

Figure 1. Amphetamine-Induced Locomotor Response.

Both saline- (**A**) and MAM-treated (**B**) rats displayed an increased sensitivity to amphetamine during proestrus/estrus (pro/est). † denotes significance from metestrus/diestrus (met/di). $n=6-10$ rats per group.

Figure 2. Pre-Pulse Inhibition to Startle.

Sensorimotor motor gating deficits were affected by prenatal treatment (Saline/MAM) but not estrous cycle stage. MAM-treated rats displayed a significant decrease in pre-pulse inhibition (PPI) when compared to saline-treated controls, † denotes significance from saline-treated rats. $n= 7-10$ rats per group.

Figure 3. Latent Inhibition.

Latent inhibition is affected by stage of the estrous cycle. Saline-treated rats in metestrus/diestrus (met/di) spend less time freezing when the tone is presented repeatedly prior to the tone-shock pairing. This effect is abolished in saline-treated rats during proestrus/estrus (pro/est) and is not observed in MAM-treated rats at any stage of the cycle. *is significantly different than saline met/di. $n=8-12$ per group.

Figure 4. Social Interaction and Elevated Plus Maze.

Social interaction is affected by both, pre-natal treatment (Saline/MAM) and estrous cycle stage (A). Behavior on the elevated plus maze is not affected by prenatal treatment (Saline/MAM) or estrous cycle stage. There are no differences between any of the groups in the open to total ratio (OTR) for time (B). In addition, there are no differences in the OTR for entries (C). $n=7-8$ rats per group. MAM-treated rats spend significantly less time interacting than saline-treated rats. Additionally, rats in proestrus/estrus (pro/est) spend less time interacting when compared to those in metestrus/diestrus (met/di). *denotes significance from met/di. † is significantly different from saline-treated rats. $n= 7-10$ rats per group.

Figure 5. Western Blot and Immunohistochemistry.

Parvalbumin (PV) protein expression in the ventral hippocampus (vHipp) is affected by prenatal treatment (Saline/MAM). During metestrus/diestrus (met/di) MAM-treated rats display a significant decrease in the expression of PV protein when compared to saline-treated controls (**A**). † is significantly different from saline-treated rats *denotes significance from saline-treated rats during met/di. $n=6-8$ rats per group. Representative western blots are depicted in (**B**). The number of PV cells in the vHipp is affected by prenatal treatment, but not estrous cycle stage. MAM-treated rats had significantly fewer PV-positive cells in the vHipp at all stages of the estrous cycle (**C**). Representative sections are depicted in (**D**). $n=4-6$ rats per group, † is significantly different from saline-treated rats. Scale bar is 20 microns.

Figure 6. Electrophysiology: Local Field Potentials.

Local field potentials in the ventral hippocampus (vHipp) were affected by prenatal treatment (Saline/MAM) and not estrous cycle stage. MAM-treated rats displayed a significant decrease in gamma oscillations (30-100 Hz) when compared to saline-treated controls (**A**). Representative trace of local field potential (top) and associated gamma (bottom) activity in MAM- and saline-treated rats (**B**). † denotes significance from saline-treated controls. $n = 5-6$ rats per group. Scale bar is 200ms.