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Alterations in dopamine system function across the estrous cycle of the MAM rodent model of schizophrenia

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Summary

Clinical studies have reported differences in the incidence and severity of schizophrenia symptoms between male and female schizophrenia patients. Unfortunately, the cause of these differences is not currently known due, in part, to the fact that preclinical studies largely focus on male subjects. Dopamine neuron activity has been previously demonstrated to change across the estrous cycle, and may therefore be of relevance, as aberrant dopamine signaling is thought to underlie the positive symptoms of schizophrenia. Here we examine dopamine neuron activity across the estrous cycle in the MAM rodent model of schizophrenia. We demonstrate that the elevation in dopamine neuron activity, consistently observed in male MAM-treated rats, is most prominent during estrus and attenuated in met-estrus. Furthermore, this appears to be mediated, in part, by progesterone in the ventral hippocampus, as increases in dopamine neuron population activity (observed in estrus) were normalized by the intra-hippocampal administration of the progesterone receptor antagonist, mifepristone (but not the estrogen receptor antagonists, fulvestrant). Taken together, these data suggest that changes in dopamine system function occur across the estrous cycle in MAM-treated rats and may contribute to the differences in symptomatology between male and female schizophrenia patients.

Keywords

Schizophrenia; Dopamine; Estrous cycle; Progesterone; Hippocampus

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Conflict of Interest

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Introduction

Schizophrenia is a devastating psychiatric condition affecting up to 1% of the US population (Bhugra, 2005; Saha et al., 2005). While this disease affects both men and women, there are reported differences between genders that suggest a hormonal component to the pathophysiology of this disorder (for review see (Leung, 2000)). Indeed, Kraepelin's initial observations suggested differences in prevalence and symptomatology between male and female schizophrenia patients (Kraepelin, 1919). Since this time, it has been demonstrated that males have an earlier onset of the disease (Aleman et al., 2003), a greater degree of premorbid deficits (Larsen et al., 1996), and significant differences in symptom severity (Leung, 2000). For example, females are reported to display relatively greater positive symptom severity (auditory hallucinations & persecutory delusions) while males show enhanced negative and cognitive dysfunction (specifically those involved in verbal processing) (Goldstein et al., 1998; Leung, 2000). In addition, female patients have been demonstrated to show a more rapid and greater response to antipsychotic medications (Szymanski et al., 1995). While this appears to be true for both typical and atypical antipsychotics, gender differences are more evident with clozapine when compared to olanzapine or risperidone (Usall et al., 2007). The consequence of this is that females are reported to require significantly lower doses, as well as, requiring longer intervals for depot administration (Seeman, 2004). Interestingly, a meta-analysis of structural imaging studies demonstrate that effect size is unrelated to gender, suggesting a similar pattern of structural alterations in male and female patients and arguing against the idea of different pathological processes in the two genders (Wright et al., 2000). Taken together, these data suggest that while the structural alterations occurring in schizophrenia patients are not related to gender, hormonal regulation of these key neuronal structures may result in differences in symptomatology and pharmaceutical efficacy.

While the pathophysiology of schizophrenia has not been conclusively demonstrated, an enhanced dopamine signaling is one of the more prominent hypotheses of the disease (Laruelle and Abi-Dargham, 1999; Abi-Dargham, 2004). Imaging studies have consistently demonstrated region specific increases in dopamine transmission in patients, whereas the efficacy of dopamine receptor antagonists in treating the disease provides further support for this theory. Consistent with this hypothesis, we have previously demonstrated a pathological increase in dopamine neuron activity in the methylazoxymethanol acetate (MAM) rodent model of schizophrenia (Lodge and Grace, 2007; Perez and Lodge, 2013; Perez et al., 2013). The MAM model is a developmental disruption model with strong face and predictive validity (Moore and Grace, 2002; Lodge and Grace, 2009). Specifically, MAM-treated rats display histological alterations consistent with those observed postmortem in schizophrenia (Moore et al., 2006; Lodge et al., 2009). In addition, MAM-treated rats display alterations in neurophysiology similar to those observed in imaging studies (Lodge and Grace, 2007; Lodge et al., 2009) and behavioral deficits analogous to those found in patients (Flagstad et al., 2004; Flagstad et al., 2005; Moore et al., 2006; Lodge et al., 2009). At present, there is only one published study examining female MAM-treated rats (Hazane et al., 2009). While, this study clearly demonstrates behavioral alterations that validate prenatal MAM

administration as a female rodent model for schizophrenia, a direct comparison between MAM-treated male and female rats remains to be examined (Hazane et al., 2009).

As mentioned above, an increase in dopamine neuron population activity is a consistent observation in the MAM rodent model (Lodge and Grace, 2007; Perez and Lodge, 2013; Perez et al., 2013). However, how (or whether) this increase in dopamine neuron activity is altered across the estrous cycle is not currently known. While, the number of reports examining changes in dopamine neuron electrophysiology across the estrus cycle is limited, there is robust evidence for gender differences in dopamine system function (for review see: (Becker et al., 2012)). This includes studies demonstrating neuroprotective effects of estrogen on midbrain dopamine neurons (Dluzen et al., 1996; Miller et al., 1998; Sawada et al., 1998; Sawada et al., 2000), increases in dopamine release/efflux across the estrus cycle (Castner et al., 1993; Walker et al., 1999), and alterations in the density of the dopamine transporter in response to changes in circulating estradiol (Le Saux and Di Paolo, 2006). In addition, a recent preclinical study has examined the electrophysiological properties of midbrain dopamine neurons across the estrous cycle and reported that rats in estrus display significant increases in dopamine neuron firing rate and burst firing (Zhang et al., 2008). Thus, hormonal alterations may affect symptom severity in schizophrenia by altering dopamine transmission.

The cause of the aberrant dopamine system function in schizophrenia has not been conclusively demonstrated; however, significant literature from both pre-clinical and clinical studies suggest that rather than a primary pathology, the dopamine system appears to be abnormally regulated in schizophrenia patients (Grace, 1991, 2000; Abi-Dargham, 2004). We have previously demonstrated, in the MAM rodent model of schizophrenia (Lodge and Grace, 2009), that the mesolimbic dopamine hyperfunction and associated behavioral alterations are directly attributed to a pathologically enhanced activity within the ventral hippocampus (vHipp) (Lodge and Grace, 2007). Specifically, a significantly higher number of spontaneously active ventral tegmental area (VTA) dopamine neurons were observed in MAM-treated rats compared to control rats. This was attributed to hyperactivity within the ventral subiculum since TTX inactivation of the vHipp normalized the augmented dopamine neuron activity, as well as reversed the behavioral hyper-responsivity to amphetamine administration (Lodge and Grace, 2007).

There have been a significant number of studies demonstrating that hippocampal function changes across the female reproductive cycle (Verrotti et al., 2010). A majority of these data stem from the observation that the susceptibility to seizures changes across the estrous cycle. Specifically, progesterone and estrogen appear to produce opposite effects on seizure generation displaying anticonvulsant and proconvulsant effects, respectively (Verrotti et al., 2010). Furthermore, a significant literature has reported electrophysiological changes in hippocampal activity across the estrous cycle with a greatly enhanced responsivity being observed during estrus (Scharfman et al., 2003). Indeed, estrogen has been demonstrated to alter the activity of hippocampal pyramidal cells and augment neuronal plasticity (Woolley, 2007). Furthermore, these physiological alterations are associated with gender related differences in dendritic morphology (Li et al., 2004) and neurogenesis (Chow et al., 2013). Given the robust literature on hormonal regulation of hippocampal function, combined with

a role for the hippocampus in the regulation of dopamine neuron activity (Lodge and Grace, 2007, 2011), we posit that female MAM-treated rats may demonstrate an augmented dopamine system function during estrus that can be modified by hormonal influences on hippocampal activity.

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 approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center.

Animals

As described previously (Moore et al., 2006; Lodge, 2013), MAM treatments were performed on timed pregnant female Sprague Dawley rats obtained from Harlan Laboratories on gestational day (GD) 16 and housed individually in plastic tubs. MAM (diluted in saline, 25 mg/kg, IP) was administered on GD17, while control rats received injections of saline (1ml/kg, IP). Pups were weaned on post-natal day 21 and housed with littermates in groups of 2–3. All experiments were performed on multiple litters of adult (>12 weeks) MAM- and saline-treated rats. Estrous cycle was determined by vaginal cytology as previously reported (Marcondes et al., 2002) and depicted in Figure 1.

Extracellular Recordings

MAM- and saline-treated rats (250–400g), were anesthetized with 8% chloral hydrate (400 mg/kg, IP), as dopamine neuron activity is not significantly depressed by this anesthetic (Hyland et al., 2002). Rats were then placed in a stereotaxic apparatus and a core body temperature of 37°C maintained by a thermostatically controlled heating pad. Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex. An extracellular glass microelectrode (impedance 6–14 M Ω) was lowered into the VTA (A/P $\pm$ 5.3, M/L $\pm$ 0.6 from bregma and D/V –6.5 to –9.0 mm ventral of brain surface) to measure dopamine neuron activity. Spontaneously active dopamine neurons were identified with open filter settings (low pass: 30Hz; high pass: 30kHz) using previously established electrophysiological criteria (Grace and Bunney, 1983). Once isolated, dopamine neuron activity was recorded for 2–3 minutes. Three parameters of dopamine neuron activity were measured: population activity (defined as the number of spontaneously active dopamine neurons encountered while making 6–9 vertical passes, separated by 200 μ m in a predetermined pattern, to sample equivalent regions of the VTA), basal firing rate, and the proportion of action potentials occurring in bursts.

Intracranial Drug Injections

A separate group of MAM-treated female rats that were in either late pro-estrus or estrus (to encompass periods of high circulating estrogen and progesterone (Staley and Scharfman, 2005; Latini et al., 2008) were administered either mifepristone (RU-486) or fulvestrant (ICI 182,780) to block progesterone and estrogen receptors, respectively. For acute administration of drugs or vehicle rats were implanted with 23 G injection cannulae 2.0 mm dorsal to the vHipp (A/P –5.6, M/L +5.0, D/V –4.6 mm from bregma). Mifepristone (5 μ g/

0.5µl) or fulvestrant (5µg/0.5µl) or vehicle (acidified DMSO in saline; 0.5 µl) were infused through a 30 G injection cannula protruding 2.0 mm past the end of the implanted guide cannula. The injection cannula was left in situ for the duration of the experiments to ensure adequate diffusion of drug into the surrounding tissue. Each rat received only one unilateral injection with a typical experiment lasting approximately 2 hours following drug infusion.

Estradiol/Progesterone ELISA

Following electrophysiological recordings, trunk blood was collected into heparin containing microfuge tubes (final concentration 10 U/ml) and centrifuged. Blood plasma was collected and stored frozen (−20°C) until required. Estradiol and Progesterone levels were determined by enzyme-linked immunosorbent assay using commercially available kits. Blood plasma for progesterone analysis was diluted 1:50 while estrogen samples were run undiluted. Absorbance readings were compared to a standard curve and samples were grouped according to estrous phase as determined by vaginal cytology.

Analysis

Electrophysiological analysis of dopamine neuron activity was performed with commercially available computer software (LabChart version 7.1; ADInstruments Ltd., Chalgrove, Oxfordshire, UK) and plotted with Prism software (GraphPad Software Inc., San Diego, CA). Electrophysiological data for MAM- and saline-treated rats across estrus cycle (as well as the effects of the inhibitors mifepristone and fulvestrant) were analyzed by a one-way ANOVA unless data failed tests of normality and/or equal variance whereby an ANOVA on Ranks was performed; post hoc comparisons were performed by Dunn's method as appropriate. Comparisons between MAM- and saline- treated rats in late pro-estrus – estrus were examined by a Mann-Whitney Rank Sum Test, as only two groups are present. ELISA plates were scanned on an iMark Microplate Absorbance Reader (BioRad, Hercules, CA, USA) and compared to a standard curve fit with a non-linear regression in Prism software (GraphPad Software Inc., San Diego, CA). Changes in hormonal levels were determined across 2 factors (estrus cycle and strain) using a two-way ANOVA with post hoc comparisons performed by the Holm Sidak method as appropriate. Data are represented as the mean ± S.E.M. unless stated otherwise, with n-values representing the number of neurons recorded or animals per experimental group where indicated. All statistics were calculated using SigmaPlot (Systat Software, Chicago, IL, USA).

Materials

MAM was purchased from Midwest Research Institute (Kansas City, MO, USA). Chloral hydrate was sourced from Sigma-Aldrich (St. Louis, MO, USA), while heparin was from PSS World Medical, Inc (Jacksonville, FL, USA). Mifepristone (RU-486) and fulvestrant (ICI 182,780) were purchased from Tocris (Minneapolis, MN, USA). The Rat Estradiol ELISA kit was obtained from Calbiotech (Spring Valley, CA, USA) and the Progesterone ELISA kit was purchased from Abnova (Walnut, CA, USA). All other chemicals and reagents were of either analytical or laboratory grade, and purchased from standard suppliers.

Results

Vaginal cytology was used to identify the current stage of the reproductive cycle for each rat (Figure 1). To verify that prenatal MAM administration did not dramatically alter plasma hormone levels, we performed ELISA on plasma obtained at the end of each experiment. It should be noted that the peak responses in circulating hormone levels may have been blunted by surgical stress-evoked release of progesterone (Nequin et al., 1975). Nonetheless, consistent with previous data (Caligioni, 2001), rats in pro-estrus displayed significant increases in circulating estradiol when compared to those in met- and di-estrus ($n=6-11$ rats/group: two-way ANOVA, $F_{(\text{cycle})}=4.614$; Holm Sidak $t=3.342$ (Pro vs Met), $t=3.007$ (Pro vs Di); $p<0.05$: Figure 2). It is important to note that MAM-treated rats did not differ from control rats in regards to the circulating plasma levels of either estradiol (two-way ANOVA; $F_{(\text{strain})}=0.314$, $F_{(\text{strain} \times \text{cycle})}=0.309$, $p>0.05$: Figure 2A) or progesterone (two-way ANOVA; $F_{(\text{strain})}=2.829$, $F_{(\text{strain} \times \text{cycle})}=1.094$, $p>0.05$: Figure 2B).

Control, saline-treated, rats displayed robust alterations in dopamine neuron burst firing across the estrous cycle consistent with previously published reports (Zhang et al., 2008). Specifically, control rats ($n=7-9$ /group) in estrus displayed a significantly enhanced burst firing when compared to those in di-estrus (ANOVA on Ranks; $H=11.220$; Dunn's Method $Q=3.346$, $p<0.05$: Figure 3C) with no significant differences in dopamine neuron population activity (one-way ANOVA; $F=0.150$, $p>0.05$: Figure 3A) or average firing rate (ANOVA on Ranks; $H=2.101$, $p>0.05$: Figure 3B). In contrast, MAM-treated rats ($n=6-11$ /group) displayed a significantly greater dopamine neuron population across the estrous cycle (ANOVA on Ranks; $H=8.126$, $p<0.05$) with significant differences observed between met-estrus and estrus (Dunn's Method $Q=2.795$, $p<0.05$: Figure 3B). However, changes in burst firing (ANOVA on Ranks; $H=6.246$, $p>0.05$: Figure 3F) or average firing rate (ANOVA on Ranks; $H=4.989$, $p>0.05$: Figure 3D) were not observed. We have previously demonstrated that male MAM-treated rats consistently exhibit elevated VTA dopamine neuron population activity when compared to saline-treated rats (Lodge and Grace, 2007; Perez et al., 2013). Here we demonstrate that female rats display a similar augmented dopamine neuron population activity; however this appears to be most prominent in rats during periods of high circulating estrogen & progesterone. Thus, MAM-treated rats in late pro-estrus - estrus displayed a significantly greater dopamine neuron population activity when compared to saline-treated rats in estrus (Mann-Whitney Rank Sum Test, $U=6.500$, $p<0.05$: Figure 4A). Consistent with data in male rats (Lodge and Grace, 2007; Perez et al., 2013) this effect was specific to dopamine neuron population activity with no differences being observed with average firing rate (Mann-Whitney Rank Sum Test, $U=7517$, $p<0.05$: Figure 4B) or dopamine neuron burst firing (Mann-Whitney Rank Sum Test, $U=7115$, $p<0.05$: Figure 4C).

Given our previous data demonstrating the role of the vHipp in the regulation of VTA dopamine neuron population activity in MAM-treated rats, we examined the contribution of vHipp estrogen and progesterone by intracranial microinjection of specific receptor antagonists to MAM-treated rats in late pro-estrus - estrus. The administration of the estrogen receptor antagonist, fulvestrant ($n=10$) failed to alter any parameter of dopamine neuron activity (Figure 5: population activity, ANOVA on Ranks, $H=13.218$; Dunn's Method, $Q=1.050$, $p>0.05$: firing rate, ANOVA on Ranks, $H=4.123$, $p>0.05$: burst firing,

ANOVA on Ranks, $H=1.364$, $p>0.05$). We do not believe this to be an issue of inadequate receptor blockade as the dose used has been previously demonstrated to block the effects of estrogen when administered directly into the hippocampus (Fernandez et al., 2008). In contrast to the lack of effect of estrogen receptor blockade, the direct intra-hippocampal administration of the progesterone receptor antagonist, mifepristone ($n=8$), significantly attenuated the elevated dopamine neuron population activity observed in MAM-treated rats (Figure 5A: population activity, ANOVA on Ranks, $H=13.218$; Dunn's Method, $Q=2.893$, $p<0.05$) to a level consistent with that seen in untreated-control animals (Figure 4A).

Discussion

Here we demonstrate changes in dopamine neuron activity across the reproductive cycle in the MAM model of schizophrenia. Specifically, increases in dopamine neuron population activity are observed in late pro-estrus or estrus during periods of high circulating estrogen and progesterone (Staley and Scharfman, 2005; Latini et al., 2008)). Furthermore, this increase in dopamine system function likely involves an effect of progesterone, but not estrogen, in the vHipp as mifepristone blockade of progesterone receptors significantly attenuated dopamine neuron activity to a level consistent with that observed in control female rats. Taken together, these data demonstrate changes in dopamine neuron activity across the reproductive cycle of the MAM model of schizophrenia which may contribute to the reported gender differences in symptomatology observed in schizophrenia patients.

An increase in dopamine system function is one of the oldest hypotheses of schizophrenia (Laruelle and Abi-Dargham, 1999; Abi-Dargham, 2004). Consistent with this hypothesis, we have previously demonstrated an augmented dopamine neuron activity in the MAM rodent model of schizophrenia (Lodge and Grace, 2007; Perez and Lodge, 2013; Perez et al., 2013). Specifically, MAM-treated rats display a significantly greater dopamine neuron population activity, defined as the number of dopamine neuron firing spontaneously. It is thought that the number of spontaneously activity dopamine neurons reflects a gain of function that can be modified to enhance dopamine signaling. Thus, a pathologically enhanced dopamine neuron population activity would likely cause a loss of this essential gain function, resulting in a high dopamine signal in response to relatively non-salient stimuli. Such aberrant dopamine signaling may therefore contribute to paranoia and other positive symptoms of schizophrenia. Here we demonstrate that MAM-treated female rats display a significant increase in dopamine neuron population activity which is consistent both with that observed in male rats (Lodge and Grace, 2007; Perez and Lodge, 2013; Perez et al., 2013) as well as with previous reports demonstrating behavioral deficits in female MAM-treated rats (Hazane et al., 2009).

Although schizophrenia affects both men and women, there are significant differences observed between genders that suggest a hormonal component to this disorder (for review see (Leung, 2000)). Unfortunately, preclinical studies of neuropsychiatric disease typically use only males due to potential confounds associated with hormonal fluctuations across the estrous cycle. However, the reasons underlying the choice of males for preclinical research is exactly the justification for which female rats should be more frequently examined. Indeed, there is increasing data demonstrating that in addition to hormonal variations,

functional and structural sex differences are observed in essentially every lobe of the brain (Cahill, 2006). Specifically related to schizophrenia, there is evidence that dopamine neuron activity varies across the estrous cycle (Zhang et al., 2008). Consistent with these data, we demonstrate that control female rats in estrus display significantly elevated dopamine neuron burst firing. Dopamine neuron burst firing is thought to promote increases in synaptic dopamine and reflects the functionally relevant signal sent to postsynaptic sites (Grace et al., 2007). In contrast, MAM-treated rats did not display differences in burst firing across the estrous cycle. This is not likely due to changes in the levels of circulating hormones in MAM-treated rats, as we did not observe alterations in either plasma estradiol or progesterone. Thus, it is probable that differences between MAM and saline treated rats reflects differential effects of sex hormones on afferent inputs to the VTA. Specifically, dopamine neuron burst firing is known to be dependent on a tonic input from the laterodorsal tegmental nucleus (Lodge and Grace, 2006b) and regulated by adjacent regions of the mesopontine tegmentum (Floresco et al., 2003; Lodge and Grace, 2006a; Chen and Lodge, 2013). These regions have been demonstrated to contain estrogen receptors (Mitra et al., 2003) and therefore may contribute to the effects of estrus cycle on dopamine neuron burst firing; however, whether this occurs remains to be elucidated.

While changes in burst firing were not observed in MAM-treated rats, a significant increase in dopamine neuron population activity was observed during estrus. Given that aberrant increases in population activity are thought to underlie psychosis (Lodge and Grace, 2007, 2011), this suggests that hormonal fluctuations may contribute to psychosis in females. Indeed, preclinical studies have demonstrated that female rats display deficits in latent inhibition during specific phases of the estrous cycle (Arad and Weiner, 2008). Deficits in latent inhibition are thought to reflect a loss of the ability to adequately gate cognitive responses to an irrelevant stimuli and are consistently observed in schizophrenia patients (Baruch et al., 1988; Braff et al., 1992). Furthermore, latent inhibition is disrupted by increases in dopaminergic transmission (Swerdlow et al., 2003), whereas disruptions in latent inhibition observed during pro-estrus–estrus (i.e. periods of the estrous cycle where we demonstrate increases in dopamine neuron population activity) are restored by antipsychotic administration (Arad and Weiner, 2008). Consistent with these preclinical data, female patients with schizophrenia demonstrate alterations in symptoms (Riecher-Rossler et al., 1994; Bergemann et al., 2007; Riecher-Rossler and Kulkarni, 2011) as well as reductions in hormonal fluctuations across the estrus cycle (Riecher-Rossler and Kulkarni, 2011). Interestingly, this literature has largely focused on the purported protective effects of estrogen in patients (Riecher-Rossler et al., 1994; Bergemann et al., 2007; Riecher-Rossler and Kulkarni, 2011) and have largely overlooked a role for progesterone. Moreover, the specific regions of the brain whereby sex hormones may alter dopamine system function has not been elucidated.

We have previously demonstrated that an augmented dopamine system function and aberrant behaviors are mediated by increased hippocampal activity in male MAM-treated rats (Lodge and Grace, 2007; Gill et al., 2011; Perez et al., 2013; Shah and Lodge, 2013). This is consistent with data from schizophrenia patients demonstrating enhanced baseline hippocampal activity that is correlated with the severity of positive symptoms (Schobel et al., 2009). Given the localization of both estrogen and progesterone receptors in the

hippocampus (Mitterling et al., 2010; Zuloaga et al., 2014), combined with previous studies demonstrating alterations in hippocampal function across the female reproductive cycle (Verrotti et al., 2010), we sought to determine whether the cyclical differences in dopamine neuron activity in MAM-treated rats were due to hormonal influences within the vHipp. We specifically examined rats in late pro-estrus-estrus as this reflects the period of high circulating estrogen and progesterone and is also the phase of the estrus cycle that displayed the most robust increases in dopamine neuron activity. Here we demonstrate that while blockade of vHipp estrogen receptors does not modulate dopamine neuron activity in MAM-treated rats during estrus, mifepristone blockade of the progesterone receptors did significantly attenuate dopamine neuron activity to a level consistent with that observed in control female rats. It should be noted that in addition to its potent affinity for the progesterone receptor, mifepristone also displays anti-glucocorticoid activity (Heikinheimo et al., 2003). We do not think this contributes to the effect of mifepristone in the current study as corticosterone has been shown to decrease hippocampal activity (Vidal et al., 1986). Blocking glucocorticoid receptors would therefore produce the opposite response than that observed (i.e. an increase rather than decrease in dopamine neuron population activity).

The effects of progesterone on hippocampal activity are complex due, in part, to effects of metabolites on GABAergic receptors (Majewska et al., 1986; Purdy et al., 1991; Paul and Purdy, 1992); however, progesterone has been reported previously to augment excitatory transmission in the hippocampus (Edwards et al., 2000). Thus, we posit that increases in progesterone, such as those occurring during the luteal phase of the estrus cycle, may augment positive symptoms of schizophrenia. Moreover, the effect of progesterone may be masked, in part, by the protective effects of estrogen (Riecher-Rossler et al., 1994; Bergemann et al., 2007; Riecher-Rossler and Kulkarni, 2011).

Taken together, we demonstrate changes in dopamine neuron activity across the reproductive cycle of the MAM model of schizophrenia which may contribute to the reported gender differences in symptomatology observed in schizophrenia patients. Moreover, we demonstrate that progesterone may augment dopamine system function in MAM-treated rats, via an action in the hippocampus and may provide a novel site of intervention for female patients. A better understanding of the neurobiology underlying gender differences in diseases such as schizophrenia, will lead to novel therapeutic approaches and adjunct treatments particularly for female patients.

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Figure 1.

Vaginal cytology was used to determine the phase of the estrous cycle of each rat. Representative photomicrographs of unstained vaginal smears depict rats in estrus (consisting primarily of cornified cells: A), met-estrus (with a mixture of cornified cells and leukocytes: B), di-estrus (mainly displaying leukocytes: C) and pro-estrus (consisting of nucleated epithelial cells: D) (Caligioni, 2001).

Figure 2.

Plasma estradiol (A) and progesterone (B) levels were measured by ELISA and are reported across the estrous cycle (determined by vaginal cytology) of both saline- and MAM- treated rats. Significant increases in plasma estrogen were observed from rats in pro-estrus when compared to met-estrus or di-estrus. There were no significant differences in plasma estrogen or progesterone levels between saline- and MAM- treated rats at any phase of the estrous cycle (n=6–11 rats/group; two-way ANOVA; † denotes significant difference ($p<0.05$) between groups as determined by Holm Sidak post-hoc analysis).

Figure 3.

Saline- and MAM- treated rats display distinct alterations in dopamine neuron activity across the estrous cycle. Saline-treated rats do not display alterations in dopamine neuron population activity (A) or average firing rate (B) across the estrus cycle; however, increases in dopamine neuron burst firing are observed in estrus (C). In contrast, MAM-treated rats demonstrated a significantly enhanced dopamine neuron population activity in estrus (D) without significant changes in average firing rate (E) or burst firing (F) (n=6–11 rats/group; one-way ANOVA/ANOVA on ranks; * denotes significant difference ($p < 0.05$) between depicted groups as determined by Dunn's post-hoc analysis).

Figure 4.

MAM-treated rats display an augmented dopamine neuron population activity (A) in late pro-estrus - estrus when compared to saline-treated controls, consistent with previous observations in male MAM-treated rats (Lodge and Grace, 2007). No significant differences were observed in either average firing rate (B) or burst firing (C) (n=8–10 rats/group; * denotes significant difference ($p < 0.05$) between depicted groups as determined by a Mann-Whitney Rank Sum Test).

Figure 5.

Hippocampal progesterone regulates the increased population activity in MAM-treated rats. Intra-vHipp administration of the progesterone antagonist, mifepristone reversed the increase in dopamine neuron population activity (A) observed in late pro-estrus - estrus in MAM-treated rats. Mifepristone did not alter average firing rate (B) or burst firing (C) of midbrain dopamine neurons. In contrast to the effects of progesterone blockade, the intra-hippocampal administration of the estrogen receptor antagonist, fulvestrant, did not alter any parameter of dopamine neuron activity in MAM-treated rats (n=8–10 rats/group; ANOVA on ranks; * denotes significant difference ($p<0.05$) between mifepristone and vehicle groups as determined by Dunn's post-hoc analysis).