

## REGULAR RESEARCH ARTICLE

# Orexin Modulation of VTA Dopamine Neuron Activity: Relevance to Schizophrenia

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## Abstract

**Background:** The hippocampus is a region consistently implicated in schizophrenia and has been advanced as a therapeutic target for positive, negative, and cognitive deficits associated with the disease. Recently, we reported that the paraventricular nucleus of the thalamus (PVT) works in concert with the ventral hippocampus to regulate dopamine system function; however, the PVT has yet to be investigated as target for the treatment of the disease. Given the dense expression of orexin receptors in the thalamus, we believe these to be a possible target for pharmacological regulation of PVT activity.

**Methods:** Here we used the methylazoxymethanol acetate (MAM) rodent model, which displays pathological alterations consistent with schizophrenia to determine whether orexin receptor blockade can restore ventral tegmental area dopamine system function. We measured dopamine neuron population activity, using *in vivo* electrophysiology, following administration of the dual orexin antagonist, TCS 1102 (both intraperitoneal and intracranial into the PVT in MAM- and saline-treated rats), and orexin A and B peptides (intracranial into the PVT in naïve rats).

**Results:** Aberrant dopamine system function in MAM-treated rats was normalized by the systemic administration of TCS 1102. To investigate the potential site of action, the orexin peptides A and B were administered directly into the PVT, where they significantly increased ventral tegmental area dopamine neuron population activity in control rats. In addition, the direct administration of TCS 1102 into the PVT reproduced the beneficial effects seen with the systemic administration in MAM-treated rats.

**Conclusion:** Taken together, these data suggest the orexin system may represent a novel site of therapeutic intervention for psychosis via an action in the PVT.

**Key Words:** Schizophrenia, paraventricular nucleus of the thalamus, orexins, dopamine

## Introduction

The ventral hippocampus (vHipp) and paraventricular nucleus of the thalamus (PVT) have been demonstrated to work in concert to regulate the activity of the mesolimbic dopamine system via a polysynaptic circuit involving the nucleus accumbens (NAc), ventral pallidum (VP), and ventral tegmental area (VTA) (Floresco et al., 2001; Perez and Lodge, 2018). Individuals with schizophrenia display baseline hyperactivity in select hippocampal subregions, which is correlated with symptom severity (Heckers,

2001, 2004; Schobel et al., 2009). Similarly, rodent models used to study this disorder also display elevated baseline activity in the vHipp, which is directly responsible for an increase in dopamine neuron population activity (defined as the number of spontaneously active dopamine neurons) in the VTA (Lodge and Grace, 2007; Aguilar, 2014; Perez et al., 2016; Perez and Lodge, 2018, 2019). The PVT, much like the vHipp, can regulate VTA dopamine neuron population activity, without affecting average firing rate

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## Significance Statement

Available pharmacological therapies for the treatment of schizophrenia are not always effective and produce undesirable side effects. For these reasons, patients with schizophrenia often discontinue antipsychotic treatments; thus, better medications are needed to adequately treat this devastating psychiatric disorder. Here, we demonstrate that the orexin system, via an action in the paraventricular nucleus of the thalamus, may represent a novel therapeutic target for the treatment of psychosis.

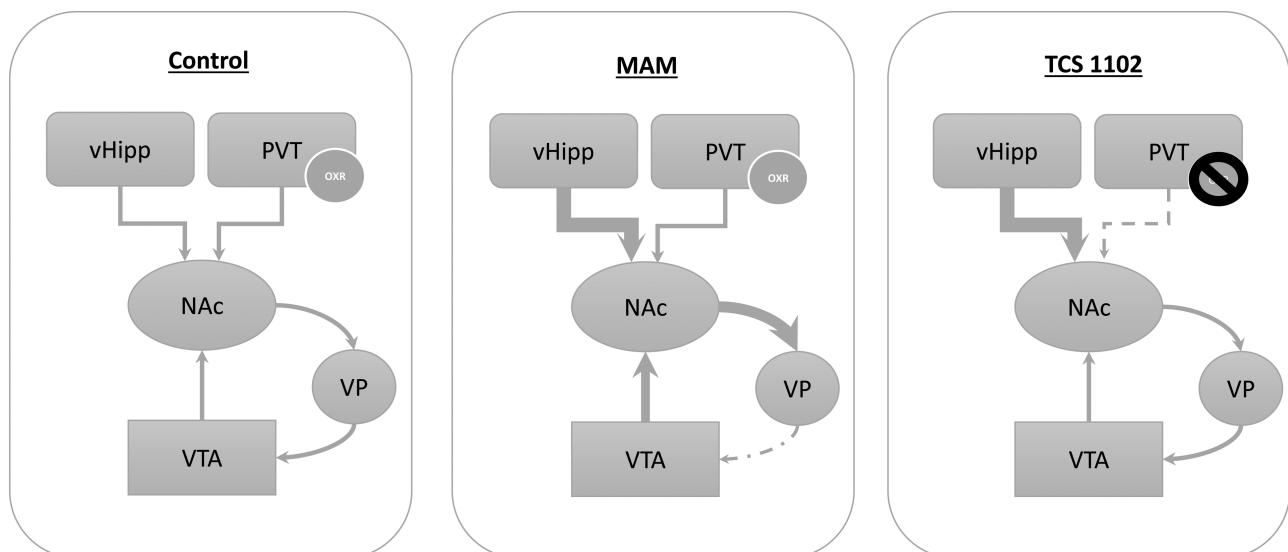
or bursting, via an indirect pathway involving the NAc and VP (see Figure 1) (Perez and Lodge, 2018). Further, activity in the vHipp is required for PVT-induced increases in dopamine neuron population activity, with the converse also being true (Perez and Lodge, 2018). Studies have demonstrated prominent glutamatergic projections from the PVT to the NAc (Su and Bentivoglio, 1990; Bubser and Deutch, 1998; Pinto et al., 2003; Dong et al., 2017). Indeed, convergent inputs from the vHipp and PVT onto medium spiny neurons of the NAc work in concert to regulate VTA dopamine activity (see Figure 1) (Perez and Lodge, 2018). In addition, the PVT can regulate dopamine system function by direct modulation of dopamine terminals within the NAc (Perez and Lodge, 2018). These data suggest that the PVT may be a novel site of intervention for the treatment of psychosis in schizophrenia. Indeed, pharmacological or chemogenetic inactivation of the PVT can restore normal dopamine system function in different rodent models used to study schizophrenia (Perez and Lodge, 2018).

A unique characteristic of the PVT is the dense and robust peptidergic innervation, including those by the orexin/hypocretin (ORX) peptides, which are of particular importance to this study (Kirouac, 2015). PVT neurons are dose dependently excited by orexin peptides A (OXA) and B (OXB) (Ishibashi et al., 2005). OXA and OXB are ligands of the G-protein-coupled receptors, orexin 1 and orexin 2, and display differential affinities such that  $OX_1R$  has a higher affinity for OXA ( $EC_{50}$  30 nM) than OXB ( $EC_{50}$  250 nM), whereas  $OX_2R$  is equally sensitive to OXA ( $EC_{50}$  34 nM) and OXB ( $EC_{50}$  60 nM). Activation of these receptors excites target neurons through various second messenger systems (Kukkonen, 2013) and can directly (Nakamura et al., 2000) and indirectly (Perez

and Lodge, 2018) modulate VTA dopamine system function. Additionally, orexin peptides produce dose-dependent increases in the firing rates of PVT neurons (Ishibashi et al., 2005), and these neurons can regulate dopamine levels in the NAc (Parsons et al., 2007). Dysfunction of the dopamine system has long been associated with positive symptoms of schizophrenia (i.e., psychosis) (Abi-Dargham et al., 1998; Laruelle and Abi-Dargham, 1999; Abi-Dargham, 2004; Howes et al., 2012). Taken together, we posit that targeting ORX receptors in the PVT can reverse the dysfunction present in the mesolimbic dopamine system commonly observed in preclinical models of the disease and, potentially, in individuals with schizophrenia. Here, we used a dual orexin receptor antagonist, TCS 1102, to evaluate whether this approach can normalize aberrant VTA dopamine neuron population activity in rats treated with methylazoxymethanol acetate (MAM), a gestational disruption that models some of the pathological alterations of schizophrenia (Moore et al., 2006; Lodge and Grace, 2009). This compound was specifically chosen because it blocks both  $OX_1R$  and  $OX_2R$  and has been shown to effectively block orexin-mediated behaviors for up to 4 hours (Bergman et al., 2008; Winrow et al., 2010). Moreover, similar compounds are already approved by the FDA for the treatment of sleep disorders. Here, we provide evidence that targeting the ORX system may be a novel approach for the treatment of psychosis in schizophrenia.

## Materials and Methods

All experiments were performed in accordance with the guidelines outlined in the USPH Guide for the Care and Use



**Figure 1.** Schematic representation of the polysynaptic circuit involved in the regulation of dopamine neuron population activity, which includes the ventral hippocampus (vHipp), paraventricular nucleus of the thalamus (PVT), nucleus accumbens (NAc), ventral pallidum (VP), and ventral tegmental area (VTA). Under “normal” conditions, GABAergic projections from the VP provide a tonic suppression of VTA activity (left). Methylazoxymethanol acetate-treated rats model schizophrenia, whereby aberrant glutamatergic inputs to the NAc from the vHipp drive a downstream hyperfunction of the dopamine system (center). Aberrant dopamine neuron activity can be reversed by blocking orexin 1 and 2 receptors ( $OX_{1,2}R$ ) in the PVT, resulting in a decreased glutamatergic transmission to the NAc (right). Adapted from (Grace et al., 2007).

of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of UT Health San Antonio.

## Animals

All experiments were performed on multiple litters of adult (>12 weeks) male MAM- and saline-treated rats, as previously described (Moore et al., 2006; Lodge, 2013). In brief, timed-pregnant female Sprague-Dawley rats were obtained from Envigo RMS Inc. (Indianapolis, IN) on gestational day (GD) 16. MAM (diluted in saline, 22 mg/kg i.p.) was administered on GD17, while control rats received injections of saline (1 mL/kg, i.p.). Pups were weaned on post-natal day 21 and housed with littermates in groups of 2–3 rats per cage. A separate set of experiments was performed on untreated adult male Sprague-Dawley rats obtained from Envigo.

## Drug Administration

For systemic drug administration, TCS 1102 (10 mg/kg; Chen et al., 2014; Ki values were 3.0 and 0.2 nM for OXR1 and OXR2, respectively) or vehicle (60% dimethyl sulfoxide in saline; 1 mL/kg) was administered i.p. (MAM- or saline-treated rats) 15 minutes prior to any electrophysiological recordings. Although the half-life of TCS 1102 is relatively short (approximately 20 minutes), behavioral responses are observed for up to 4 hours following administration (Bergman et al., 2008), which is consistent with the average time to complete VTA recordings for a single animal (2–3 hours). For unilateral intracranial drug administration, a 26-gauge guide cannula (Plastics One, Roanoke County, VA) was lowered into/immediately adjacent to the PVT (A/P –2.0 mm, M/L +0.4 mm from bregma and D/V –5.8 mm ventral of the brain surface) to avoid damage to the relatively small structure as previously reported (Perez and Lodge, 2018). An internal cannula (Plastics One), extending 1 mm past the end of the guide cannula, was used to deliver a 1-time injection of vehicle, peptides (OXA or OXB: 3 µg/750 nL; untreated SD rats), or TCS 1102 (1.5 µg/0.5 µL; MAM- or saline-treated rats) at a rate of approximately 0.5 µL/min at 10 minutes prior to any electrophysiological recording.

## Extracellular Dopamine Neuron Recordings

Male rats (approximately 300–450 g) were anesthetized with 8% chloral hydrate (400 mg/kg, i.p.), as this anesthetic does not significantly depress dopamine neuron activity (Hyland et al., 2002). Anesthesia was maintained by supplemental administration of chloral hydrate as required to maintain suppression of limb compression withdrawal reflex. Rats were positioned in a stereotaxic apparatus (Kopf, Tujunga, CA), and a core body temperature of 37°C was maintained by a thermostatically controlled heating pad (Kent Scientific, Torrington, CT). Extracellular glass microelectrodes (impedance: 6–14 MΩ) were lowered into the VTA (A/P ±5.3 mm, M/L ±0.6 mm from bregma and D/V –6.5 to –9.0 mm ventral of the brain surface) using a hydraulic micro-positioner (Kopf, Model 640) to measure dopamine neuron activity. Spontaneously active dopamine neurons were recorded for a period of 2–3 minutes and identified with open filter settings (low pass: 30 Hz; high pass: 30 kHz) using previously established electrophysiological criteria (Grace and Bunney, 1983). We measured 3 parameters of dopamine neuron activity: (1) population activity (defined as the number of spontaneously active dopamine neurons encountered while making 6–9 tracks or dorsal/ventral vertical passes), separated by 200 µm in a pre-determined pattern to sample equivalent regions of the VTA);

(2) basal firing rate; and (3) the proportion of action potentials occurring in bursts. At the cessation of all electrophysiological recordings, rats were rapidly decapitated.

## Analysis

Electrophysiological analysis of dopamine neuron activity was performed using 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 was analyzed by 1-way ANOVA, 2-way ANOVA (strain × treatment) with post hoc comparisons performed using the Holm-Sidak method, or Kruskal-Wallis 1-way ANOVA on ranks with post hoc comparisons performed using Dunn's method. Data are represented as the mean ± SEM unless otherwise stated, with n values representing the number of rats per group or neurons recorded per experimental group where indicated. Significance was determined at  $P < .05$ . All statistics were calculated using SigmaPlot (Systat Software, Chicago, IL).

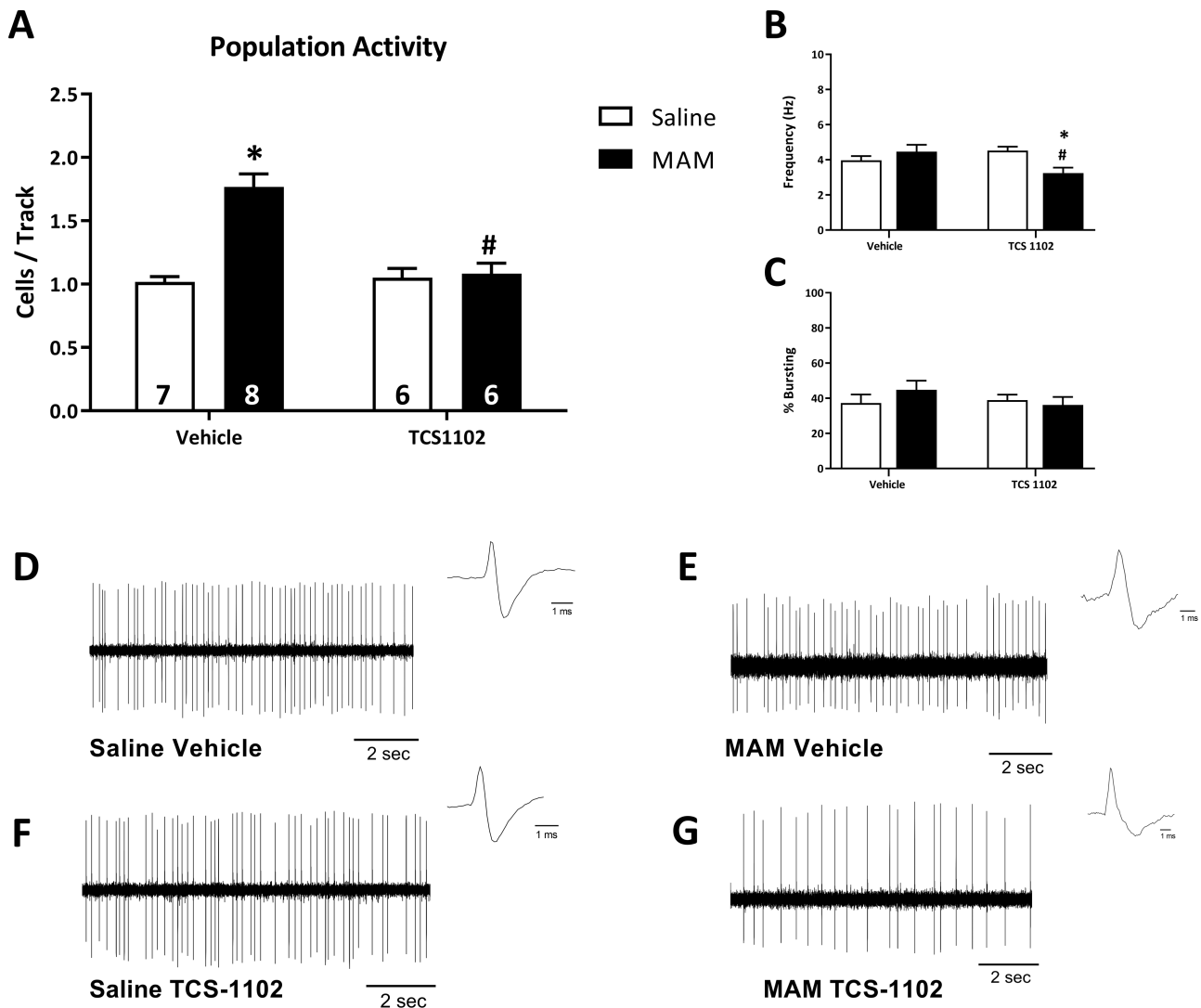
## Materials

MAM was purchased from Midwest Research Institute (Kansas City, MO). TCS 1102 was purchased from Tocris (Cat. No. 3818; Minneapolis, MN). Orexin B was purchased from R & D systems (Catalog # 1457; Minneapolis, MN), and Orexin A was sourced from Cayman Chemicals (Item No. 15073; Ann Arbor, MI). Chloral hydrate was sourced from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were of either analytical or laboratory grade and purchased from standard suppliers.

## Results

### TCS 1102, a Dual Orexin Antagonist, Reverses Aberrant VTA Dopamine Neuron Population Activity

We used TCS 1102 to examine whether blockade of orexin signaling could modulate VTA dopamine neuron activity in MAM-treated rats (Figure 2A). Similar to previous reports, SD control rats ( $n=7$  rats) displayed a population activity of  $1.02 \pm 0.04$  cells per track, and MAM-treated rats ( $n=8$  rats) displayed a significantly higher population activity ( $1.77 \pm 0.10$  cells per track; 2-way ANOVA;  $F_{(1,26)Strain} = 22.38$ ;  $P < .001$ ;  $F_{(1,26)Treatment} = 15.52$ ;  $P < .001$ ;  $F_{(1,26)Interaction} = 18.73$ ;  $P < .001$ ; Holm-Sidak post hoc;  $t = 6.79$ ;  $P < .001$ ). The i.p. administration of TCS 1102 had no effect on saline-treated rats ( $n=6$  rats;  $1.05 \pm 0.07$  cells per track); however, it restored normal VTA dopamine system function in MAM-treated rats ( $n=6$  rats;  $1.08 \pm 0.08$  cells per track; Holm-Sidak post hoc;  $t = 5.93$ ;  $P < .001$ ). Consistent with previous reports, saline-treated vehicle rats displayed an average firing rate of  $3.97 \pm 0.24$  Hz ( $n=45$  neurons; Figure 2B). The average firing rate of MAM-treated rats also treated with TCS 1102 ( $n=38$  neurons;  $3.24 \pm 0.31$  Hz) displayed a significant decrease compared with control MAM-treated rats ( $n=83$  neurons;  $4.52 \pm 0.23$  Hz; 2-way ANOVA;  $F_{(1,202)Interaction} = 8.53$ ;  $P = .004$ ; Holm-Sidak post hoc;  $t = 3.19$ ;  $P = .002$ ) and saline-treated rats administered TCS 1102 ( $n=37$  neurons;  $4.46 \pm 0.40$  Hz; Holm-Sidak post hoc;  $t = 2.59$ ;  $P = .01$ ). No significant differences were observed in the average percent bursting between any of the groups (Figure 2C; saline-vehicle:  $n=45$  neurons;  $36.12 \pm 3.93\%$  bursting; MAM-vehicle:  $n=83$  neurons;  $38.97 \pm 3.08\%$  bursting; saline-TCS 1102:  $n=37$  neurons;  $44.40 \pm 4.77\%$  bursting; MAM-TCS 1102:  $n=38$  neurons;  $36.15 \pm 4.56\%$  bursting).



**Figure 2.** Administration of the dual orexin receptor antagonist, TCS 1102, restores normal dopamine system function in the MAM rodent model of schizophrenia (A). MAM-treated vehicle rats display a significant increase in ventral tegmental area (VTA) dopamine neuron activity compared with saline-treated vehicle rats. \* $P < .001$ . Further, TCS 1102 administration restores normal dopamine system function in MAM-treated rats. \* $P = .01$  denotes significance from MAM-treated vehicle rats. The average firing rate of VTA dopamine neurons is attenuated by TCS 1102 administration (B). \* $P = .002$  denotes significance from MAM-treated vehicle rats. \* $P = .01$  denotes significance from saline-treated rats administered TCS 1102. No differences were observed in the average percent bursting between any groups (C). Representative dopamine recording and action potential from a saline-treated vehicle (D), MAM-treated vehicle (E), saline-treated TCS 1102 (F), and MAM-treated TCS 1102 (G) rats.

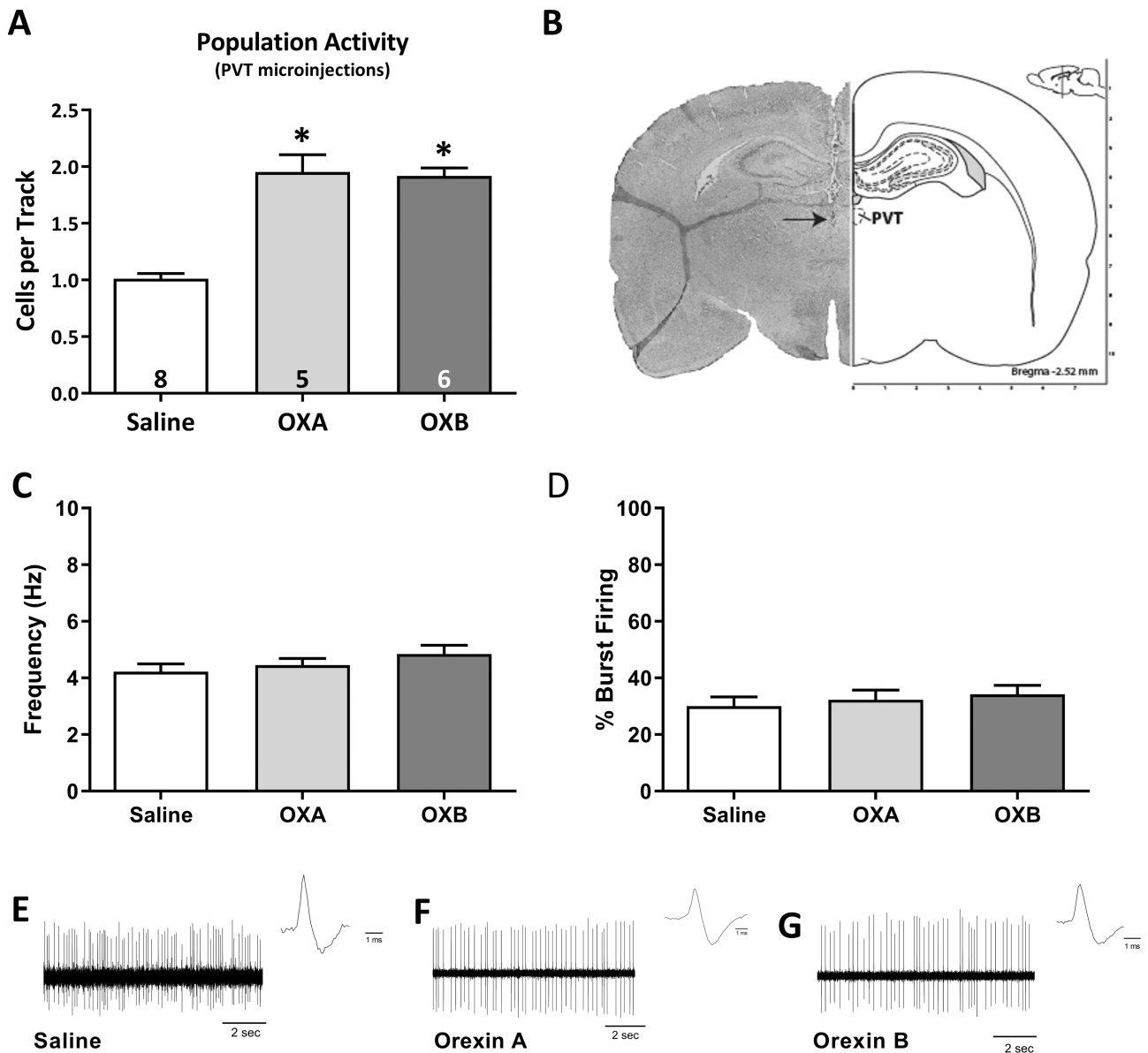
### Orexin Peptides A and B in the PVT Increase VTA Dopamine Neuron Population Activity

To determine whether orexin modulation of the PVT regulates VTA dopamine neurons, we unilaterally injected exogenous OXA and OXB peptides into the PVT (Figure 3A). Consistent with previous reports in Sprague-Dawley control rats, rats injected with vehicle displayed an average population activity of  $1.01 \pm 0.05$  cells per track ( $n = 8$  rats). Intra-PVT administration of the exogenous peptides, OXA ( $n = 5$  rats;  $1.95 \pm 0.15$  cells per track) or OXB ( $n = 6$  rats;  $1.92 \pm 0.07$  cells per track), caused a significant increase in population activity compared with control rats (1-way ANOVA;  $F_{(2,18)} = 42.69$ ;  $P < .001$ ; Holm-Sidak post hoc; OXA:  $t = 7.69$ ;  $P < .001$ ; OXB:  $t = 7.81$ ;  $P < .001$ ). No significant differences were observed in the average firing rate (Figure 3B; Kruskal-Wallis 1-way ANOVA on ranks; control:  $n = 56$  neurons;  $4.22 \pm 0.27$  Hz; OXA:  $n = 60$  neurons;  $4.45 \pm 0.24$  Hz; OXB:  $n = 65$  neurons;  $4.85 \pm 0.31$  Hz) or average percent bursting (Figure 3C; Kruskal-Wallis 1-way

ANOVA on ranks; control:  $n = 56$  neurons;  $30.03 \pm 3.27\%$  bursting; OXA:  $n = 60$  neurons;  $32.26 \pm 3.43\%$  bursting; OXB:  $n = 65$  neurons;  $34.19 \pm 3.23\%$  bursting) between any of the groups.

### TCS 1102 in the PVT Restores VTA Dopamine Neuron Population Activity

To determine whether orexin receptor blockade in the PVT could reverse aberrant VTA dopamine neuron population activity in MAM-treated rats, we unilaterally injected TCS 1102 or vehicle into the PVT of MAM- or saline-treated rats (Figure 4A). Consistent with previous reports in Sprague-Dawley control rats, saline-treated rats injected with vehicle displayed an average population activity of  $1.01 \pm 0.05$  cells per track ( $n = 8$  rats), and MAM-treated rats injected with vehicle displayed a significant increase in population activity ( $n = 6$  rats;  $1.67 \pm 0.06$  cells per track) compared with saline-treated controls (2-way ANOVA;  $F_{(1,23)\text{Strain}} = 13.79$ ;  $P = .001$ ;  $F_{(1,23)\text{Treatment}} = 12.74$ ;  $P = .002$ ;



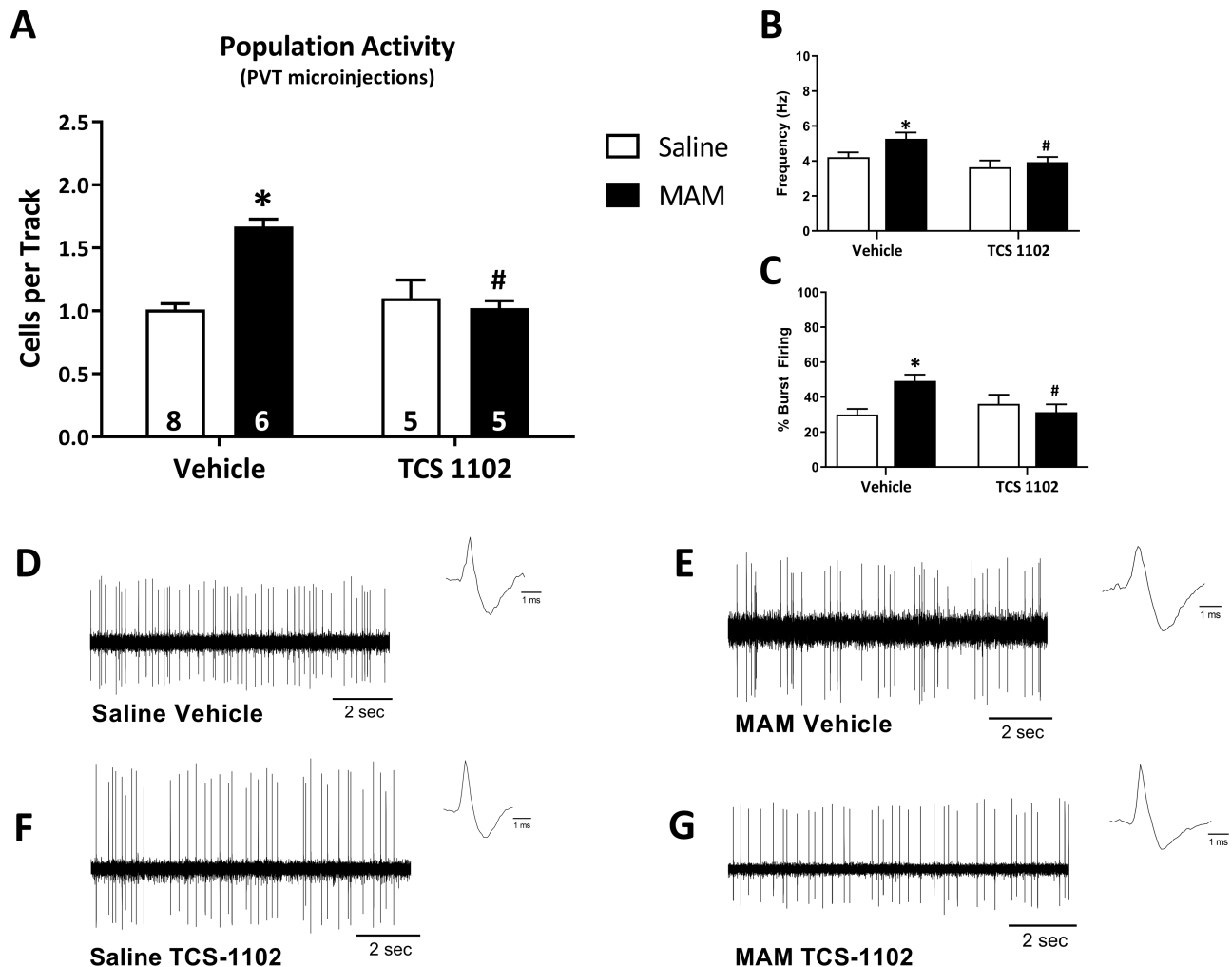
**Figure 3.** Intracranial microinjection of exogenous orexin A (OXA) or orexin B (OXB) peptides into the paraventricular nucleus of the thalamus (PVT) causes a significant increase in ventral tegmental area (VTA) dopamine neuron activity (A). \* $P < .001$  denotes significance from vehicle control. No differences were observed in the average firing rates (C) or average percent bursting between any groups (D). B depicts a representative brain slice (left) with a canula track indicated by an arrow in the PVT and corresponding schematic of the brain section (right). Representative dopamine recording and action potential from a control saline (E), OXA (F), and OXA (G) microinjected rats.

$F_{(1,23)Interaction} = 22.18$ ;  $P < .001$ ; Holm-Sidak post hoc;  $t = 6.49$ ;  $P < .001$ ). Intra-PVT administration of TCS 1102 caused a significant decrease in population activity in MAM-treated rats ( $n = 5$  rats;  $1.02 \pm 0.06$  cells per track) compared with MAM-treated control rats (Holm-Sidak post hoc;  $t = 5.69$ ;  $P < .001$ ). No changes in population activity were observed in saline-treated rats injected with TCS 1102 in the PVT ( $n = 5$  rats;  $1.10 \pm 0.15$  cells per track). Vehicle MAM-treated rats ( $n = 60$  neurons;  $5.27 \pm 0.36$  Hz;  $49.27 \pm 3.57\%$  burst firing) displayed an elevated firing rate (Figure 4B) and bursting pattern (Figure 4C) compared with saline-treated vehicle control rats ( $n = 56$  neurons;  $4.22 \pm 0.27$  Hz; 2-way ANOVA; Holm-Sidak post hoc;  $t = 2.50$ ;  $P = .01$ ;  $30.03 \pm 3.27\%$  burst firing;  $t = 3.90$ ;  $P < .001$ ) and MAM-treated rats injected with TCS 1102 in the PVT ( $n = 33$  neurons;  $3.94 \pm 0.29$  Hz; 2-way ANOVA; Holm-Sidak post hoc;  $t = 2.73$ ;  $P = .007$ ;  $31.37 \pm 4.54\%$  burst firing;  $t = 3.11$ ;

$P < .001$ ). TCS 1102 injected in the PVT had no effect on saline-treated rats ( $n = 29$  neurons;  $3.65 \pm 0.39$  Hz;  $36.11 \pm 5.31\%$  burst firing).

## Discussion

The thalamus serves as a major point of convergence for various neuronal circuits and is composed of several nuclei, each with distinct afferent and efferent projections (Andreasen et al., 1994; Byne et al., 2009). Moreover, thalamic abnormalities have been previously implicated in schizophrenia (Pergola et al., 2015). Specifically, a decrease in the thalamic volume and reduced gray matter has been reported in schizophrenia patients compared with healthy controls (Andreasen et al., 1994; Buchsbaum et al., 1996; Gilbert et al., 2001; McDonald et al., 2005; Glahn et al., 2008;



**Figure 4.** Intracranial microinjection of the dual orexin antagonist, TCS 1102, into the paraventricular nucleus of the thalamus (PVT), restored normal dopamine system function in MAM-treated rats (A). \* $P < .001$  denotes significance from saline-treated vehicle. # $P < .001$  denotes significance from MAM-treated vehicle. TCS 1102 administration attenuated the average firing rate (B) and average percent bursting (C) in MAM-treated vehicle rats. \* $P < .05$  denotes significance from saline-treated vehicle. # $P < .05$  denotes significance from MAM-treated vehicle. Representative dopamine recording and action potential from a saline-treated TCS 1102 (D) and MAM-treated TCS 1102 (E) rats.

Fornito et al., 2009). Furthermore, positron emission tomography studies performed in individuals with schizophrenia report abnormal activation of the thalamus during active auditory hallucinations (Andreasen et al., 1992) and decreased thalamic blood flow (Andreasen et al., 1996; Andreasen, 1997). However, the exact role of discrete thalamic nuclei in schizophrenia remains unclear, and it is not currently known whether the structural and functional alterations observed in this disease are a consequence of, or contribute to, the symptoms of schizophrenia (Sim et al., 2006; Keedy et al., 2009; Pergola et al., 2015).

Of importance to this study is the PVT and its innervation of the NAc (Moga et al., 1995; Pinto et al., 2003; Kirouac, 2015; Dong et al., 2017). The PVT sends direct and indirect projections to dopamine neurons within the VTA (Zahm and Heimer 1990; Zahm 2000) and synapse on both medium spiny neurons and dopamine terminals within the NAc (Pinto et al., 2003; Perez and Lodge, 2018). Thus, in addition to direct modulation of NAc neurons, the PVT can regulate presynaptic dopamine release (Pinto et al., 2003; Parsons et al., 2007). Indeed, it has been reported that glutamate release from PVT terminals can act on ionotropic glutamate receptors to induce dopamine efflux in

the NAc (Parsons et al., 2007). Thus, a decrease in PVT activity, by orexin receptor blockade, may decrease dopamine signaling multiple mechanisms, including (1) a direct effect on pre-synaptic release, and (2) an indirect effect on dopamine neuron activity in the VTA. We recently demonstrated that pharmacological (N-methyl-D-aspartate) activation of the PVT induces an increase in VTA dopamine neuron population activity without affecting the firing rate or bursting pattern of these neurons (Perez and Lodge, 2018), as those measures are altered by manipulations to other brain regions (Murase et al., 1993; Floresco et al., 2003). Indeed, this is consistent with previous studies examining the regulation of VTA dopamine neurons by the vHipp, in which activation of the vHipp produced selective increases in dopamine neuron population activity (Lodge and Grace, 2007). Interestingly, this PVT-induced increase in population activity is dependent on glutamatergic projections to the NAc, as chemogenetic activation of the PVT-NAc pathway produces a similar increase in dopamine neuron population activity (Perez and Lodge, 2018). Further, inactivation of the PVT was able to reverse aberrant dopamine neuron activity thought to contribute to psychosis in schizophrenia (Perez and Lodge, 2018).

The PVT receives a dense innervation from the ORX system (Kirouac, 2015) and can directly (via direct innervation of dopaminergic neurons of the VTA (Nakamura et al., 2000) or indirectly (via projections to the NAc (Perez and Lodge, 2018) modulate dopamine system function. Thus, we used the MAM rodent model, which displays neurophysiological and behavioral deficits consistent with schizophrenia to examine whether targeting the ORX system could reverse the mesolimbic dopamine system dysfunction, commonly observed in individuals with schizophrenia. As mentioned previously, numerous rodent models used to study the neurobiology of schizophrenia exhibit a significant increase in dopamine neuron population activity (Lodge and Grace, 2007; Shah and Lodge, 2013; Aguilar, 2014; Boley et al., 2014; Perez et al., 2016, 2019a, 2019b). We observed a similar increase in MAM-treated vehicle rats, as they displayed a significant increase in VTA dopamine neuron population activity compared with saline-treated vehicle rats. Importantly, TCS 1102 administration was able to restore normal dopamine system function in MAM-treated rats, demonstrating that the ORX system may be a therapeutic target (Figure 2A). It should be noted that MAM-treated rats treated with TCS 1102 displayed a slight decrease in the average firing rate of VTA dopamine neurons compared with MAM-treated vehicle rats and saline-treated rats administered TCS 1102 (Figure 2B). Although we did observe this significant difference, it is likely that only subpopulations of dopamine neurons are affected. The ORX system has been demonstrated to facilitate dopamine neuron activity and fluctuations in ORX levels and can influence the excitability of VTA dopamine activity (Moorman and Aston-Jones 2010). Thus, direct effects of the ORX antagonist on dopamine neurons are possible; however, the dramatic effects on population activity are likely attributable to indirect regulation.

Orexin peptides play a role in wakefulness and have been demonstrated to increase arousal and locomotor activity (Hagan et al., 1999; Alexandre et al., 2013). Although some antipsychotics have sedative effects on patients, this is often related to the dose and this sedative effect is not thought to contribute to the mechanism of action (Miller 2004; Muench and Hamer, 2010). Indeed, other compounds that affect arousal, such as benzodiazepines, are not effective monotherapies (Dell'osso and Lader 2013); therefore, it is likely that the results here are indicative of changes in the regulation of dopamine system function rather than nonselective changes in arousal. Further, it should be noted that the PVT has been shown to be activated after stressful or aversive events (Bubser and Deutch, 1999; Penzo et al., 2015). Interestingly, stress is also a known risk factor for a number of psychiatric conditions, including schizophrenia, suggesting that the PVT might be a potential site of convergence for stress-related psychiatric disorders (Day et al., 1987; Malla et al., 1990). To determine whether ORX system modulation could increase VTA dopamine neuron population activity via the PVT, we performed direct microinjections of the OXA or OXB peptides and recorded VTA dopamine neuron activity (Figure 3). We evaluated the efficacy of OXA and OXB in modulating VTA dopamine neuron activity, as they differentially target  $OX_1R$  (binds OXA) and  $OX_2R$  (binds OXA and OXB), both of which are similarly expressed in the PVT (Marcus et al., 2001). Studies have reported activation of the dopamine system by orexins (Nakamura et al., 2000) and that these peptides produce excitatory effects in the PVT (Ishibashi et al., 2005). Interestingly, infusion of either OXA or OXB into the PVT produced a significant increase in dopamine neuron population activity (Figure 3A), similar to what has been reported in rodent models used to study schizophrenia (Lodge and Grace, 2007; Perez et al., 2016, 2019a, 2019b). This increase

was not observed in rodents that received vehicle infusions in the PVT, consistent with previous observations in control rats (Lodge and Grace, 2007; Perez et al., 2016, 2019a, 2019b). The ORX peptides were microinjected into or immediately adjacent to the PVT (Figure 3B) to avoid damage to the relatively small structure; thus, it is important to note that it is possible that other structures adjacent to the PVT may have been affected. Specifically, diffusion of the ORX peptides into the medial dorsal nucleus of the thalamus, immediately adjacent to the PVT, was likely; however, given that the density of ORX receptors in this region are very low, we do not believe this region to be modulated by ORX peptide infusion (Marcus et al., 2001). These data support previous chemogenetic studies targeting the PVT-NAc pathway (Perez and Lodge, 2018) and suggest that orexins can modulate mesolimbic dopamine neuron population activity via the PVT.

Lastly, to demonstrate that the effects of TCS 1102 on VTA dopamine neuron activity were indeed mediated by antagonizing orexin receptors, specifically in the PVT, we microinjected TCS 1102 into the PVT of MAM- and saline-treated rats and recorded the activity of VTA dopamine neurons. The significant increase in dopamine neuron population activity consistently observed MAM-treated rats was indeed reversed by local orexin receptor blockade in the PVT. This is consistent with a previous study that used chemogenetics to activate the PVT-NAc or the PVT-mPFC pathway and found that PVT-NAc activation was able to increase VTA dopamine neuron activity (Perez and Lodge, 2018). Thus, we posit that the effect observed in the current study is indeed specific to an action in the PVT. Further, it is likely that the ORX modulation occurs through an indirect pathway (Figure 1) involving the PVT and the NAc-VP-VTA polysynaptic circuit. These data validate the action of ORX in modulating and restoring dopamine system function in a model with relevance to schizophrenia.

Collectively, these data suggest that targeting the ORX system could be beneficial in restoring normal dopamine system function and eliminating symptoms of psychosis linked to dysfunction of that system. Further, data collected in this study support the idea that the FDA-approved dual orexin receptor antagonist, Suvorexant, may also treat symptoms of psychosis in individuals with schizophrenia. Suvorexant, at high doses, is effective at treating insomnia (Rhyne and Anderson 2015); however, lower, non-sedative doses could be effective at treating psychotic symptoms. Future studies will be aimed at observing the consequences of ORX modulation on behaviors associated with positive, negative, and cognitive symptom domains displayed by rodent models of schizophrenia. Indeed, activation of orexin neurons has been previously linked to improvements in working memory (Hahn et al., 2003; Pasumarthi and Fadel, 2008; Stanojlovic et al., 2019) as well as symptoms often comorbid with schizophrenia, such as anxiety (Li et al., 2010; Heydendael et al., 2014), depression (Arendt et al., 2013), and sleep disorders (Mieda and Sakurai 2013) (for orexin system review, see Chieffi et al., 2017). Further studies will explore the relative contribution of  $OX_1R$  and  $OX_2R$  separately, using an antagonist specific for each receptor. Therefore, this study provides evidence that the PVT is a potential site of intervention in schizophrenia and that targeting the ORX system may be a novel therapeutic approach for the treatment of psychosis.

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## Statement of Interest

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