Chapter

Orexin 2 Receptor Antagonists from Prefrontal Cortical Circuitry to Rodent Behavioral Screens

Gerard J. Marek, Stephen Chaney and Mark J. Benvenga

Abstract

Orexin is a neuropeptide contained in neurons from several hypothalamic nuclei that project throughout the forebrain analogously to monoamines synthesized by brainstem nuclei. Orexin, like 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (DA), histamine and acetylcholine (ACh) exerts prominent effects on the sleep-wake cycle of all mammals. Activation of the orexin$_2$ receptor appears to induce spontaneous excitatory synaptic currents (EPSCs) on layer V pyramidal neurons due to release of glutamate from thalamocortical terminals similar to activation of 5-HT$_{2A}$ and α_1-adrenergic receptors. Layer V pyramidal cells are the major descending output cell in the prefrontal cortex with projections to the thalamus, striatum, amygdala, brainstem and spinal cord. In keeping with salient modulation of prefrontal cortical physiology, orexin$_2$ receptor antagonists exert similar effects to 5-HT$_{2A}$ receptor antagonists in suppressing hallucinogen (e.g., DOI)-induced head twitches and producing antidepressant-like effects on the differential-reinforcement-of-low-rate 72-s (DRL 72-s) schedule of reinforcement. Currently, there is both negative and some preliminary positive evidence that blocking orexin$_2$ receptors may result in antidepressant efficacy in patients with major depressive disorder. Overall, the treatment of mood disorders is an additional potential indication for orexin receptor antagonists beyond simply improving sleep.

Keywords: antidepressant drug screens, excitatory postsynaptic potential currents (EPSCs), DOI-induced head twitches, differential-reinforcement-of-low-rate 72-s (DRL 72-s) behavior, LSN2424100, layer V pyramidal neurons, prefrontal cortex, thalamocortical axons

1. Introduction

Only approximately 50–60% of patients experience an antidepressant response when treated with selective reuptake inhibitors (SSRIs) or serotonin-norepinephrine reuptake inhibitors (SNRIs) [1–3]. Even those patients that do respond often continue to experience residual symptoms such as insomnia and cognitive dysfunction [4–7]. Thus, novel antidepressant medications are needed that treat a broader expanse of symptoms or are effective in patients that have failed several different classes of antidepressants drugs.

The primary well-documented augmentation treatment for depressed patients already on SSRIs or SNRIs are atypical antipsychotics (aripiprazole, quetiapine,

risperidone or olanzapine) and less so for mirtazapine/mianserin [8–12]. The common pharmacological action shared by these medications is blockade of 5-HT_{2A} receptors [13]. Blockade of 5-HT_{2A} receptors may also be a key pharmacological feature for most tricyclic antidepressant drugs which explain their greater antidepressant efficacy compared to SSRIs [14–17]. However, side effects especially problematic for augmentation of SSRIs/SNRIs with atypical antipsychotic drugs are weight gain and extrapyramidal symptoms. Thus, discovery of a drug targeted on key neurocircuitry modulated by 5-HT_{2A} receptors is one strategy to develop a novel antidepressant medication.

Given that pathophysiology of mood disorders appears to involve the prefrontal cortex and associated macrocircuits, an obvious candidate brain region to provide a context for 5-HT_{2A} receptor blockade at augmenting the effects of SSRIs/SNRIs is the prefrontal cortex [18–21]. In particular, layer V pyramidal neurons can effectively modulate important cortical circuits (including corticothalamic, corticostriatal, cortico-amydalar and cortico-brainstem) that impact mood, cognition/executive function, sleep and appetite [22, 23]. One aspect of 5-HT_{2A} receptor function largely restricted to layer V pyramidal cells is increasing the frequency of spontaneous excitatory postsynaptic currents/potentials (EPSC/EPSPs) onto the dendritic branches [24]. This effect appears to be mediated by AMPA receptor stimulation of directly on the layer V pyramidal cells [24, 25]. Lesion studies have suggested that 5-HT_{2A} receptor activation is releasing glutamate from thalamocortical terminals arising from the "non-specific" midline and intralaminar thalamic nuclei [26, 27]. There appear to be hot spots in layer I and layer Va where focal 5-HT-induced release of glutamate sensitive to the sodium channel blocker tetrodotoxin (TTX) occurs, although an amplification of postsynaptic currents, including TTX-sensitive sodium currents [24]. A number of G_i/G_o-coupled GPCRs (including $mGlu_2$, $mGlu_4$, μ-opioid, adenosine A_1 receptors) also suppresses 5-HT- or DOI-induced glutamate release from these terminals [28–33]. Several other G_q/G_{11}-coupled GPCRs (α_1-adrenergic receptors and $mGlu_5$ receptors) also appear to induce glutamate release onto layer V pyramidal neurons that are suppressed by the sodium channel blocker TTX, μ-opioid agonists, and AMPA receptor antagonists [34, 35]. This rich pharmacological modulation of 5-HT_{2A} receptor-mediated electrophysiological effects on dendritic integration for the principle output neurons in the prefrontal cortex provides heuristic promise for drug discovery efforts with respect to major psychiatric disease, including mood disorders and schizophrenia [36, 37].

The increase in spontaneous EPSC/EPSPs upon layer V pyramidal cells induced by 5-HT_{2A} receptor activation may be associated with other electrophysiological, biochemical and behavioral effects involving the medial prefrontal cortex (mPFC). On an electrophysiological level, electrical stimulation of the white matter below the cortex appears to result in an induction of "late" EPSC/EPSPs during washout after application of 5-HT or when the phenethylamine hallucinogen DOI is bath-applied to the cortical slice [38]. These late EPSCs are also suppressed by a range of neurotransmitter receptors that suppress spontaneous 5-HT-induced EPSCs such as agonists for $mGlu_2$, μ-opioid, and adenosine A_1 receptors [30, 32]. There are also some differences between these two electrophysiological responses as NMDA receptor stimulation appears important for the electrical stimulation/DOI-evoked responses unlike the spontaneous 5-HT-induced EPSC/EPSPs [39].

Secondly, systemic DOI administration also induces a range of immediate-early gene-like signals in the prefrontal cortex/neocortex that are also suppressed by activation of $mGlu_2$ autoreceptors and appear dependent on glutamate release from thalamocortical terminals [40–45]. This effect of prefrontal cortical 5-HT_{2A} receptor activation is relatively sparsely studied compared to the electrophysiological or behavioral sequelae.

Third, either systemic administration or local prefrontal cortical administration of agonists for 5-HT_{2A} receptors induces a robust increase in the frequency of head twitches (a behavior infrequently observed under baseline condition) [46, 47]. Agonists or positive allosteric modulators of $mGlu_2$, $mGlu_4$, μ-opioid, adenosine A_1 receptors also suppress DOI-induced head twitches [28, 31, 48–52]. Naturally, these head twitches induced by direct 5-HT_{2A} receptor agonists are also suppressed by a number of antidepressant drugs that potently block 5-HT_{2A} receptors or down-regulate 5-HT_{2A} receptors such as mirtazapine [53], mianserin [54–57], trazodone [55, 58–60], nefazodone [58, 61] and tricyclic antidepressants [55, 57, 62–68] Some of the tricyclic antidepressants are active only with chronic daily administration. While the antidepressant and monoamine oxidase inhibitor (MAOI) tranylcypromine does not directly bind to 5-HT_{2A} receptors, chronic daily administration of this antidepressant has been found to suppress 5-methoxy-N,N-dimethyltryptamine-induced head twitches under conditions associated with a down-regulation of 5-HT_{2A} receptors [63]. The clinical lore regarding μ-opioid receptor agonists and potential antidepressant action is intriguing in light of effects for this class of drugs on DOI-induced head twitches have been discussed elsewhere [36].

Finally, an argument was advanced recently that the basis for detecting antidepressant-like drug effects on the operant differential-reinforcement-of-low-rate 72-s (DRL 72-s) schedule may be related to the biology of a range of neurotransmitter systems that interact with the 5-HT_{2A} receptor in the prefrontal cortex to modulate motor impulsivity [69, 70]. As expected from the similar effects of 5-HT_{2A} receptor antagonists compared to $mGlu_2$ receptor positive allosteric modulators (PAMs) and also to adenosine A_1 receptor agonists for the prefrontal electrophysiology discussed above, 5-HT_{2A} receptor antagonists, $mGlu_2$ receptor PAMs and adenosine A_1 receptor agonists all test similar to known antidepressant drugs in rats performing under the DRL 72-s schedule [51, 71–77].

The underlying thesis of this chapter is that understanding how other neurotransmitter systems interact with 5-HT_{2A} receptors in the medial prefrontal cortex on an electrophysiological, biochemical and behavioral scale may help discover novel antidepressant drugs. Orexin (OX) receptor agonists/antagonists appear to be one such neurotransmitter system that interacts with critical biological aspects of 5-HT_{2A} receptor activation/blockade in thalamocortical pathways influencing the principle output (layer V pyramidal cells) of the prefrontal cortex in a manner suggesting that OX_2 receptor antagonists are putative antidepressant medications.

2. Orexin-2 receptor blockade and putative antidepressant action

The orexins are two peptide neurotransmitters produced in several nuclei within the lateral hypothalamus which are intimately involved in arousal and reward [78]. The name "orexin" was originally coined from the Greek word "orexis" when the orexin/hypocretin peptides were studied for effects on appetite. However, the more salient biological aspect of the orexin system later was realized to be altering sleep and arousal. More specifically, mutations of genes for the orexin-2 (OX_2) receptor, orexin peptides, and loss of orexin-containing hypothalamic cell bodies were demonstrated to be the genetic cause of narcolepsy in canines, mice and humans. The first approved medication targeting the orexin system, suvorexant, blocks both orexin-1 (OX_1) and OX_2 receptors as a dual orexin receptor antagonist (DORA) and is indicated for the treatment of insomnia [78, 79]. Several other DORAs have been shown to be efficacious in treating primary insomnia [80–82]. The overlapping and diverging distribution for the OX_1 and OX_2 mRNA and protein has inspired several decades of past/ongoing research exploring these receptors for sleep, arousal, feeding,

alcohol and drug self-administration, stress, anxiety and depression models [83]. The involvement of OX_2 receptors in arousal together with the presence of OX_2 receptor mRNA in the non-specific midline and intralaminar thalamic nuclei and the interactions of the orexin system with brainstem nuclei with overlapping monoamine projections makes the OX2 receptor an especially interesting target for mood disorder therapeutics [78, 83]. As discussed below, OX_2 or hypocretin-2 receptor blockade appears to be a mechanism of action that provides a means of testing the hypothesis discussed above where a drug appropriately modifying multiple levels of biological effects for 5-HT_{2A} receptor activation in the mPFC would be a putative antidepressant medication.

Electrophysiological effects of OX_2 receptor activation in the prefrontal cortex appear to parallel certain effects of 5-HT_{2A} receptor activation when recording from layer V pyramidal neurons. The orexin-B (hypocretin-2) peptide was found to increase spontaneous EPSC/EPSPs in layer V pyramidal neurons of the prefrontal cortex that were blocked by postsynaptic AMPA receptor antagonists as well as by TTX and u-opioid agonists on the presynaptic side similar to the case for 5-HT_{2A} receptor stimulation [84]. Experiments to delineate the origin of afferents in the PFC from which orexin induced glutamate release from suggested that the cells of origin were in the midline and intralaminar thalamic nuclei [84]. Further, the relative potency for orexin-B compared to orexin-A (hypocretin-1) at inducing spontaneous OX-induced EPSCs/EPSPs in PFC layer V pyramidal cells is similar to that found in the intralaminar and midline thalamic nuclei with OX_2, not OX_1, receptor responses [84–86]. The tetrodotoxin sensitivity of the orexin-induced EPSCs/EPSPs is in keeping with earlier studies suggesting that thalamocortical projections from these "non-specific" thalamic nuclei associated with arousal were prone to the generation of terminal spikes as previously suggested [87, 88]. This dependence on thalamocortical pathways originating in the midline and intralaminar thalamic nuclei and terminating in layers I and Va of the prefrontal cortex is consistent with features for the spontaneous 5-HT-induced EPSCs/EPSPs [26, 27]. One difference between OX-induced spontaneous EPSCs and 5-HT-induced EPSCS is that OX does not appear to induce postsynaptic depolarization (consistent with absence of OX_2 mRNA in layer V pyramidal cells) unlike the case for 5-HT_{2A} receptor activation in the majority of layer V pyramidal cells [84, 89]. However, studies characterizing the ability of orexin-B induced EPSCs/EPSPs to be blocked with selective OX_2 receptor antagonists or selective OX_1 receptor antagonists would be useful to unambiguously identify the OX receptor subtype involved in this response.

Limited work has been done exploring effects of OX_2 receptor antagonists on immediate early gene (IEG-like) responses in the prefrontal cortex. However, the OX_2 receptor antagonist LSN2424100 did suppress restraint stress-induced increases in c-Fos protein expression without having any effects on baseline Fos protein expression in the home cage [90]. These effects of the OX_2 receptor antagonist LSN2424100 on restraint stress-induced increases Fos expression in the prelimbic cortex are similar to an effect of the $mGlu_2$ receptor agonist LY354740 on restraint stress-induced increases in Fos expression [45]. As discussed above, 5-HT_{2A} receptor agonists induce a number of immediate IEG-like responses in the prefrontal cortex. Activation of $mGlu_2$ receptors appears to suppress the DOI-induced increases in a number of IEG-like responses in the prefrontal cortex [40, 41, 44, 91].

Modulation of 5-HT_{2A} receptor agonist-induced head twitches is a behavioral measure that is suppressed by a range of antidepressants blocking/regulating 5-HT_{2A} receptors as discussed above; these DOI-induced head twitches are also suppressed by the selective OX_2 receptor antagonist LSN2424100 (**Figure 1**). LSN2424100 possesses approximately 200-fold functional OX_2 receptor antagonist activity at both human recombinant OX_2 vs. OX_1 receptors or rat OX_2 vs. OX_1

receptors [90]. Administration of LSN2424100 (10 mg/kg, i.p.) 30 min prior to administration of DOI (3 mg/kg, i.p.) with behavioral observations beginning 5 min later for a 30 min period resulted in over a 67% statistically significant reduction in the frequency of DOI-induced head twitches in CD-1 mice (n = 8/group; **Figure 1**) using conditions/methods/statistical analyses reported elsewhere in greater detail [52]. Head twitches were observed in 8/8 vehicle/DOI treated mice but in only 3/8 LSN2424100/DOI treated mice ($p < 0.05$, Fisher's Exact Test). This experiment demonstrating that a G_q/G_{11}-coupled GCPR OX_2 receptor antagonist (like 5-HT_{2A} receptor antagonists) suppress DOI-induced head twitches fits in with evidence that agonists or positive allosteric modulators of G_i/G_o-coupled GCPRs ($mGlu_2$, $mGlu_4$, adenosine A_1, and μ-opioid receptors) similarly suppress DOI-induce head twitches [28, 31, 48, 50, 52, 92, 93]. Thus, the effects of these drugs on spontaneous EPSCs/EPSPs upon layer V pyramidal neuron apical dendrites in layers I and Va of the prefrontal cortex all produce directionally consistent effects on DOI-induced head twitches [37]. These results imply that adequate orexin, glutamate, adenosine and endogenous opioid release is present from or onto thalamocortical afferents under the in vivo experimental conditions employed to engender salient changes in dendritic integration of the principle output layer V pyramidal cells.

OX_2 receptor antagonists also appear to modulate at least certain aspects of executive function mediated by the prefrontal cortex, namely impulsivity and biasing operant responding for DRL schedules in rodents [69, 90]. The OX_2 receptor antagonist LSN2424100 increased reinforcers obtained and decreased total responses by Sprague-Dawley rats performing under a DRL 72-s schedule of reinforcement (**Figure 2**) [90]. These antidepressant-like responses were largely replicated in wild-type CD-1 mice and OX_1 receptor knockout mice responding on a DRL 36-s schedule of reinforcement rate [90]. However, no changes in the reinforcement rate or response rate

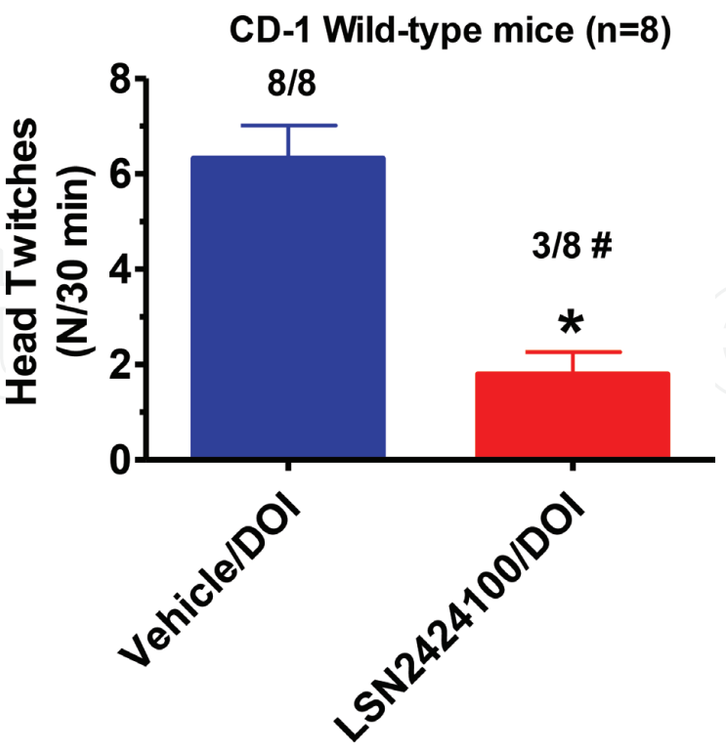

Figure 1.
*The effect of (±)-DOI (3 mg/kg, i.p.) and the selective OX_2 receptor antagonist LSN2424100 (10 mg/kg, i.p.) on head twitches in CD-1 wild-type mice observed for 30 min following drug administration. LSN2424100 was administered 30 min prior to DOI. Each bar represents the mean (± SEM) of eight mice. Significantly different from the mean number of head twitches for the vehicle/DOI group, * $p < 0.05$. Significantly different from the number of mice displaying head twitches for the vehicle/DOI group, # $p < 0.05$ by the Fisher exact test.*

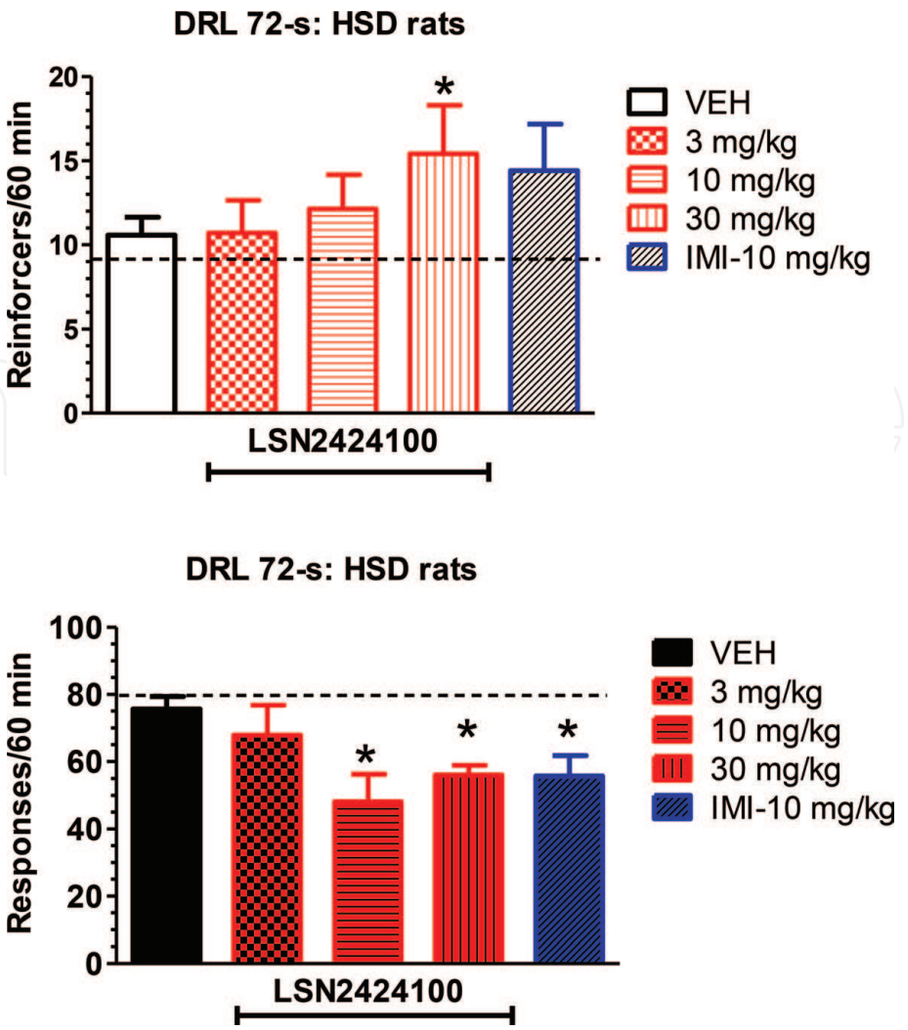

Figure 2.
*The antidepressant-like effect of LSN2424100 on male Sprague-Dawley rats (n = 7) stably performing under a DRL 72-s schedule. The top graph shows the effects of LSN2424100 (3–30 mg, i.p.) and imipramine (10 mg/kg, i.p.) on the number of reinforcers obtained after vehicle/drug was administered 1 hour prior to the daily session. The bottom graph shows the effect of LSN2424100 (3–30 mg, i.p.) and imipramine (10 mg/kg, i.p.) on the total number of responses. The dotted line shows the control reinforcement and response rate and * denotes data points significantly different from control ($p < 0.05$) (this figure was adapted from data presented by Fitch et al. [90]).*

were observed in OX_2 receptor knockout mice when testing LSN2424100 doses up to twice as large as those used for wild-type and OX_1 receptor knockout mice [90]. A similar antidepressant-like profile was observed in rats, wild-type CD-1 mice, and OX_1 receptor KO mice with the non-selective OX_1/OX_2 receptor antagonist almorexant [90]. In contrast, a selective OX_1 receptor antagonist failed to produce an antidepressant-like response in rats performing on a DRL 72-s schedule or wild type mice or OX_2 receptor knockout mice responding on a DRL 36-s schedule [90]. However, the well-established tricyclic antidepressant drug imipramine tested as expected in these experiments as a positive control (e.g., antidepressant-like effects) in Sprague-Dawley rats, wild-type mice, OX_1 receptor knockout mice, or OX_2 receptor KO mice trained to lever press under a DRL 72-s schedule (rats) or a DRL 36-s (mice) schedule.

3. Clinical trials with orexin receptor antagonists in patients with MDD

Thus far only a single small double-blind, placebo-controlled, diphenhydramine-controlled, parallel group, phase 1b/2a trial of a selective OX_2 receptor antagonist, JNJ-42847922/MIN-202 or seltorexant, has been conducted [94].

Only 47 men and women with a diagnosis of MDD (DSM-IV) were randomized to received either diphenhydramine, 25 mg q.d. (n = 13), seltorexant, 20 mg q.d. (n = 22) or placebo (n = 12) for 10 nights. Sleep polysomnography was also performed to provide objective assessment of improvements on sleep. There were improvements from baseline in the seltorexant treatment group for the HAMD-17 total score (−3.6 points) as well as the HAM-17 adjusted total score accounting for sleep improvement in addition to changes in the HAMD-6 item score (−1.5 points). This resulted in effect sizes of −0.48, −0.55 and − 1.05 for the OX_2 receptor antagonist compared to placebo. However, one caveat is that the subjects assigned to the histamine H_1 receptor antagonist diphenhydramine showed highly comparable improvement compared to placebo as did seltorexant. To answer these questions/concerns, a phase 2b randomized, double-blind parallel group, placebo-controlled, adaptive dose-finding trial for seltorexant adjunctive treatment to antidepressants scheduled to enroll about 280 adult subjects at 85 US, European, Russian and Japanese sites began in September 2017 (NCT03227224).

The only other MDD clinical trial for an OX receptor antagonist was negative [95]. Filorexant (MK-6096), a dual orexin receptor antagonist, was evaluated in a 6-week, double-blind, placebo-controlled, parallel-group phase 2a proof-of-concept trial where subjects with MDD were randomized 1:1 to once-daily oral filorexant 10 mg or matching placebo. Subjects on antidepressants continued to take their prescribed antidepressant for the duration of the trial. This study was stopped after enrolling 129 (40%) of a planned 326 subjects. Less than a 1 point numerical improvement was observed for filorexant compared to placebo using the mean change from baseline to week 6 MADRS total score. Exploratory analyses also failed to reveal statistically significant changes in the Insomnia Severity Index (ISI). Regarding safety, there were no deaths, drug-related serious adverse events (SAEs) and only one discontinuation due to AEs in both treatment groups. There were no other problematic safety issues reported.

This negative filorexant MDD study may be related to an issue of inadequate power as the planned study was designed with 80% power to detect a 3.5-point difference between treatments with a 2-sided 5% level of significance and a fully enrolled trial. However, the enrollment of only 129 subjects while using 61 sites (United States, Canada, Finland, France, Germany, Norway and Sweden) speaks to the recruitment challenges in this study. The dose chosen for this MDD trial appears reasonable based on positive effects reported for filorexant in a phase 2 randomized, double-blind, placebo-controlled adaptive crossover polysomnography dose-ranging study evaluating approximately 80 subjects each at nightly doses of 2.5, 5 and 10 mg [81]. All doses showed significant effects on sleep efficiency and wakefulness after persistent sleep onset while the two higher doses demonstrated significant effects on sleep onset. Filorexant was also well tolerated in this insomnia trial as well [81].

Preclinical results suggest that the combined OX_1/OX_2 receptor antagonism should not have compromised potential antidepressant action in patients with MDD. Namely, the OX_1/OX_2 receptor antagonist almorexant acted similarly to the OX_2 receptor antagonist LSN2424100 and the known tricyclic antidepressant imipramine in rats and mice performing on DRL 72-s or DRL 36-s schedules [90]. In addition, the non-selective OX receptor antagonist almorexant also tested similarly to known antidepressants in mice subjected to unpredictable chronic mild stress (UCMS) and then evaluated with the tail suspension test, the resident-intruder test, and the elevated plus maze [96]. However, opposing antidepressant-like and "pro-depressant"-like effects were observed in OX_1 and OX_2 receptor knockout mice, respectively, studied with the forced swim paradigm [97]. In this same study, the selective OX_1 receptor antagonist SB-334867 also exerted an antidepressant like

effect in the forced swim test. No data has been published suggesting that selective OX_2 receptor antagonists test as antidepressants in rodent forced swim tests. Nevertheless, the balance of data are consistent with the hypothesis that adequate blockade of both OX_1 and OX_2 receptors, or OX_2 receptors alone, should improve depressive symptoms in patients with MDD.

4. Conclusions

Activation of 5-HT_{2A} receptors or OX_2 receptors appears to induce glutamate release from thalamocortical terminals with cell bodies originating in the midline and intralaminar thalamic nuclei when recording from prefrontal cortical layer V pyramidal neurons (**Figure 3**). This 5-HT and orexin-B-induced glutamate release appears to dependent action potentials in the presynaptic terminals judging from the TTX-induced blockade of the 5-HT- or orexin-induced EPSC/EPSPs as

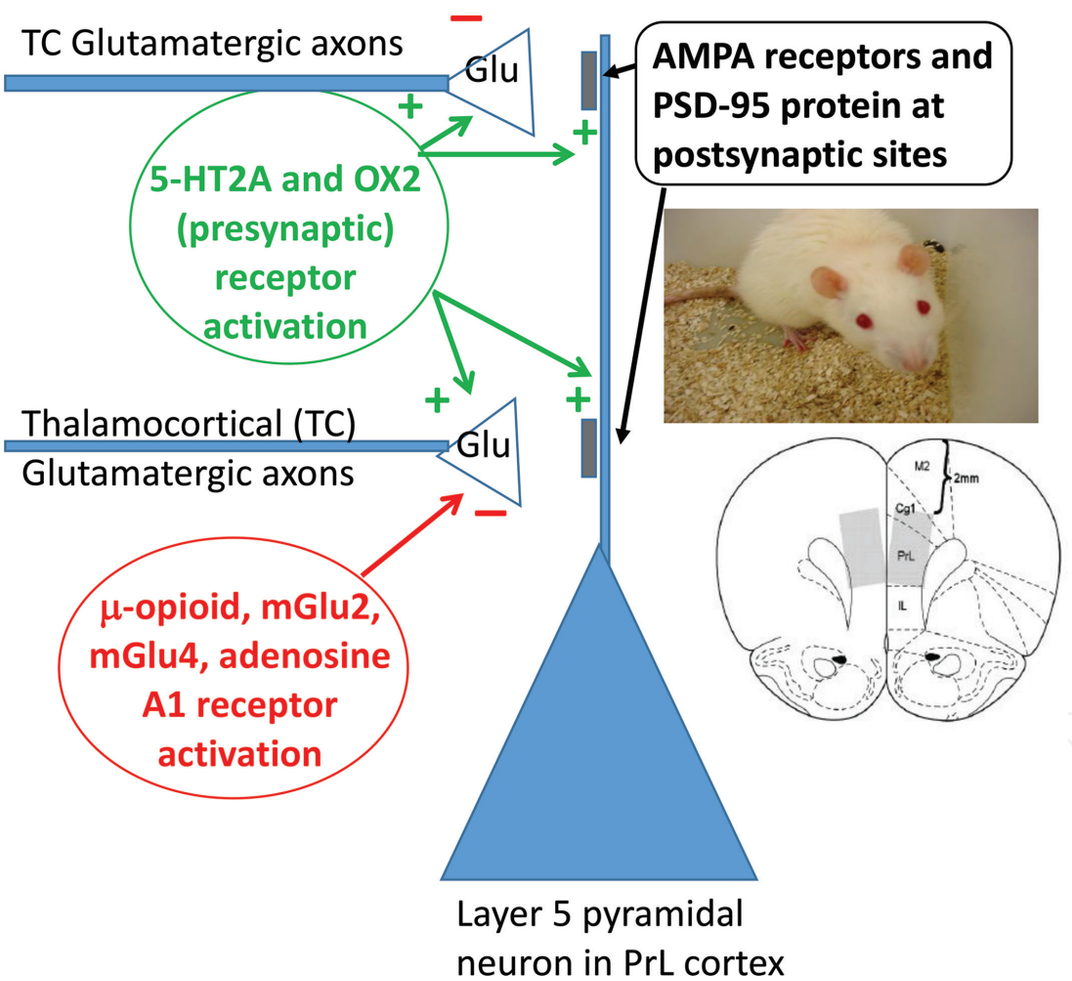

Figure 3.
The model where activation of 5-HT_{2A} or OX_2 receptors depolarizes and releases glutamate from non-specific thalamocortical inputs to layer I and Va of the apical dendrites from layer V pyramidal neurons. The majority of 5-HT_{2A} receptors, apart from a minority of presynaptic receptors and those on GABAergic interneurons, are present on and also directly depolarize layer V pyramidal neurons. Other glutamatergic receptors ($mGlu_2$ and $mGlu_4$), μ-opioid receptors and adenosine A_1 receptors that suppress the EPSCs/EPSPs induced by activation of 5-HT_{2A} and OX_2 receptors appear to be present on non-specific thalamocortical afferents. This circuitry (with additional positive modulator receptor such as $mGlu_5$ and NK_3 receptors and also additional negative modulators such as β_2-adrenergic receptors) appears to underlie a similar valence of action for all these receptors for a behavior mediated by activation of 5-HT_{2A} receptors in the prefrontal cortex, DOI-induced head twitches. This circuitry also appears to underlie impulsive behavior (DRL 72-s behavior) where a similar valence of GPCR mediated effects appears to drive antidepressant-like effects on this screening behavior as DOI-induced head twitches and 5-HT-induced EPSCs.

suggested previously for non-specific thalamocortical axons. Apical dendritic layer V pyramidal AMPA receptors appear to be activated postsynaptic to the thalamic terminals. The 5-HT or DOI-induced spontaneous EPSCs/EPSPs or DOI/electrically evoked EPSC/EPSPs also appear suppressed by $mGlu_2$, $mGlu_4$, adenosine A_1, 5-HT-$_{1\text{-like}}$ and β_2-adrenergic receptors.

Future work is required to establish that orexin-B-induced glutamate release from non-specific thalamic afferents is also suppressed by $mGlu_2$, $mGlu_4$, adenosine A_1, 5-HT-$_{1\text{-like}}$ and β_2-adrenergic receptors. Blockade of OX_2 and 5-HT_{2A} receptors also both appear to suppress DOI-induced head twitches, a behavioral response that appears to be mediated by activation of prefrontal cortical 5-HT_{2A} receptors. A selective OX_2 receptor antagonist tested similar to the tricyclic antidepressant imipramine in rats and mice responding under an operant DRL 72-s schedule of reinforcement. Another question for future preclinical research with rodent DRL behavior is whether blockade of OX_2 receptors is additive/synergistic with tricyclic antidepressants or SSRIs in the same manner as blockade of 5-HT_{2A} receptors. The ongoing clinical antidepressant trial with the OX_2 receptor antagonist seltorexant are important to understanding whether the circuitry involving orexin-containing cells in the hypothalamus together with orexin-containing axon terminals in the intralaminar and midline thalamic nuclei and the prefrontal cortex are necessary and sufficient by themselves to augment the antidepressant effects of tricyclic antidepressants and SSRIs. If this ongoing and other clinical antidepressant trials with selective OX_2 receptor antagonists or additional adequately powered clinical trials testing OX_1/OX_2 receptor antagonists are negative, then future work will be required to begin to ask whether additional actions of OX_2 receptor antagonists in other circuitry are functionally opposed to the brainstem/thalamic/prefrontal cortical circuits.

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Conflict of interest

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