Research Paper

Differential Effects of the Lateral Hypothalamus Lesion as an Origin of Orexin and Blockade of Orexin-1 Receptor in the Orbitofrontal Cortex and Anterior Cingulate Cortex on Their Neuronal Activity

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ABSTRACT

Introduction: Introduction: Several studies have demonstrated that orexins may regulate different forms of affective and cognitive processes during wakefulness. The Orbitofrontal Cortex (OFC) and Anterior Cingulate Cortex (ACC), as an essential part of the Prefrontal Cortex (PFC), have a crucial role in cognitive processes such as reward and decision-making. They also have a high amount of orexin receptor type 1 (OX1Rs).

Methods: In the present study, we inhibited OX1Rs in this area after a 10-min baseline recording to find out the role of OX1Rs in the OFC neuron's firing rate. Next, we inhibited the lateral hypothalamus (LH) as the primary source of orexinergic neurons. Afterward, using a single-unit recording technique in rats, we detected the effects of the lateral hypothalamus on the firing rate and activity pattern of the ACC or OFC neurons.

Results: Data showed that the blockade of OX1Rs in the OFC could excite 8 and inhibit 1 neuron(s) out of 11. In addition, the blockade of OX1Rs in the ACC could excite 6 and inhibit 3 neurons out of 10. LH inactivation excited 5 out of 12 neurons and inhibited 6 in the ACC. It also excited 8 and inhibited 6 neurons out of 14 in the OFC. These data suggest that the blockade of OX1Rs excites 72% of the neurons, but LH inactivation had a stimulating effect on only 50% of neurons in two main subregions of the PFC.

Conclusion: Accordingly, PFC neurons may receive the orexinergic inputs from the LH and indirectly from other sources.

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Highlights

- Blockade of orexin receptors 1 in ACC alter neural activity in this region.

- Blockade of orexin receptors 1 in OFC alter neural activity in this region.

- Lateral hypothalamus inhibition increase neural activity of only 50% neurons in ACC and OFC.

Plain Language Summary

The discovery of the neuropeptide orexin/hypocretin in 1998 and subsequent research during the past 20 years revealed an important role for the lateral hypothalamus (LH) in driving the reward pathway, sleep and awake circuits, decision making and psychotic disorders. But our knowledge towards the function of this neurotransmitter on neural activity in specific areas of the brain is limited. In this study we decided to clear the specific role of orexin receptors placed in the two crucial areas of medial prefrontal cortex (mPFC) and the orexin projections from LH on neural firing rates in those regions. In the present study, we investigated the following items by using an in vivo extracellular single-unit recording technique in rats and our data shown that the effect of blocking of orexin receptors1 in mPFC caused a different results than inhibition of the origin of orexin projection in LH as a source of it.

1. Introduction

O rexin A and orexin B are expressed by lateral hypothalamus (LH) neurons and have various functions, such as feeding and energy homeostasis (Ganjavi & Shapiro, 2007), regulation of arousal conditions (de Lecea, Sutcliffe, & Fabre, 2002; Sutcliffe & de Lecea, 2002), natural and drug rewards processing (Harris & Aston-Jones, 2006; Harris, Wimmer, & Aston-Jones, 2005), and decision-making (Karimi, Hamidi, Fatahi, & Haghparast, 2019). Because orexin neurons project their axons throughout the brain (Nambu et al., 1999; Peyron et al., 1998a), LH, as a large and complicated area in the brain, has different regions with different functions. Anatomical evidence shows that the frontal cortex receives a direct projection from the LH (Kievit & Kuypers, 1975). Also, as mentioned above, LH is the main origin of orexin A and orexin B, containing neurons that are key regulators of different physiological functions, such as wakefulness (Chen, de Lecea, Hu, & Gao, 2015; Li, Hu, & Lecea, 2014). Recent studies have revealed that cognitive processes, such as associative learning and memory, control feeding behavior (Petrovich, 2018), and decision-making (Karimi et al., 2017) require LH function. LH may serve as a motivation-cognition interface processor in the brain. Behavioral and electrophysiological studies have suggested the significance of the functional connections between the PFC and the LH (Arikuni, 1976; Kita & Oomura, 1981; Oomura & Takigawa, 1981).

The medial prefrontal cortex (mPFC) plays a crucial role in many executive functions such as attention, judgment, decision-making, and working memory. Anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) are two main parts of the mPFC (Moreira, Marques, & Magalhães, 2016).

The OFC is crucial in detecting and tracking the value of the stimulus (Rolls, 2000). It plays a key role in processing rewards and integrating multiple sources of information about the reward outcome to originate a value signal (Wallis, 2007). The OFC is activated when an expected reward is not obtained and when behavior should be changed (Rolls, 2000).

In the mPFC, ACC is located in an unequaled S and has a good connection with the limbic system, as the emotional and the prefrontal cortex are known as cognition sites (Euston, Gruber, & McNaughton, 2012). Several researchers believe that the ACC plays a crucial role in initiation, motivation, and goal-directed behaviors (Devinsky, Morrell, & Vogt, 1995). Also, this area has an essential role in maximizing rewards and minimizing threats or punishments (Monosov, 2017).

Earlier studies have shown an extensive distribution of orexin receptors (OXRs) throughout the central nervous system, including the mPFC (Marcus et al., 2001; Peyron et al., 1998b). Also, orexinergic neurons in the LH send a direct projection to mPFC (Jin et al., 2016). The published documents reveal that mPFC's layers II/III and V have a much higher density of Orexin Receptor

Type 1 (OX1Rs) than orexin receptor type 2 (OX2Rs); accordingly, OX1Rs play a more crucial role in the orexin-mediated function in the mPFC (Li et al., 2009). The orexin system is probably essential for maintaining the excitability of mPFC neurons, supporting the high efficiency of cognitive functions (Chen, de Lecea, Hu, & Gao, 2015; Li, Hu, & Lecea, 2014). Additionally, we have previously shown that the OX1Rs of the mPFC are necessary for having a proper decision on the cost and benefit of decision-making (Karimi, et al., 2019). The study results show that the blockade of OX1Rs by its antagonist (SB334867) in the ACC and or OFC alters the preference of the animals from high reward to low reward in the cost and benefit decision-making.

All mentioned documents provide sufficient evidence to confirm the relationship between an orexinergic projection from the LH and mPFC. However, there is no clear evidence for claiming the specific role of OX1Rs, placed in the orbitofrontal cortex, on neural firing rates in this region. Also, the role of the projection from LH to mPFC on its neural firing rate has not been studied yet. In the present study, we investigated the following items by using an in vivo extracellular single-unit recording technique in rats: the effect of reversible inactivation of the LH on the electrical properties of ACC or OFC neurons, as two important regions of the mPFC and the blockade of OX1Rs in the OFC on the electrical properties of this area.

2. Materials and Methods

Summary of the experimental method

In this study, we applied an in vivo single-unit recording technique on anesthetized rats during the whole experiment. Neural activation in the ACC or OFC region of the medial prefrontal cortex was extracellularly recorded for 10 min. Next, Lidocaine 4% (0.5 μL per side) or SB334867 (30 nM in 0.5 μL per side) (Sigma-Aldrich, USA) was infused into the LH or OFC, and the recording was continued from ACC or OFC areas for 40 min. The same volumes of the respective drug-free vehicles (saline or dimethyl sulfoxide (DMSO) 12%) instead of lidocaine or SB334867 were microinjected in control animals. The alteration in the firing pattern and activity of the recorded neurons in these cortical areas were determined and reported as the functional connectivity between this area and the ACC or OFC neurons before and after the inactivation of the LH or OX1Rs in the OFC.

Animal preparation and stereotactic surgery

In this study, we used 35 male Wistar rats weighing about 270 g. Urethane (1.5 g/kg, IP, with supplemental doses as required; Sigma-Aldrich, Germany) was applied to anesthetize them. The surgical tracheostomy was processed to lessen the respiratory effects and keep the airway open during the recording. Then, the tracheostomized rat was gently put on a stereotactic frame (Stoelting, USA), the scalp was removed to expose the cranial surface, and the bregma was identified and used as the stereotactic reference point.

Single-unit recording from anterior cingulate or orbitofrontal cortex after LH reversible inactivation

23-gauge stainless steel guide cannulae were bilaterally implanted 1 mm above the LH. The coordinates for the target locations (LH) were determined from a rat brain atlas (Paxinos & Watson, 2007): 2.65 mm posterior to bregma; 1.3 mm lateral to the midline; and 8.2 mm ventral to the skull. After securing the guide cannulae in place, dental acrylic cement was applied to attach the implants over the lateral hypothalamus (-2.65 mm AP, 1.3 mm ML) or to infuse drugs. For electrode placement, two small burr holes were drilled in the skull above the anterior cingulate (1.8 mm AP, 0.6 mm ML) or orbitofrontal cortex (3.2 8 mm AP, 2.2 mm ML). A polyethylene tube (PE-20) filled with lidocaine 4% or saline connected a 30G injecting needle to a Hamilton microsyringe. The injected needle was advanced into the LH (8 or 8.5 mm below the skull surface) and stayed in the LH for subsequent infusion. A heating pad (Int. Biomedical Inc., USA) was used to preserve the rat's body temperature during the experiment.

Single-unit recording from anterior cingulate or orbitofrontal cortex after OX1Rs inactivation

The coordinates for the target locations (ACC or OFC) were determined from a rat brain atlas (Paxinos & Watson, 2007). For ACC and OFC, the following coordinates were used: For ACC, 1.8 mm anterior to bregma, 0.6 mm lateral to the midline; and for OFC, 3.2 8 mm anterior to bregma, 2.2 mm lateral to the midline. The small burr holes were drilled in the skull above the described coordination for electrode placement. After securing the guide cannulae in place, dental acrylic cement (Paladur) was applied to attach the implants over the lateral hypothalamus (-2.65 mm AP, 1.3 mm ML) or to infuse drugs. Two small burr holes were drilled in the skull above the anterior cingulate (-1.8 mm AP, -0.6 mm ML) or orbitofrontal cortex (3.2 8 mm AP, 2.2 mm ML) for electrode position.

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Extracellular single-unit recording

In this study, we used the methods described in previous studies (Moaddab, Kermani, Azizi, & Haghparast, 2013) for single-unit recordings. A parylene-coated tungsten microelectrode (impedance 4.5-5.5 MΩ, FHC Company) was used for recording from ACC or OFC after LH inactivation.

A single-barrel electrode, made in the Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran, has been used to record from OFC or ACC after the OX1Rs blockade.

The electrode was carefully driven through the determined region, and then a manual Microdrive was used for fine sorting spike activity with a justifiable signal-to-noise ratio. Signals from the electrode were pre-amplified for impedance matching with a unity gain preamplifier, amplified 10000 times using a differential amplifier (DAM-80; WPI, Sarasota, FL), bandpass filtered at 0.3–10 kHz, and digitalized at 50 kHz sampling rate and 12-bit voltage resolution using a home-made data acquisition system (D3109; WSI, Tehran, Iran). All-or-none spike events were detected using a window discriminator (W3205; WSI, Tehran, Iran) based on the spike amplitude. The recording data (undetected spikes and background activities) were saved on a computer device for later offline analyses by Plexon offline sorter software (Plexon Inc., Dallas, TX).

A baseline recording was carried out for about 10 min after the recognition of the neurons with fixed firing frequency and stable spike amplitude and waveforms. Then, the lidocaine was infused into the LH in 2 min. The recording was continued for around 40 min. In other groups, SB334867 (30 nM/0.5 µL) was infused in the OFC or ACC in the same place as the electrode was stabled for 2 min. After the recording, we marked the recording site with a negative direct current (DC) of 50 µA for 16 s via the recording electrode.

Data analysis

The Plexon Offline Sorter software was used to analyze the data from the recorded neurons. Threshold adjustment at a suitable potential for spike detecting occurred after applying a low-cut filter of approximately 12 to 25 Hz. The T-distribution expectation-maximization method based on the first to third principal components was used for spike sorting. Neuroexplorer software (Nex-Technologies, Littleton, MA) was used to draw the rate histograms of the spike firing per time block of 1 min over the entire recording period for all single units. Accordingly, clusters of spikes with acceptable inter-spike interval histograms were exported to Neuroexplorer software. A significant response to the applied drug was defined as an alteration in the firing rate more than the Mean±SD of the baseline firing for at least 3 successive bins (Riahi, Arezoomandan, Fatahi, Haghparast, & memory, 2015). For all experimental and control groups, duration, magnitude, and latency of the response in each neuron and also the number of excited or inhibited neurons were used for statistical analysis.

Histological verification

At the end of the experiment by using an overdose of pentobarbital, the rats were euthanized and then normal saline was perfused transcardially, followed by 10% formaldehyde. LH microinjection (Figure 1A) and electrical recording sites (Figure 1B) were histologically verified using the brain atlas (Paxinos & Watson, 2007).

Statistical analysis

All data were presented as Mean±SEM. In all control and experimental groups, the Kolmogorov-Smirnov was tested for normality of the data and Gaussian distribution. A change in activity was defined as an increase (excitation) or decrease (inhibition) in the firing rate more or less than the mean baseline activity±two standard deviations (Mean±SD), sequentially. A Chi-squared test was used for comparing the ratio of neurons with inhibitory or no response to those with excitation between different groups. The paired Student's t test was used for evaluating the drug's effect on the neural firing rate. An independent sample t test was used for comparing a significant difference in the percentage of change between the control and experimental groups. A two-tailed probability value of P<0.05 was considered statistically significant.

3. Results

The effect of the OX1Rs blockade on OFC neuron's firing rate

To investigate the role of OX1Rs in controlling the electrical firing in the OFC, SB334867 (30 nM/0.5 µL) was infused into the OFC regions after recording the baseline. Next, in 40 min, the neural firing was recorded (Figure 2A). Eleven neurons from 6 individual rats were recorded. The data have shown that OX1Rs inactivation could excite 8 (72%), and inhibit 1 (9%) neuron. Also, SB 334867 did not affect 2 neurons (18%). Five
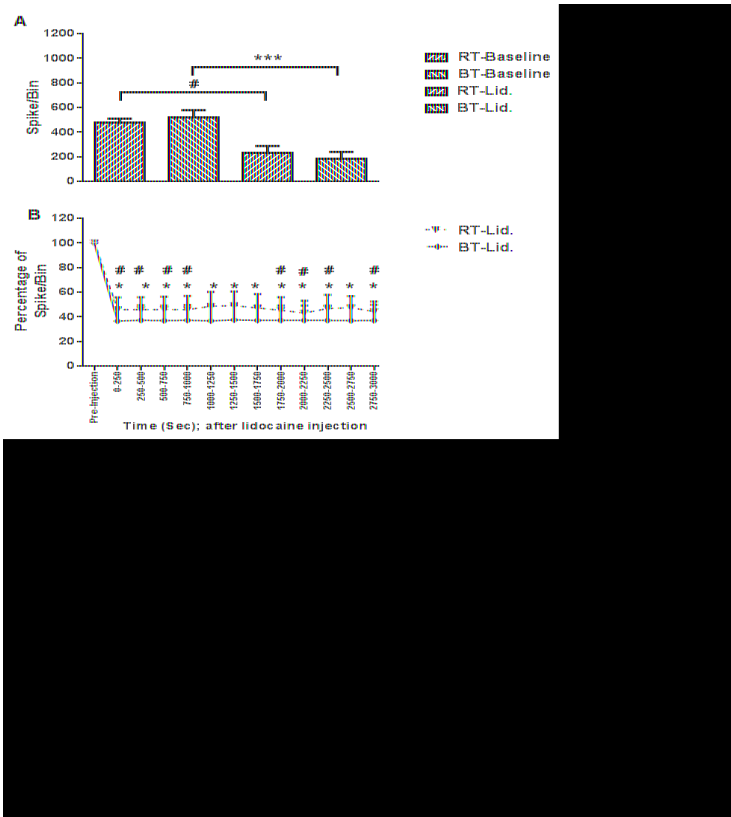
B) Electrical Recording Site

Anterior Cingulate Cortex

Orbitofrontal Cortex
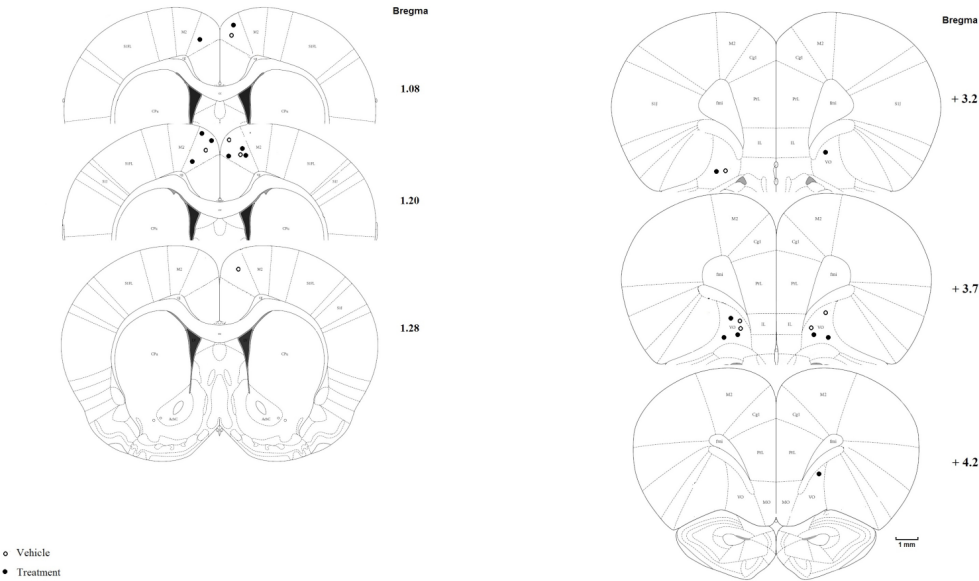
○ Vehicle
● Treatment

Figure 1. Histological Verification

Representative images show the microinjection site located on the LH (A) and the electrophysiological recording site (B), located in the ACC and OFC.
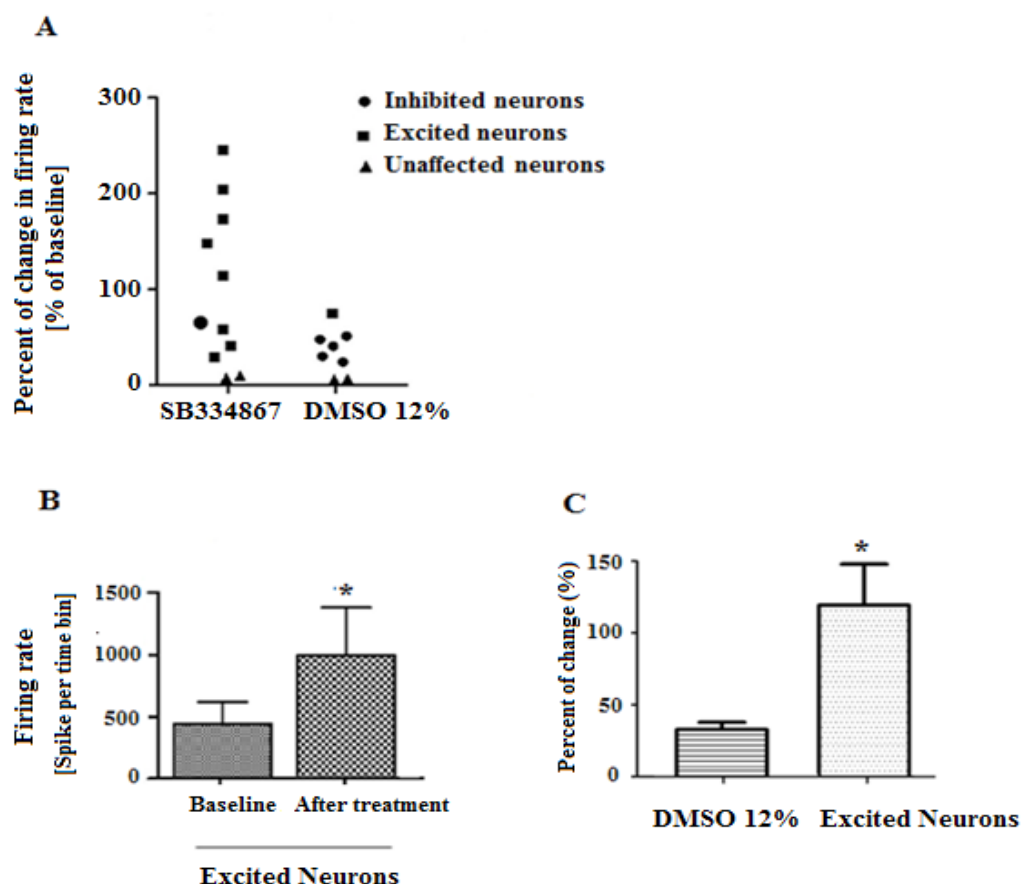
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Figure 2. The effect of orexin receptor type 1 (OX1Rs) anterior cingulate cortex neural activity
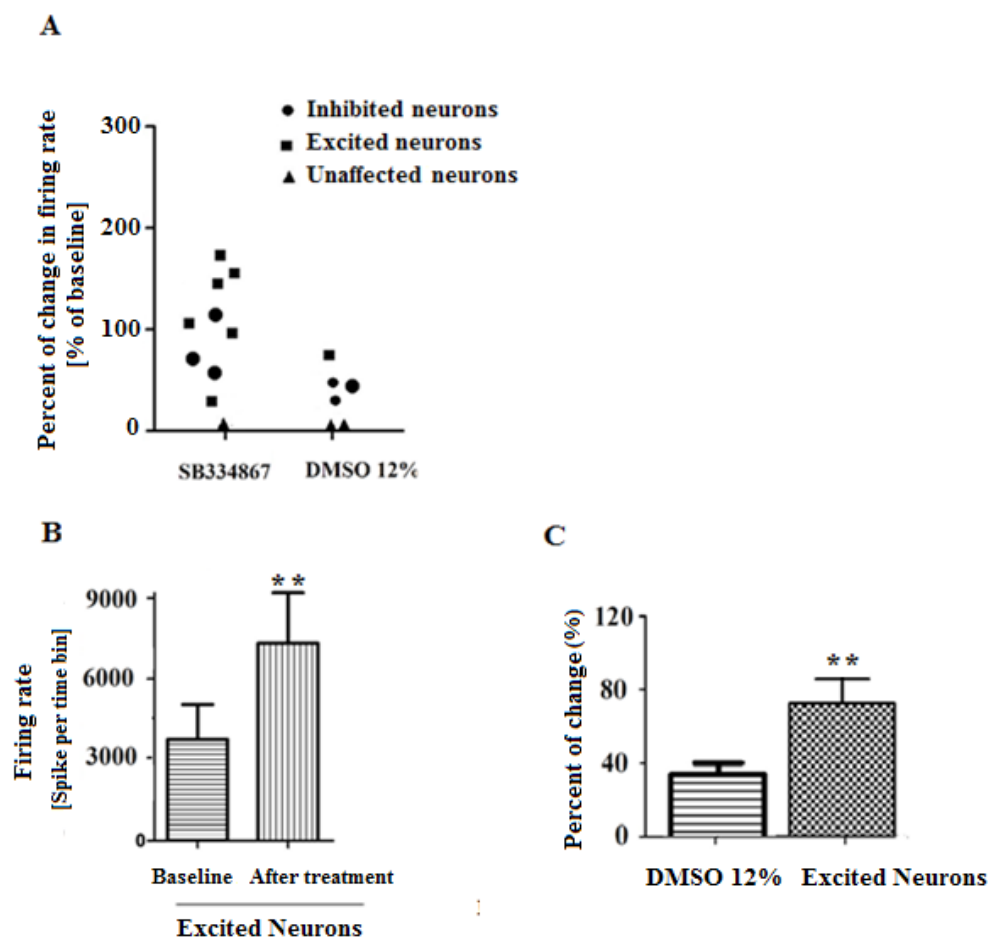
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SB334867 (30 nM/0.5 µL) infusion into the OFC elicited an excitatory effect in most neurons. A) The histogram represents the spike count per time bins of 60 s over the entire recording; blockade of OX1R could increase the firing frequency of one recorded neuron. B) The scatter plot is illustrating OFC neurons' responses after SB334867 (30 nM/0.5 µL) administration. C) SB334867 (30 nM/0.5 µL) caused excitation in 8 neurons and could diminish the inhibitory effects of dimethyl sulfoxide (DMSO) 12%, P=0.04. D) In the subclass of excitatory neurons (n=7), the firing frequency was increased significantly relative to the baseline firing, P=0.0078.

rats received DMSO 12% with the same protocol as the control group. Of 8 neurons recorded in this group, DMSO 12% could inhibit 5 (62%) neurons, excite 1 neuron (12%), and have no effect on 2 neurons (25%) (Figure 2B). A Chi-squared test was used for comparing the ratio of neurons with inhibitory or irresponsiveness to those with excitation between the SB334867 and the control group. The test revealed a significant difference between these groups ($X^2[1]$=6.68, N=18, P=0.035). The excitation response was initiated 1 min after treatment administrations and persisted 9 to 20 min after the administration in the main group. DMSO 12% could initiate an inhibitory effect approximately 1 to 2 min after the administration, and could persist for 5 to 20 min. Analyzing the percentage of change between neurons showing excitatory response to OX1Rs blockade and DMSO 12% group determined a significant increase in the average of firing rate in OFC neurons after OX1Rs inactivation (121.1±32.24 vs, 31.81±5.661, sequentially;

t[10]=2.29, P=0.044; Figure 2C). In these neurons, the Wilcoxon matched-pairs test of the average of the firing rate between pre and post-injection of SB334867 into the OFC revealed that blockade of the OX1Rs could significantly increase the firing rate of some neurons compared to their baseline activity (P=0.0078; Figure 2D). Accordingly, the blockade of OX1Rs in the OFC has a more excitatory than inhibitory effect.

The effect of the OX1Rs blockade on ACC neuron's firing rate

SB334867 (30 nM/0.5 µL) was infused into the ACC regions after the baseline recording (10 min) to detect the effect of the OX1Rs blockade on the electrical firing rate of this region. The neural firing record has been done for 40 min recording after treatment (Figure 3A). Ten neurons from 4 individual rats were selected, and the data have shown that OX1Rs inactivation could excite 6
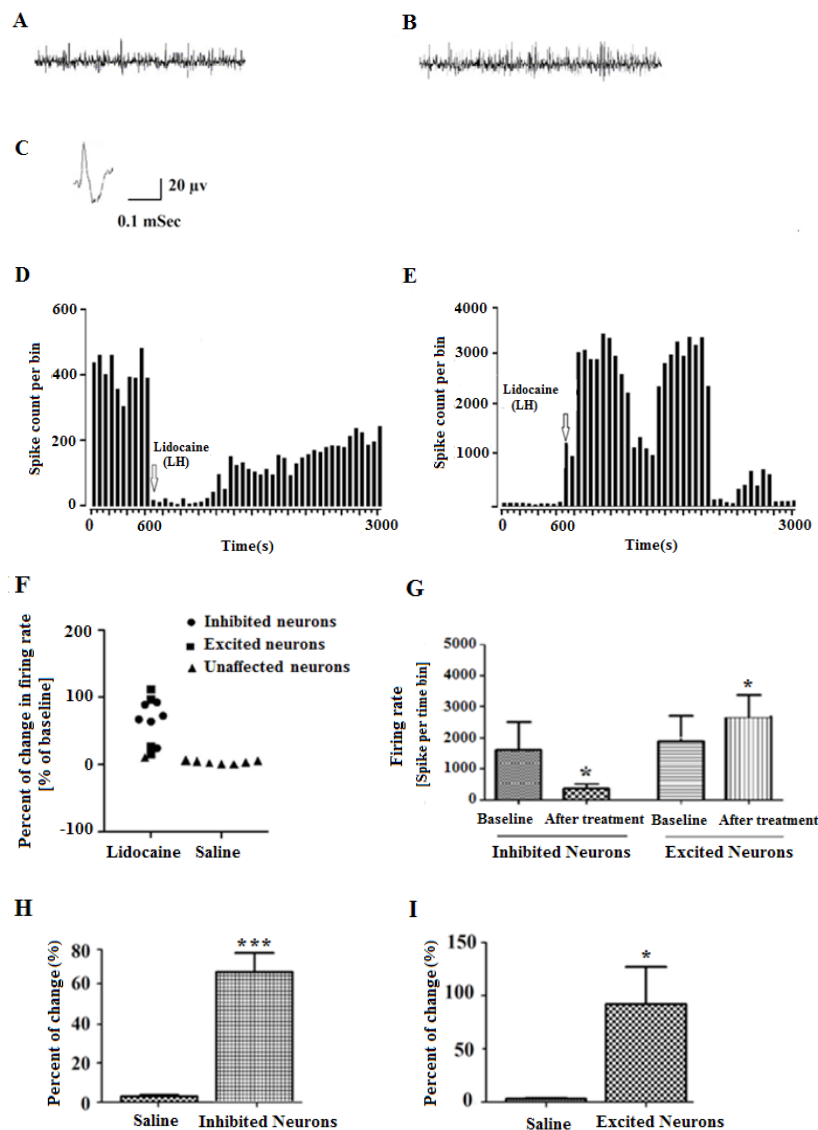
Figure 3. The Effect of Orexin Receptor Type 1 (OX1Rs) Blockade on Orbitofrontal Cortex (OFC) Neural Activity

SB334867 (30 nM/0.5 μL) infusion into the anterior cingulate cortex (ACC) elicited excitatory and inhibitory effects in the neurons of this area. A) The histogram represents the spike count per time bins of 60 s over the entire recording; blockade of OX1R could increase the firing frequency of one recorded neuron.B) The scatter plot is illustrating ACC neurons' responses after SB334867 (30 nM/ 0.5 μL) administration. C) A comparison of the percentage of alterations in the firing rate between the experimental and the control group. D) In the subclass of excitatory neurons (n=6), the firing frequency was increased significantly relative to the baseline firing, P=0.03.

(60%) and inhibit 3 (30%) neurons. SB334867 had no effect on 1 neuron (10%). The control group (n=4 rats) received DMSO 12% (vehicle) similar to the main group. Six neurons were recorded in this group and DMSO 12% could inhibit 3 (62%), excite 1 (12 %) and no effect on 2 neurons (25%) (Figure 3B). A Chi-squared test was used for comparing the ratio of neurons with inhibitory or irresponsiveness to those with excitation between the SB334867 and the DMSO group. The test revealed a significant difference between these groups [χ^2 (1)= 6.19, N=18, P=0.045]. The excitation response was initiated 1 to 2 min after treatment and persisted for 15 to 25 min after the administration in the main group. DMSO 12% could initiate inhibitory affects approximately 1 to 2 min after administration, and could persist for 9 to 15 min. Analyzing the percentage of alteration between neurons showing excitatory response to OX1Rs blockade and

DMSO 12% group determined a significant increase in the average of the firing rate in ACC neurons after OX1Rs deactivation (32.3±19.79 vs. 83.2±18.65, sequentially; t[10]=4.25, P=0.0017; Figure 3C). In these neurons, the Wilcoxon matched-pairs test of the average of the firing rate between pre and post-injection of SB334867 into the OFC revealed that the blockade of OX1Rs could significantly increase the firing rate of some neurons compared to their baseline activity (P=0.03; Figure 3D left panel).

For inhibited neurons, analyzing the percentage of the alteration between these neurons and DMSO 12% group showed no significant change in the average of the firing rate in ACC neurons after OX1Rs deactivation (54.57±6.67 vs, 31.03±8.1, respectively; t[8]=2.022, P=0.07; Figure 3D right panel). It seems that the block-

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Figure 4. The Effect of Lateral Hypothalamus (HL) Inactivation on Anterior Cingulate Cortex (ACC) Neural Activity
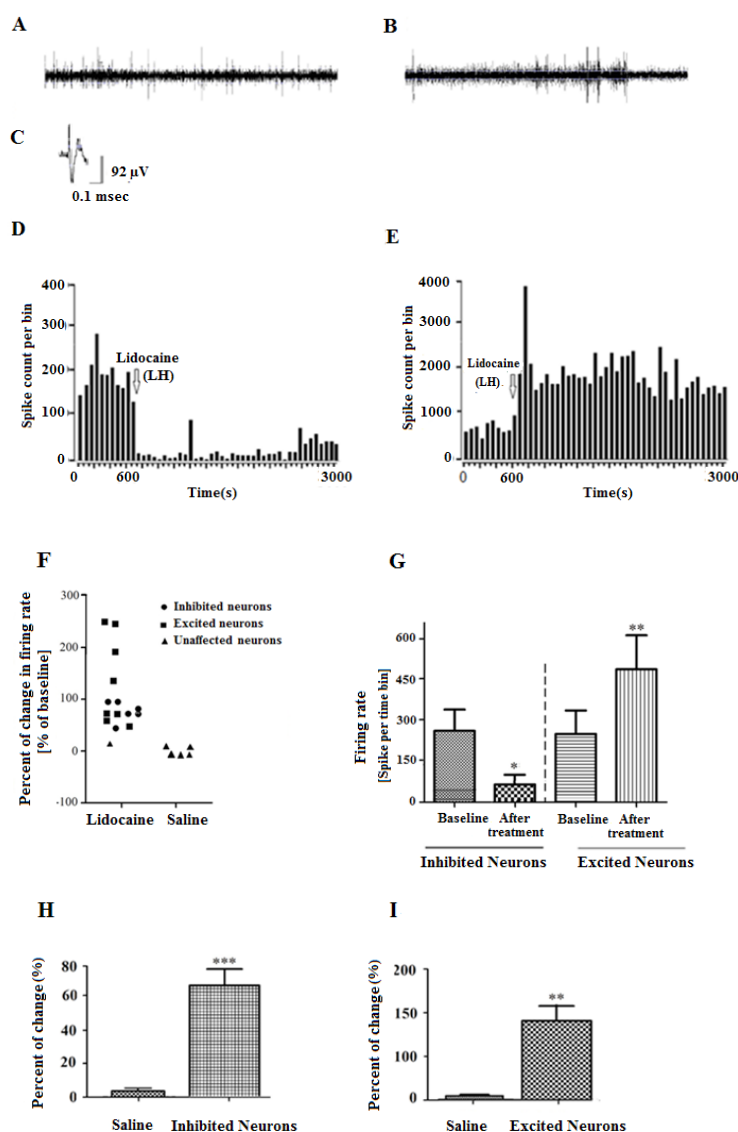
Lidocaine 4% infusion into the lateral hypothalamus elicited an inhibitory effect in most of the ACC neurons. A) The representative pattern of the baseline shows the spontaneous firing recorded simultaneously from ACC neurons (timeframe: 0.172 s). B) Firing pattern of the same neurons recorded after intra-LH administration of lidocaine 4% (timeframe: 0.330 s). C) An expanded waveform of the spikes generated from the ACC neurons. D and E) Histograms representing spike count per time bins of 60 s over the entire recording. Reversible inactivation of LH could decrease the firing frequency of one recorded neuron (D) and, at the same condition, increase the firing rate in the other neuron (E). F) The scatter plot illustrates ACC neurons with different responses to intra-LH administration of saline (n=7) or Lidocaine 4% (n=12) injection. G) Lidocaine-induced excitation (n=5) was dramatically greater than saline effect, P=0.01. H) In the subclass of neurons with inhibitory response to intra-LH lidocaine 4% infusion (n=6), the firing frequency was decreased significantly relative to the baseline firing, P=0.03. I) Intra-LH inactivation-induced inhibition (n=6) was significantly different from saline effect (n=6), P≤0.0001.

ade of OX1Rs in the ACC has a more excitatory than inhibitory effect.

LH inactivation altering ACC firing rate

To survey the role of LH's projections in the electrical firing of the ACC, lidocaine 4% in 0.5 μL per side was infused into the LH, following the baseline recording. Then, the neural firing was recorded for 40 min from ACC (Figure 4A-E). The data were collected from 12 neurons of 6 individual rats, and LH inactivation could excite 5 (41%), inhibit 6 (50%), and elicit no response in 1 (8.33%) neuron(s). Saline was administered to 5 rats, similar to the prior group. Saline did not affect 8

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Figure 5. The Effect of Lateral Hypothalamus (LH) Inactivation on Orbitofrontal Cortex (OFC) Neural Activity

Reversible inactivation of the lateral hypothalamus elicited an excitatory effect in most OFC neurons. A) Representative pattern of baseline spontaneous firing recorded simultaneously from OFC neurons (frame duration: 1.755 s). B) Firing pattern of the same neurons recorded after intra-LH administration of lidocaine 4% (frame duration: 1.755 s). C) An expanded waveform of the spikes generated from the OFC neurons. D and E) Histograms representing spike count per time bins of 60 sec over the entire recording. Reversible inactivation of the LH could decrease the firing frequency of one recorded neuron (D) and, under the same condition, increase the firing rate in the other neuron (E). F) Scatter plot illustrating OFC neurons with different responses to intra-LH administration of saline (n=5) or Lidocaine 4% (n=14) injection. G) Lidocaine-induced excitation (n=8) was dramatically greater than saline effect, P=0.0083. H) In the subclass of neurons with inhibitory (n=6) (left panel) and excitatory (n=8) (right panel) response to intra-LH lidocaine 4% infusion, the firing frequency was changed significantly relative to the baseline firing, P=0.031 (inhibitory response), P=0.0078 (excitatory response). I) intra-LH inactivation-induced inhibition (n=6) was significantly different from saline effect (n=5), P=0.0002.

(100%) neurons, recorded from 8 ACC neurons in this group (Figure 3F). A Chi-squared test was used to compare the ratio of neurons with inhibitory or no response to those with excitation of the LH inactivated group and saline group. The test revealed a significant difference between these groups ($\chi2[1]$=16.30, N=12, P=0.003). The excitatory response began 1 to 2 min after lidocaine administration and persisted for 9 to 37 min. Analyzing the percentage of the alteration between neurons shows the excitatory response to lidocaine administration. Also,

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the control group that received saline showed a significant increase in the average firing rate in ACC neurons after the LH deactivation (91.91±34.92 vs 3.089±0.83, sequentially; t[10]=3.070, P=0.0118; Figure 4G). Also, the inhibitory response was initiated only 1 min after lidocaine administration, and in 5 neurons, this response persisted for 28 to 38 min. In 1 neuron, this inhibition lasted for 10min. In these neurons, the statistical analysis of the average firing rate between pre and post-injection of lidocaine into the LH (W=21, P=0.03; Figure 4H) revealed that LH transient inactivation could significantly decrease the firing rate of the neurons compared to their baseline activity. An unpaired Student's t test (t (11)=7.1, P < 0.0001) revealed that in neurons in which LH inactivation could inhibit, a significant difference exists in the percentage of the decrease compared to the saline control group (Figure 4I).

LH inactivation altering OFC firing rate

After 10 min baseline recording, lidocaine 4% in 0.5 μL per side was infused into the LH to investigate the effects of LH's projection on the electrical firing of OFC neuron activation. The neural firing was recorded for 40 min after treatment (Figure 5 A-E). We collected our data from 14 neurons of 7 individual rats. LH inactivation could excite 8(57.14%), inhibit 6(42.85%), and elicit no response in 1 (7.14 %) neuron(s). In 5 rats as the control group, saline was micro-infused similar to lidocaine administration. Saline did not affect all (100%) of the 5 recorded neurons (Figure 5 F). A Chi-squared test was administered to compare the ratio of neurons with inhibitory or no response to those with excitation in the LH inactivated and saline group. The test revealed a significant difference between these groups (χ^2[1]=15.56, N=14, P=0.0004). Either excitatory or inhibitory response was initiated 1 min after lidocaine administration in 12 neurons, and 2 neurons were started 5 min after the treatment. Both excitatory and inhibitory responses persisted until the end of the recording in 10 neurons. However, in 4 neurons, this response could be observed 15 to 22 min after the treatment. The Wilcoxon matched-pairs test (P=0.0078; Fig. 5H, left panel) between neurons show excitatory response to the administration of lidocaine in LH. It revealed that LH transient inactivation could significantly increase the firing rate of neurons compared to their baseline activity. Analysis of the alterations between neurons showing excitatory response to lidocaine administration and the control group suggested significant increases in the neuron's firing rate after LH inactivation compared to the control group (6.378±2.832 N=5 vs 127.4±29.24 N=8, Sequentially; t(11)=3.212, P=0.0083; Figure 5G).

Data analyses of inhibitory neurons have shown that LH transient inactivation could significantly decrease the neuron's firing rate compared to their baseline activity (W=21, P=0.031; Figure 5H, right panel). The Student's t test revealed that in the population of neurons with inhibitory response to LH inactivation, the percentage of the decrease was significant compared to the saline group (Figure 5I) (t[9]=5.902, P=0.0002).

4. Discussion

The principal findings of this study were that the blockade of the OX1Rs had an excitatory effect in the OFC and also in ACC in most of the recorded neurons. On the other hand, DMSO 12% had an inhibitory effect on OFC and ACC's neural firing rate. Besides, reversible inactivation of the lateral hypothalamus elicited excitatory and inhibitory responses in the OFC and ACC, two essential areas of the mPFC. In the previous behavioral and electrophysiological studies, it has been clear that DMSO could affect the neural cells (Castro et al., 1995; Maclennan, Smith, & Darlington, 1996; Ogura, Shuba, McDonald, & Therapeutics, 1995) by increasing or decreasing the effects of the drug for which DMSO is a solvent (de la Torre, 1995). It has been reported that DMSO as a vehicle decreases the firing rate of medial vestibular nucleus neurons (Maclennan et al., 1996). However, in this study, the inhibitory effect of DMSO could decrease or may abolish with SB334867.

Our previous research showed that LH inactivation disrupts the effort and delay-based decision-making task, and this document clarified a relationship between ACC or OFC, as the two important sites for decision-making, and LH (Karimi et al., 2017). The alteration in the neural firing rate in ACC or OFC could also be related to the elimination of neurotransmitters in the LH. However, relatively little attention has been paid to the role of LH neurotransmitters-promoting systems in regulating neural activity in the PFC. In recent years growing evidence has highlighted the importance of the orexinergic system in cognitive functions and its crucial role in the mPFC for modulating cognitive behaviors. Recent evidence indicates that orexins could directly regulate the mammalian PFC (Lambe & Aghajanian, 2003; Lambe, Olausson, Horst, Taylor, & Aghajanian, 2005; Li, Li, Wei, Wang, Sui, & Kirouac, 2010; Liu & Aghajanian, 2008).

Several lines of evidence indicate that these new neuropeptides may also be involved in regulating a variety of affective and cognitive processes during wakefulness. Also, LH sends its projections to mPFC, which has a higher density of OX1Rs, (Peyron et al., 1998b), and it receives

much more fiber than other neocortical areas (Fadel & Deutch, 2002; Lambe & Aghajanian, 2003; Lambe et al., 2005; Li et al., 2010; Liu & Aghajanian, 2008).

It seems that OX1Rs have an essential role in orexin-mediated functions in the OFC than ACC (Dias, Li, Nattie, & neurobiology, 2010; Hirose et al., 2016) because although the OFC is densely connected with LH (Hirose et al., 2016), ACC receives modest density of fibers from LH (Hoover, Vertes, & Function, 2007). In addition, Aracri et al. suggested the direct regulatory actions of orexins in the frontal areas are much brighter than the prior. Also, they have shown that orexin could control synaptic transmission in this area (Aracri, Banfi, Pasini, Amadeo, & Becchetti, 2013). Regardless of the brain area, orexin via 2 G protein-coupled receptors (Sakurai et al., 1998) produces an excitatory effect on the cellular function (Bayer et al., 2004; Hagan et al., 1999; Yamanaka et al., 2002). Previous studies have revealed a postsynaptic excitatory effect of orexin-A on PFC neurons in layers 5 and 6. (Xia et al., 2005; Xia et al., 2009; Yan, He, Xia, Zhang, & Hu, 2012). Additionally, previous research demonstrates that orexin can target the glutamatergic terminals, and orexin 1 stimulates glutamate release onto fast spike cells in Fr2 layer V. Furthermore, orexin could release GABA onto Fr2 layer V pyramidal cells and increase GABA onto regular spiking pyramidal cells (Aracri et al., 2013). These results suggest that PFC activity may regulate via direct innervations of the PFC neurons by the hypothalamic orexin system. In addition, some studies indicate that orexin has an excitatory effect on the neural firing rate in multiple brain regions, including GABAergic neurons in pars reticulate (Korotkova, Eriksson, Haas, & Brown, 2002), VTA (Korotkova, Sergeeva, Eriksson, Haas, & Brown, 2003), arcuate nucleus (Burdakov, Liss, & Ashcroft, 2003), the cholinergic neurons of the basal forebrain (Eggermann et al., 2001), histaminergic neurons in the ventral tuberomammillary nuclei (Bayer et al., 2001; Eriksson, Sergeeva, Brown, & Haas, 2001)

In the present study, inhibition of some neurons may be related to the reduction in orexinergic projections' activation of excitatory neurotransmitters. In addition, the elimination of orexins' excitatory effect on GABA releases may cause the increase in firing rate in some neurons.

Furthermore, growing evidence points to a cross-talk between endocannabinoid and orexinergic systems. Anatomical studies have found an overlapping distribution in several areas of the CNS between cannabinoid receptors 1 (CB1) and OXRs (Hervieu, Cluderay, Harrison, Roberts, & Leslie, 2001; Marcus et al., 2001; Van

Sickle et al., 2005). The ventral tegmental area (VTA), the nucleus accumbens (NAc), and the PFC, as the mesocorticolimbic area, have both receptors (Aston-Jones et al., 2010; Maldonado, Valverde, & Berrendero, 2006; Plaza-Zabala, Flores, Maldonado, & Berrendero, 2012). Firstly in 2003, direct CB1-OX1Rs interaction was proposed (Hilairet, Bouaboula, Carrière, Le Fur, & Casellas, 2003). Moreover, the activation of OX1Rs stimulates the synthesis of the most important endocannabinoids, 2-arachidonoylglycerol, and could inhibit the neighboring cells via its retrograde inhibition effect. This phenomenon suggests that endocannabinoids could contribute to some hypocretin effects. Stress could activate the presynaptic CB1 and suppress inhibitory, excitatory, and monoaminergic neurotransmitter release in the corticolimbic brain regions, including the prefrontal cortex, hippocampus, amygdala, and hypothalamus (Freund, 2003). In this study the blockade of OX1 may cause to stop the retrograde inhibition effects of CB1 on other the neighboring cells.

Finally, the LH orexinergic projections that directly and transsynaptically converge on the ACC and the OFC, as two important regions of the medial prefrontal cortex, regulate the firing rate of these areas. As a result LH is associated with cognitive functions such as decision-making and reward processing.

Ethical Considerations

Compliance with ethical guidelines

All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Kashan University of Medical Sciences and also Shahid Beheshti University of Medical Sciences, Tehran, Iran. Additionally, all efforts were made to minimize animal suffering and to reduce the number of animals used to obtain reliable results.

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Authors' contributions

Conceptualization: Sara Karim; Methodology and Data analysis: Sara Karimi and Mohammad Ismail Zibaii; Writing – original draft and Data collection: Sara Karimi; Writing – review & editing: Abbas Haghparasr and Gholam Ali Hamidi; Funding acquisition and Resources: Gholam Ali Hamidi; Supervisors: Gholam Ali Hamidi and Abbas Haghparast.

Conflict of interest

The authors declared no conflict of interest.

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