Title: 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

Several studies revealed that orexins may take part in the regulation of the different forms of affective and cognitive processes during wakefulness. The orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) as an important part of the prefrontal cortex (PFC) have a crucial role in cognitive processes such as reward and decision-making and has a high density of orexin receptor type 1 (OX1Rs). In the present study, to find out the role of OX1Rs in the OFC neurons firing rate, the OX1Rs were inhibited in this area after a 10-min baseline recording. In the second part, the lateral hypothalamus (LH) as the main source of orexinergic neurons was inhibited and its effect on the firing rate and activity pattern of the ACC or OFC neurons were detected by using single-unit recording technique in the rats. Results showed that blockade of OX1Rs in the OFC could excite 8 and inhibit 1 neuron out of 11. Besides, the blockade of OX1Rs in the ACC could excite 6 and inhibit 3 neurons out of 10. Also, LH inactivation excited 5 out of 12 neurons and inhibited 6 neurons in the ACC. It excited 8 and inhibited 6 neurons out of 14 in the OFC. These data suggested that blockade of the OX1Rs excited 72% of the neurons, but LH inactivation had an exciting effect on just 50% of neurons in two main subregions of PFC. It seems that the PFC neurons receive the orexinergic inputs from the LH and indirectly from other sources.

Keywords: Neuronal activity, Orexin-1 receptor, Lateral hypothalamus, Orbitofrontal cortex, Anterior cingulate cortex, Single-unit recording
1. Introduction

Orexin A and orexin B are expressed notably by lateral hypothalamic (LH) neurons, and have different functions such as feeding and energy homeostasis (Ganjavi & Shapiro, 2007), the regulation of arousal condition (de Lecea, Sutcliffe, & Fabre, 2002; Sutcliffe & de Lecea, 2002), natural and drug rewards processing (Harris & Aston-Jones, 2006; Harris, Wimmer, & Aston-Jones, 2005) as well as, 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. There is a piece of anatomical evidence that the frontal cortex receives a direct projection from the LH (Kievit & Kuypers, 1975). Besides, as mentioned above, LH is the main origin of orexin A and orexin B containing neurons that are the key regulators of the different physiological functions such as wakefulness (Chen, de Lecea, Hu, & Gao, 2015; J. Li, Hu, & Lecea, 2014). More recent works have revealed cognitive processes, such as associative learning and memory, control feeding behavior (Petrovich, 2018) and, decision-making (Karimi et al., 2017) need the LH function and it seems that it 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, 1976).

The medial prefrontal cortex (mPFC) plays key roles 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 mPFC (Moreira, Marques, & Magalhães, 2016). OFC has a crucial role in detecting and tracking the value of the stimulus (Rolls, 2000). The OFC plays a key role in processing rewards. It integrates multiple sources of information about the reward outcome to originate a value signal (Wallis, 2007). The OFC is also activated when an expected reward is not obtained and when behavior should be changed (Rolls, 2000).

In the mPFC, ACC is located in an unequalled S, and it has a good connection with the limbic system as the emotional and the prefrontal cortex as known as a cognition sites (Euston, Gruber, & McNaughton, 2012). Currently, there are several views which
show that in initiation, motivation, and goal-directed behaviors the ACC plays a crucial role (Devinsky, Morrell, & Vogt, 1995). Also, this area has an important role in the maximization of rewards and also, the minimization of threats or punishments (Monosov, 2017).

Earlier studies have shown an extensive distribution of orexin receptors (OXRs) all over the central nervous system, including the mPFC (Marcus et al., 2001; Peyron et al., 1998b). Also, orexinergic neurons in LH send a direct projection to mPFC (Jin et al., 2016). The published documents revealed that mPFC’s layers II/III and V have much higher density of orexin receptor type 1 (OX1Rs) than orexin receptor type 2 (OX2Rs), so it seems that OX1Rs has a more crucial role in orexin-mediated function in the mPFC (B. 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; J. Li, Hu, & Lecea, 2014). Additionally, we have previously shown the mPFC’s OX1R, are necessary for having a proper decision on the cost and benefit decision-making (Karimi, Hamidi, Fatahi, Haghparast, & Psychiatry, 2019). The results of that study showed that blockade of the OX1Rs by its antagonist (SB334867) in the ACC and/or OFC altered the preference of the animals from high reward to low reward in the cost and benefit decision-making.

All mentioned documents are sufficient evidence for confirming the relationship between an orexinergic projection from the lateral hypothalamus and mPFC. But there is no clear evidence for claiming the specific role of OX1Rs, which are placed in the orbitofrontal cortex on neural firing rates in this region. Also, the role of the projection from LH to mPFC on the neural firing rate in the mPFC has not been working yet. In the present study, the effect of reversible inactivation of the LH on the electrical properties of ACC or OFC neurons, two important regions of the mPFC, and also blockade of OX1Rs in the OFC on the electrical properties of this area was investigated by using in vivo extracellular single-unit recording technique in the rats.
2. Materials and methods

2.1. Summary of the experimental method

In vivo single-unit recording was applied in this study and all the animals were anesthetized rat during whole experiment. Neural activation in the ACC or OFC region of the medial prefrontal cortex was extracellularly recorded around 10 min. Next, Lidocaine 4% (0.5 μl per side) or SB334867 (30nM 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 a 40 min period. The same volumes of the respective drug-free vehicles (saline or 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 aforementioned cortical areas, before and after inactivation of the LH or OX1R, in the OFC, were determined and reported as the functional connectivity between this area and the ACC or OFC neurons.

2.2. Animal preparation and stereotactic surgery

35 male Wistar rats weighing about 270 g were used in this study. Urethane (1.5 g/kg, i.p., with supplemental doses as required; Sigma–Aldrich, Germany) was utilized to anesthetize them. The surgical tracheostomy was processed to lessen the respiratory effects and keep open airway 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.

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

23 gauge stainless steel guide cannulae were implanted bilaterally 1 mm above LH. The coordinates for target locations (LH) were determined from a rat brain atlas (Paxinos& Watson, 2007) as follows: 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 for infusing drug. 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 the lidocaine 4% or saline connected a 30-G injecting needle to a Hamilton microsyringe. The injected needle was lowered 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) used to preserve the rat body temperature during whole experiment.

B) Single-unite recording from anterior cingulate or orbitofrontal cortex after OX1R inactivation

The coordinates for target locations (ACC/ OFC) were determined from a rat brain atlas (Paxinos& Watson, 2007). For ACC the coordination was used as follows: 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 for drug infusion. 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.

2.3. Extracellular single-unit recording

In this study, the methods which described in previous studies were used (Moaddab, Kermani, Azizi, & Haghparast, 2013) were used 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, which was made in the Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran, has been used or recoding from OFC or ACC after OX1R blocker.

The electrode was carefully driven through the determined region and then a manual Micorodrive until 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 10,000 times using a differential amplifier (DAM-
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. All recordings 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 recognition of the neurons with fixed firing frequency and stable spike amplitude and waveforms, then the lidocaine was infused into LH in 2 min, the recording was continued around 40 min. In other groups, SB334867 (30nM/0.5µL) was infused in the OFC or ACC in the same place as the electrode was stabled in 2 min. Next to the end of the recording, we marked the recording site by a negative direct current (DC) of 50 µA for 16 s via the recording electrode.

2.4. Data analysis

The Plexon offline sorter software was used to analyze datum from the recorded neurons. Threshold adjustment at a suitable potential for spike detecting was occurred after applying a low-cut filter of approximately 12–25 Hz. The T-distribution Expectation-Maximization method based on the first to third principal components was used for Spike sorting. Neuroexplorer software (NexTechnologies, Littleton, MA) was used to draw the rate histograms of spike firing per time blocks of 1 min over the entire recording period, for all single-units. So, clusters of spikes with acceptable inter-spike interval histograms were exported to Neuroexplorer software. A significant response to the applied drug was defined as a change in the firing rate more than mean ± twice of the standard deviation (Mean ± 2SD) 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 used for statistical analysis.

2.5. Histological verification

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

2.6. Statistical analysis

All data were showed as Mean ±SEM. In all control and experimental groups, the Kolmogorov-Smirnov was tested for normality of Data and Gaussian distribution. A change in activity was defined as an increase (excitation) or decrease (inhibition) in firing rate more or less than the mean baseline activity ± two standard deviations (mean ± 2SD), sequentially. For comparing the ratio of neurons with inhibitory or no response to those with excitation between different groups a chi squared test was used. The paired student t-test was used for evaluating drug 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

3.1. The effect of the OX1Rα blockade on OFC neurons firing rate

To investigate the role of OX1Rα to control of the electrical firing in the OFC, SB334867 (30 nM/ 0.5µl) was infused into the OFC regions after recording the baseline. Next, in 40 min the neural firing was recorded (Fig. 2A). Eleven neurons from 6 individual rats were recorded, and the data have shown that OX1Rα inactivation could excite, 8 (72%), inhibit 1 (9%) and SB 334867 did not affect 2 neurons (18%). 5 rats received DMSO 12% with the same protocol as control group. From 8 neurons recorded in this group, DMSO 12% could inhibit 5(62%) neurons, excited 1 neuron (12 %) and it had no effect on 2 neurons (25%) (Fig.2B). For comparing the ratio of neurons with inhibitory or no response to those with excitation between the SB334867 and control group a chi-squared test was used and it revealed a significant difference between these groups [X2 (1) = 6.68, N = 18, p = 0.035]. The excitation response was initiated after treatment administrations and persisted 9–20 min after administration in the main group. DMSO 12% could initiate an inhibitory effect approximately 1-2 min after administration and it could persist for 5–20 min. Analysis of the percentage of change
between neurons which showing excitatory response to OX1R<sub>s</sub> blockade and DMSO 12% group determined that there was a significant increase in the average of firing rate in OFC neurons after OX1R<sub>s</sub> inactivation [121.1 ± 32.24 vs, 31.81 ± 5.661, sequentially; t (10) = 2.29, p =0.044; Fig. 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 OX1R<sub>s</sub> could increase the firing rate of some neurons significantly compared to their baseline activity [p = 0.0078; Fig. 2D). It seems that the effect of blockade of OX1R<sub>s</sub> in the OFC is more excitatory than inhibitory.

3.2. The effect of the OX1R<sub>s</sub> blockade on ACC neurons firing rate

SB334867 (30 nM/0.5µl) was infused into the ACC regions after the baseline recording (10 min) for detecting the effect of the OX1R<sub>s</sub> blockade on the electrical firing rate of this region. The neural firing record has been done for 40 min recording after treatment (Fig. 3A). Ten neurons from 4 individual rats were selected, and the data have shown that OX1R<sub>s</sub> inactivation could excite, 6 (60%), inhibit 3 (30%) and SB 334867 had no effect on 1 neuron (10%). The control group (n=4 rats) received DMSO 12% (vehicle) as same as a main group, 6 neurons recorded in this group and it seems that DMSO 12% could inhibit 3 (62%) neurons, excited 1 neuron (12 %) and it had no effect on 2 neurons (25%) (Fig.3B). For comparing the ratio of neurons with inhibitory or no response to those with excitation between the SB334867 and DMSO group a chi squared test was used and it revealed a significant difference between these groups [X<sup>2</sup> (1) = 6.19, N = 18, p = 0.045]. The excitation response was initiated 1-2 min after treatment administrations and persisted 15-25 min after administration in the main group. DMSO 12% could initiate inhibitory affects approximately 1-2 min after administration and it could persist for 9-15 min. Analysis of the percentage of change between neurons that showing excitatory response to OX1R<sub>s</sub> blockade and DMSO 12% group determined there is a significant increase in the average of the firing rate in ACC neurons after OX1R<sub>s</sub> deactivation [32.3 ± 19.79 vs, 83.2 ± 18.65, sequentially; t (10) = 4.25, p =0.0017; Fig. 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 blockade of the OX1R<sub>s</sub> could increase the firing rate of some neurons significantly compared to their baseline activity [p = 0.03; Fig. 3D left panel].
For inhibited neurons, analysis of the percentage of change between these neurons and DMSO 12% group showed that there was no significant change in the average of the firing rate in ACC neurons after OX1R s deactivation [54.57 ± 6.67 vs, 31.03 ± 8.1, respectively; \( t (8) = 2.022, \ p = 0.07; \) Fig. 3D right panel]. It seems that the effect of blockade of OX1R s in the ACC is more excitatory than inhibitory.

### 3.3. LH inactivation could alter ACC firing rate

To survey the role of LH’s projections in the electrical firing of the ACC, lidocaine 4% in 0.5µl per side following to the baseline recording was infused into the LH. Then the neural firing was recorded for 40 min from ACC (Fig. 4A-E). The data were collected from twelve neurons from 6 individual rats, and LH inactivation could excite 5 (41%), inhibit 6 (50%), and elicit no response in 1 (8.33%) neuron. Saline was administrated in 5 rats with as same as prior group. Saline did not affect 8 (100%) neurons which recorded from 8 ACC neurons in this group (Fig. 3F). For comparing the ratio of neurons with inhibitory or no response to those with excitation the LH inactivated group and saline group a chi-squared test was used and it revealed a significant difference between these groups \([X^2 (1) = 16.30, \ N = 12, \ p = 0.003]\). The excitatory response was began1-2 min after lidocaine administrations and persisted for 9–37 min. Analysis of the percentage of change between neurons that showing excitatory response to lidocaine administration and the control group that received saline showed that there is a significant increase in the average of the firing rate in ACC neurons after LH deactivation \([91.91± 34.92 \text{ vs}, 3.089 ± 0.83, \text{ sequentially}; \ t (10) = 3.070, \ p = 0.0118; \text{ Fig. 4G}]\). Also, the inhibitory response was initiated just 1min after lidocaine administration, and in 5 neurons this response persisted 28-38 min and in 1 neuron this inhibition lasted for a 10-min period. In these neurons, the statistical analysis of the average of the firing rate between pre- and post-injection of lidocaine into the LH \([W=21, \ p = 0.03; \text{ Fig. 4H}]\) revealed that LH transient inactivation could decrease the firing rate of the neurons significantly compared to their baseline activity. Student unpaired \(t\)-test \([t (11) = 7.1, \ P< 0.0001]\) revealed that in the neurons which LH inactivation could inhibit them, there was a significant difference in the percentage of decrease as compared to saline-control group (Fig. 4I).
3.4. LH inactivation could alter OFC firing rate

After 10 min baseline recording lidocaine 4% in 0.5 μl per side was infused into the LH to investigate the effect of LH’s projection on the electrical firing of OFC neuron activation. The neural firing was recorded for 40 min after treatment (Fig. 5A-E). Our data was collected from fourteen neurons from 7 individual rats, and LH inactivation could excite 8 (57.14%), inhibit 6 (42.85%), and elicit no response in 1 (7.14 %) neuron. In 5 rats as a control group saline was micro infused as same as lidocaine administration. Saline did not affect in all (100%) neurons in 5 neurons recorded (Fig. 5F). For comparing the ratio of neurons with inhibitory or no response to those with excitation the LH inactivated group and saline group a chi squared test was used and it revealed a significant difference between these groups [X2 (4) = 15.56, N = 14, p = 0.0004]. Either excitatory or inhibitory response was initiated 1min after lidocaine administrations in 12 neurons and 2 neurons were stared 5 min after treatment. Both excitatory and inhibitory response persisted until the end of the recording in 10 neurons, but in 4 neurons this response could see just 15-22 min after treatment. The Wilcoxon matched-pairs test [p = 0.0078; Fig. 5H, left panel] between neurons showing excitatory response to administration of lidocaine in LH revealed that LH transient inactivation could increase the firing rate of the neurons significantly compared to their baseline activity. Analysis of the percent of changes between neurons that showing excitatory response to lidocaine administration and the control group informed that there were significant increases in the neurons’ firing rate after LH inactivation in comparison with control group [6.378 ± 2.832 N=5 vs, 127.4 ± 29.24 N=8, sequentially; t (11) = 3.212, p =0.0083; Fig. 5G].

Data analyzing of inhibitory neurons has shown LH transient inactivation could significantly decrease the neurons’ firing rate compared to their baseline activity [W=21, p = 0.031; Fig. 5H, right panel]. The Student’s t-test revealed that in the population of neurons with the inhibitory response to LH inactivation, the percentage of decrease was significant compared with the saline group, (Fig. 5I) [t (9) = 5.902, p=0.0002].
4. Discussion

The principal approaches of this study were that the blockade of the OX1Rs in the OFC and also in ACC in most of the recorded neurons had an excitatory effect. 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 as two important areas of the mPFC. In previous behavioral and electrophysiological studies, it has been cleared that DMSO by increasing or decreasing the effects of the drug for which it is a solvent (de la Torre, 1995) could affect the neural cells (Castro et al., 1995; Maclellan, Smith, & Darlington, 1996; Ogura, Shuba, McDonald, & Therapeutics, 1995). It has been reported that DMSO as a vehicle led to a decrease in the firing rate of medial vestibular nucleus neurons (Maclellan et al., 1996). However, in this study, it seems that the inhibitory effect of DMSO could decrease or may abolish with SB334867.

Our prior research showed that LH inactivation disrupts effort and delay-based decision-making task and this document clarified there is a relationship among ACC/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 due to the elimination of neurotransmitters in the LH. However, relatively little attention was paid to the role of LH neurotransmitters-promoting systems in the regulation of neural activity in the PFC. In recent years ascending evidence has highlighted the importance of the orexinergic system in cognitive functions and also its crucial role in the mPFC for modulating cognitive behaviors. Recent evidence indicates that orexins could regulate the mammalian PFC directly (Lambe & Aghajanian, 2003; Lambe, Olausson, Horst, Taylor, & Aghajanian, 2005; Y. Li et al., 2010; Liu & Aghajanian, 2008). Several lines of evidence indicate that these new neuropeptides may also involve in the regulation of a variety of affective and cognitive processes during wakefulness. Also, as we know, LH sent their projections to mPFC, which have 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; Y. Li et al., 2010; Liu & Aghajanian, 2008).

It seems that OX1Rs has an important 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), and. Also, Aracri and colleges suggested the direct regulatory actions of orexins in the frontal areas are much more bright than prior also they have shown 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. (Jianxia Xia et al., 2005; JX Xia et al., 2009; Yan, He, Xia, Zhang, & Hu, 2012). Additional previous research demonstrated 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 in Fr2 layer V pyramidal cells and also, increase GABA onto regular spiking pyramidal cells (Aracri et al., 2013). These results recommend that PFC activity may regulate via direct innervations of the PFC neurons by hypothalamic orexin system. In addition, some studies indicated that orexin has had 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) and 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 due to the reduction in orexinergic projections’ activation on excitatory neurotransmitters. In addition, the elimination of orexins’ excitatory effect on GABA releases my cause of increase in firing rate in some neurons.

Furthermore, ascending evidence points to a cross-talk between endocannabinoid and orexinergic systems. Anatomical studies have found there is an overlapping distribution in several areas of 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 mesocorticolumbic area, have both receptors
(Aston-Jones et al., 2010; Maldonado, Valverde, & Berrendero, 2006; Plaza-Zabala, Flores, Maldonado, & Berrendero, 2012). Firstly in 2003, direct CB1- OX1R interaction was proposed (Hilairet, Bouaboula, Carrière, Le Fur, & Casellas, 2003). Moreover, the activation of OX1R stimulates the synthesis of the most important endocannabinoids, 2-arachidonyl glycerol, and could inhibit the neighboring cells via its retrograde inhibition effect. This phenomenon suggests that endocannabinoids could contribute to some hypocretin effects. Stress could active the presynaptic CB1 and leads to the inhibition of inhibitory, excitatory, and monoaminergic neurotransmitter release in the cortico-limbic brain regions including the prefrontal cortex, hippocampus, amygdala and hypothalamus (Freund, 2003). It seems that in the present study the absence of orexin release this retrograde inhibition was omitted; however, it needs more investigations to confirm this hypothesis.

Taken together, it seems that the LH orexinergic projections that directly and transynaptically converge on the ACC and also OFC as two important regions of the medial prefrontal cortex regulate the firing rate of these areas, and as a result of this cooperation LH associates with cognitive functions such as decision-making and reward processing.
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A) Microinjection Site

Lateral Hypothalamus

- Vehicle
- Treatment (SB334867 OR Lidocaine 4%)
- Misplacement
Fig. 1. Histological verification
Representative images showing the microinjection site located on the LH (A) and the electrophysiological recording site (B) located in the ACC and OFC.
Fig. 2. The effect of OX1Rs blockade on OFC neural activity

SB334867 (30nM/ 0.5µl) infusion into the OFC elicited an excitatory effect in most of the neurons. A, histogram is representing spike count per time bins of 60sec over the entire recording. Blockade of OX1r could increase the firing frequency of one recorded neuron, B) scatter plot illustrating OFC neurons’ responses after SB334867 (30nM/ 0.5µl) administration. C, SB334867 (30nM/ 0.5µl) caused excitation in 8 neurons and, it could diminish inhibitory effects of DMSO 12%, P= 0.04. D, in the subclass of excitatory neuron (n = 7), the firing frequency was increased significantly relative to the baseline firing, P=0.0078.
Fig. 3. The effect of OX1Rs blockade on OFC neural activity SB334867 (30nM/ 0.5µl) infusion into the ACC elicited an excitatory and inhibitory effects in the neurons of this area. A, histogram is representing spike count per time bins of 60sec over the entire recording. Blockade of OX1r could increase the firing frequency of one recorded neuron, B) scatter plot illustrating ACC neurons’ responses after SB334867 (30nM/ 0.5µl) administration. C, A comparison of percent of change in the firing rate between experimental and control group. D, in the subclass of excitatory neuron (n = 6), the firing frequency was increased significantly relative to the baseline firing, P=0.03.
Fig. 4. The effect of LH inactivation on ACC neural activity

Lidocaine 4% infusion into the lateral hypothalamus elicited an inhibitory effect in most of the ACC neurons. A, representative pattern of baseline spontaneous firing recorded simultaneously from ACC neurons (time frame: 0.172 s). B, firing pattern of the same neurons recorded after intra LH administration of lidocaine 4% (time frame: 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 60sec over the entire recording. Reversible inactivation of LH could decrease the firing frequency of one recorded neuron (D) and, at the same condition, increased the firing rate in the other neuron (E). F, scatter plot illustrating 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.
Fig. 5. The effect of LH inactivation on OFC neural activity

Reversible inactivation of the lateral hypothalamus elicited an excitatory effect in most of the 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 LH could decrease the firing frequency of one recorded neuron (D) and, at the same condition, increased 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.