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Orexin 1 receptors in the anterior cingulate and orbitofrontal cortex regulate cost and benefit decision-making

Sara Karimi[a], Gholamali Hamidi[a,*], Zahra Fatahi[b], Abbas Haghparast[b,*]

[a] Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran
[b] Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Orexin neurons are discretely localized within the lateral hypothalamus and have widespread projections into all areas of the brain. In addition, several lines of evidence specify that orexins may also participate in the regulation of a variety of affective and cognitive processes. The Orexin-1 receptor (OX1r) is distributed extensively throughout the prefrontal cortex (PFC). Delay-based decision- making is mediated largely by the orbitofrontal cortex (OFC) while effort- based decision-making is controlled by the anterior cingulated cortex (ACC). Hence, in the present study, a series of experiments were conducted to clarify the role of OX1r in the mPFC (ACC and/or OFC) in cost and benefit decision-making. The rats were trained in a delay and/or effort-based form of cost-benefit T-maze decision-making task. Two goal arms were different in the amount of accessible reward and cost. Before surgery, all animals were selecting the high reward arm and pay the cost on almost every trial. During the test days, the rats received local injections of either DMSO 20% /0.5 µl, as a vehicle, or SB334867 (3, 30 and 300 nM/0.5 µl), as a selective OX1r antagonist, within the ACC and/or OFC. The results of this study showed that the bilateral microinjection of SB334867 into ACC and/or OFC changed the preference to a low reward arm with no cost, indicating the role of OX1 receptors in cost and benefit decision- making. From these results, it can be implied that OX1 receptors in the mPFC play a crucial role for allowing the animal to evaluate and pay the cost to acquire greater rewards.

1. Introduction

The orexins are neuropeptide transmitters made exclusively in hypothalamic neurons that have extensive central nervous system (CNS) projections. The orexins constitute two peptides (orexin A and orexin B) which have two receptors (OX1r and OX2r). While the Orx2r has an equal affinity for both ligands, the Orx1r binds OrxA with a tenfold greater affinity relative to OrxB (Sakurai et al., 1998). Orexin is mainly expressed by neurons located in the posterior of the hypothalamus. Despite being small in number, these neurons release orexin through the CNS and affect a variety of physiological functions including sleep, hunger, and drug abuse (Sakurai et al., 1998; Mahler et al., 2012; Ritchie et al., 2010; Thannickal et al., 2000). Orexin neurons can be putatively organized into three cell-clusters in the hypothalamus: a cluster in the dorsomedial hypothalamus, perifornical area (PeF), and the lateral hypothalamus (LH). PeF predominantly contains neurons that are activated during cortical activations (Kostin et al., 2012). There are several reasons to believe that orexin might also be important in motivating cost-benefit decisions. The orexin system has also been implicated in reward behaviors (Aston-Jones et al., 2010), in arousal (Sutcliffe and de Lecea, 2002; Sakurai, 2007). Additionally, orexin is thought to play an important role in different forms of learning and memory (Akbari et al., 2006; Akbari et al., 2007; Aou et al., 2003; Jaeger et al., 2002).

"Decision-making is an adaptive behavior that takes into account several internal and external input variables and leads to the choice of a course of action over other available and often competing alternatives" (Khani et al., 2015). The costs and benefits must be weighed before deciding on which course of action to choose and recent studies have shown the relationship between some brain regions and calculating the cost of actions. The anterior cingulate cortex (ACC) is located in a unique position in the brain and is connected to both the limbic system as well as the "emotional" and the prefrontal cortex "cognition" (Euston et al., 2012). ACC plays a crucial role in initiation, motivation, and goal-directed behaviors (Devinsky et al., 1995). A number of studies indicate that the ACC is involved in cost evaluation (Rushworth et al., 2004). Recent studies have demonstrated that the ACC plays a basic role in effort-based decision-making (Walton et al., 2003; Schweimer

* Corresponding authors.
E-mail addresses: Hamiidi@yahoo.com (G. Hamidi), Haghparast@yahoo.com (A. Haghparast).

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and Hauber, 2005). The ACC has a fundamental role in both learning and using extended action-outcome histories to optimize voluntary choice behavior (Kennerley et al., 2006).

The orbitofrontal cortex (OFC) is the portion of the prefrontal cortex that is important for detecting and tracking the value of a stimulus (Rolls, 2000). The OFC is also activated when an expected reward is not obtained and when behavior should be changed (Rolls, 2000). The OFC plays a key role in processing reward. It integrates multiple sources of information about the reward outcome to originate a value signal. In effect, OFC calculates how rewarding a reward is (Wallis, 2007). In 2006, Rudebeck et al. reported that OFC is involved in delay-based decision-making (Rudebeck et al., 2006). A previous study showed that the systemic administration of SB334867 reduced the subjects' willingness to expend effort for high-fat chocolate food over regular food in effort-based decision-making task (Borgland et al., 2009). An earlier study showed that there is a direct projection from LH to mPFC with rostro-caudally gradient and the expression of OX1r in the ACC (Jin et al., 2016). The OFC is densely connected to the hypothalamus and the lateral OFC is an important region for the delay-based decision-making (Hirose et al., 2016). Since there is limited knowledge about the role of the hypothalamic-cortical orexinergic system in the decision-making process, this study aimed to determine the involvement of OX1r in the ACC and OFC regions in the reduction of the preference for expanding an effort to obtain a high reward in T-maze decision–making task in rats.

2. Materials and methods

2.1. Animals

42 male Wistar rats (Pasteur Institute, Iran) were used as subjects. Rats were 8 weeks old at their arrival to the animal facility. Rats were housed in groups of three per cage under standard conditions in a temperature-controlled room and maintained on a standard 12/12 h light/dark cycle (lights on at 07:00 am). Water was available ad libitum. The animals were handled on a daily basis and food was adjusted for initial body weights of about 85% of the free feeding weight during the beginning of the behavioral experiment (190–220 g) and after this a controlled weight gain of about 6–12 g per week. All animals were naïve to the current tests and had no experience in any behavioral experiments.

2.2. Drugs

In this study, the drugs used were as follows: SB334867 (Tocris Bioscience, Bristol, UK), as an OX1r antagonist, was dissolved in 20% dimethyl sulfoxide (DMSO; Sigma Aldrich, Germany). Control animals received DMSO 20% as a vehicle.

2.3. Apparatus

T-mazes were used adopted with parameters from the study by Denk in 2005 (Denk et al., 2005). The Plexiglas mazes had three arms each 60 cm long, 10 cm wide and 40 cm high. Four retractable doors were built in the goal arms of the maze. One door was placed just before the food at each arm, 5 cm from the end of the arm and the other after the entrance into each arm, 12.5 cm from the entrance point. The doors were used in delay-based decision-making task to delay the access of the animals to rewards (Fig. 1). Furthermore, there were grooves at the beginning of the entrance to each goal arm in both mazes, where a barrier of 10 cm width and 40 cm height could be placed on certain trials to force the animal to go to one of the goal arms (“forced” trials).

2.4. Behavioral training

Before the start of training, the rats were handled every day for one week to familiarize them with human contact and were put on a restricted feeding schedule. When they reached 85% of their free-feeding weight, the rats were introduced to the T-maze.

2.4.1. Habituation phase

On the two days, the animals were placed in the start arm in cages of three and were allowed to explore the maze for 10 min. Plentiful food was left in both feeding wells in the goal arms. The third and fourth days of habituation were identical except for the fact that each animal investigated the maze individually. At the end of these 2 days, all of the rats were eating the pellets in the food wells (45 mg food-reinforcement pellets, Formula A/I; P. J. Noyes, Lancaster, NH).

2.4.2. Discrimination phase

The first phase of discrimination training involved putting ten pellets in the feeding well of one goal arm [high food arm (HRA)] and two pellets in the other goal arm [low food arm (LRA)]. For half of the rats, the HRA was to the left, and, for the others, it was to the right. The side of the maze that served as the HRA was the same for each subject throughout all experiments. Initially, each rat was placed in the start arm and was allowed to choose both food arms on each trial. Five trials ran each day over 2 d. For the next 2 d, the rats were moved onto the second phase in which access to one of the goal arms was prevented by placing a door at its entrance (forced trials), thus forcing the rat to sample a particular arm on each trial. The order of the forced trials was determined pseudo randomly so that they never had more than two consecutive turns to either side. There were 10 trials run per day for each animal. On each day, at first each rat received two forced trials, one to each goal arm, then 8 choice trials, with an inter-trial interval of approximately 5 min. The rats were removed from the maze after eating the food in the selected arm without being able to sample from the other arm. This protocol was used throughout all experiments.

2.4.3. Effort-based decision-making task

After rats had learned the unequal size of the reward, they then underwent barrier training. When 80% of choices were HRA with the 10-cm barrier for each rat the barrier height was increased to 20 cm. Rats were given three training days with a 20-cm barrier and 3 days with a 30-cm barrier. In experiment 1a (n = 16) the barrier introduced just for high reward arm (differential effort) but for experiment 1b (n = 6) the barriers introduced for both arms (equal effort).

2.4.4. Delay-based decision-making task

After the rats had learned the different size of reward, a delay of 5 s was introduced into the HRA, meaning that in the LRA the rat received it immediately two food pellets, whereas in the HRA it had to wait5 s, confined in the arm by the movable gates, before receiving ten food pellets. Each day rats received ten trials, two forced and eight choice trials. Once rats chose the HRA on at least 80% of trials in 3 days, the delay was increased to 10 s, and then to 15 s after the same criteria was met. In experiment 2a (n = 14) the delay introduced for high reward arm (differential delay) and in experiment 2b (n = 6) delay introduced for both arm (equal delay).

2.5. Surgery

Anesthesia was achieved using a mixture of Ketamine (100 mg/kg) and Xylazine (8 mg/kg) intraperitoneal (i.p.) injection, and then rats were immobilized in a stereotaxic device (Stoelting Co., USA). An incision was made along the midline on the head of the rats and the skull was exposed. 23 gauge stainless steel guide cannulae were implanted bilaterally 1 mm above the target locations. The coordinates for target locations were determined from a rat brain atlas (Paxinos and Watson, 2007) as follows: 3.7 mm anterior to bregma, 2.2 mm lateral to the midline, and 5 mm ventral to the skull for OFC and 1.2 mm anterior to bregma, 0.7 mm lateral to midline and 3 mm ventral to the skull is the

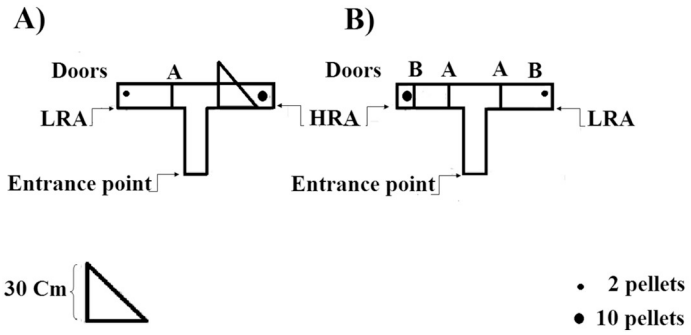

Fig. 1. Schematic illustration of the apparatus. T-maze decision-making task (main task). The apparatus has three arms including start, high reward and low reward arms.(A) Effort-based T-maze decision-making task. The animals could choose between a low reward and a high-reward arm. They could climb over a wire mesh barrier (30 cm) to obtain ten pellets in the HRA. (B) Delay-based the animals could choose to wait (15 s) in order to obtain a higher reward (10 pellets) and/or choose to receive low reward (2 pellets) immediately. (C) Transient time was calculated from entrance point to end of entrance arm and decision time from end of entrance point to A point. HRA, High reward arm; LRA, Low reward arm.

coordination of the ACC. After securing the guide cannulae in place, dental acrylic cement (Paladur) was applied to fix the implants. Following the surgery, rats were housed individually, were monitored on a daily basis, and were allowed to recover from the surgery at least for 10 days before restarting any behavioral training. For 2 days before the surgery and during the recovery period, rats had free access to food and water.

2.6. Experimental design

Following recovery from the surgery and reestablishment of the restricted food regime, the animals completed two “forced decision” trials before eight “choice” trials of T-maze decision-making task per day, until they reached the pre-surgery performance rate. To ensure steady behavior, the training was continued for 5 days. During the test days, after the “forced decision” trials, the animals received bilateral microinjection of either SB334867 (OX1r antagonist) or DMSO 20% (0.5 µl/per side) in the OFC or ACC in a counterbalanced manner; the animals that received local SB334867treatment on the first test day were subjected to local vehicle treatment and vice versa. The animals performed the task for 3 days without any manipulation between test days and microinjection day.

All microinjections were administered in a volume of 0.5 µl per side over 60 s using a stainless steel needle (30gauge) which was directly inserted into the guide cannula, protruding 1 mm beyond the tip of the cannula. Polyethylene tubing (PE-20) connected the injector cannula to a 1-µl Hamilton syringe. The injectors were left in place for 60 s after the injection to allow diffusion and were then replaced by the stylets. After the microinjections, the behavioral testing was continued in exactly the same way as before and the animals' behavior was recorded.

In experiment 1 after the completion of all behavioral testing with microinjection in the effort-based decision task, the animals were trained on a control task in which a similar 30 cm height barrier was newly introduced also to the LRA (equal effort), to test possible involvement of locomotor activity, spatial preference or memory in the decisions of the animals. The training continued for about 10 days to ensure that the changed rule was well established for the animals. On the test days, the animals received microinjections of SB334867 or DMSO 20% (0.5 µl/per side) in the ACC in a counterbalanced manner and behavioral parameters were analyzed as above.

For experiment 2 after the completion of all behavioral testing with microinjection in the delay-based decision task, the animals were trained on a control task in which a similar 15 s delay was newly introduced also to the LRA (equal delay), to test possible involvement of locomotor activity, spatial preference or memory in the decisions of the animals. The training continued for about 10 days to ensure that the changed rule was well established for the animals. On the test days, the animals received microinjections of SB334867 or DMSO 20% (0.5 µl/per side) in the OFC as a counterbalance manner and behavioral parameters were analyzed as above.

2.7. Transient time

For all trials in both experiments, a transient time was calculated. Transient time was defined as a time from a start point to decision point before chose an arm (Fig. 1).

2.8. Decision time

In both experiments and for all trials a time spending during making a decision (Decision time) was calculated. Decision time was defined as a time from a decision.

2.9. Locomotor activity

In all cost-benefit decision-making tasks, the effect of factors such as memory, locomotor ability, or possible spatial preferences in the make a decision is controlled by the equal cost control experiments. However, in this investigation, we added another control experiment for surveying the role of locomotor activity in the decision making. Changes in the activity level as a result of orexinergic system deactivation may bias animals' choice in the presence of differential costs. Two separate groups of rats were implanted with cannulae aimed at the ACC or OFC. The coordinates and surgery procedure were the same as described above. Both groups received SB334867 in the ACC/OFC. Five minutes after microinjection, rats were placed in the center of an open field measuring 30 cm × 30 cm with 30 cm walls and were allowed to freely move inside the arena for 10 min. The activity was recorded through a 3CCD camera (Panasonic Inc., Japan) mounted 2 m above the open field and was analyzed offline using Ethovision video tracking software (version 3.1, Noldus Information Technology, The Netherlands).The distance traveled for 10 min were analyzed.

2.10. Histology

After completion of behavioral testing, the animals were deeply anesthetized with Ketamine and Xylazine. Then, they were transcardially perfused with 0.9% saline and 10% formalin solution. The brains were removed, blocked and cut coronally in 50 m sections through the cannulae placements. The neuroanatomical location of cannulae tips placements were confirmed using a rat brain atlas. Only the animals with correct cannulae placements were included in the data analysis. Schematic illustrations of coronal sections of the rat brain showing the approximate location of the OFC and ACC injection sites in the experiments (Fig. 2). The numbers indicate anterioposterior coordinates relative to bregma. Atlas plates are adapted from Paxinos and Watson (Paxinos and Watson, 2007).

2.11. Statistics

Data is expressed as mean ± SEM (standard error of the mean). The data were analyzed by commercially available software GraphPad

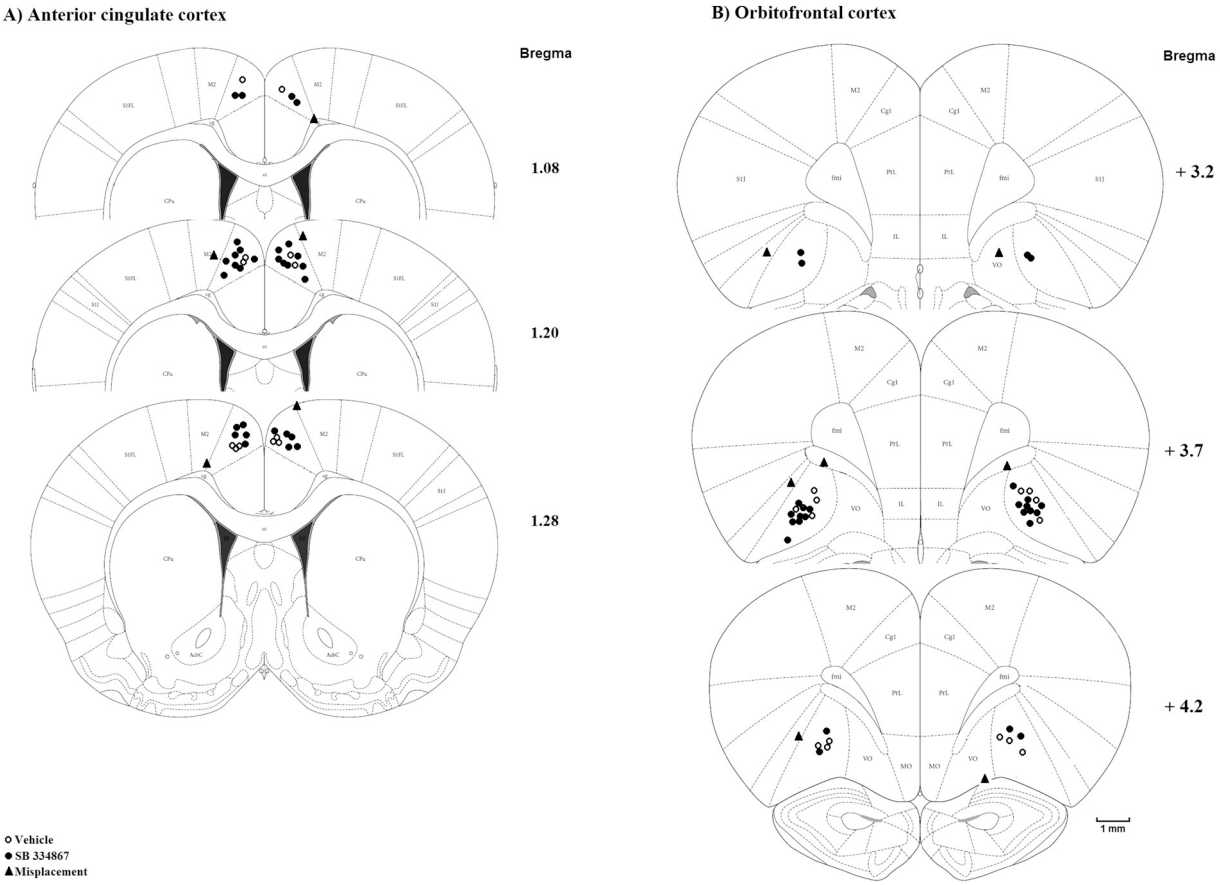

Fig. 2. Coronal schematic sections show the injection sites in A) the ACC and B) OFC.
[○ Vehicle; ● SB334867; ▲ Misplacement]. CPu, Caudate putamen (striatum); cc, Corpus callosum; cg, Cingulum; M2, Secondry motor cortex; S1FL, Primary somatosensory cortex, forelimb region; S1J, Primary somatosensory cortex, jaw region; AcbC, Accumbens nu, core; cg1, Cingulate cortex area 1; PrL, Prelimbic cortex; Fmi, Forceps minor of corpus callosum; IL, Infralimbic cortex; VO, Ventral orbital cortex; MO, Medial orbital cortex. Adopted from "The Rat Brain in Stereotaxic Coordinates" (Denk et al., 2005).

Prism® 5.0. In order to compare the percentage of high reward choice (HRC), transient time and decision time in all groups (vehicle and experimental groups), two-way analysis of variance (ANOVA) followed by post hoc Bonferroni was used as needed. *P*-values < .05 ($P < .05$) were considered to be statistically significant.

3. Results

3.1. Effect of ACC OX1 receptors blockade on the effort-based decision-making

3.1.1. Differential effort

After recovery from surgery, the animals were trained until the percentage of HRC reestablished to its level on the day before surgery for three consecutive days. On test day, different doses of SB334867 (3, 30 and 300 μM/0.5 μl DMSO) or DMSO 20% (0.5 μl/per side)were administrated into the ACC. Two-way repeated measure ANOVA followed by Bonferroni test [Treatment effect: $F(3,23) = 4.54$, $P = .0122$; Day effect: $F(3,69) = 18.75$, $P < .0001$; Treatment × Day: $F(9,69) = 2.58$, $P = .0126$)] revealed that blockade of ACC OX1r significantly decreased tending to HRC and in these animals, HRC percent significantly decreased compared to vehicle-control group (Fig. 3A). The maximum effect was at the doses of 30 ($P < .001$). Thus, the activation of OX1r in the ACC affected the preference of animals from deciding to pass the barrier for getting a large reward to choose to receive a small reward without any cost.

3.1.2. Equal effort

In addition, we trained the SB334867 (30 μM)-treated animals on an additional equal control task to determine possible involvement of spatial preference or memory in the decisions of the animal. In this task, there was the equal cost (30 cm barrier) but different reward (10 pellets vs 2 pellets) in both HRA and LRA. Two-way repeated measure ANOVA followed by Bonferroni test [Treatment effect: $F(1,9) = 2.12$, $P = .179$; Day effect: $F(3,27) = 0.24$, $P = .37$; Treatment × Day: $F(3,27) = 1.09$, $P = .86$)] revealed that in equal control task,SB334867 -treated animals preferred high/delay reward and there was no significant reduction in HRC percentage compared to vehicle-control group in this task (Fig. 3B).

3.2. Effect of ACC OX1 receptors on transient and decision-making time in the effort-based decision-making

Two-way repeated measure ANOVA followed by Bonferroni test [Treatment effect: $F(3,18) = 0.80$, $P = .51$; Day effect: $F(3,54) = 2.88$, $P < .04$; Treatment × Day: $F(9,54) = 0.89$, $P = .5437$)] showed that administration of different doses of SB334867 into the ACC had no effect on transient time compared to vehicle-control group (Fig. 4A). The results showed that ACC administration of SB334867had no effect on decision time [Treatment effect: $F(3,17) = 0.84$, $P = .4921$; Day effect: $F(3,51) = 14.2$, $P < .0001$; Treatment × Day: $F(9,51) = 1.27$, $P = .2748$); Fig. 4B].

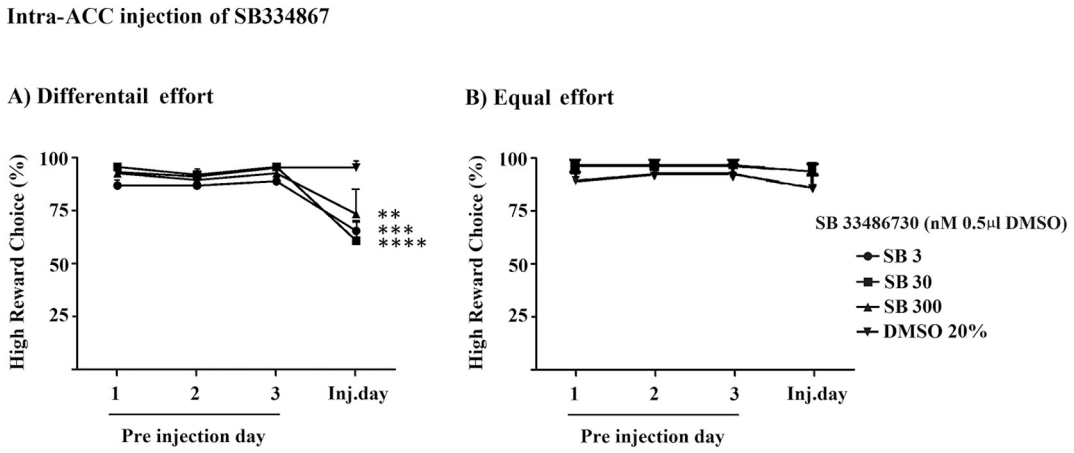

Fig. 3. *(A)* Intra- ACC injection of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) on test day decreased percent of high reward choice. *(B)* The performance on a control task in which the same group of rats had passed the barrier to receive reward in both goal arms. Data show as mean ± SEM for 6–8 rats.
** $P < .01$; *** $P < .001$; **** $P < .0001$ different from the vehicle-control group.

3.3. Effect of OFC OX1 receptors blockade on the delay-based decision-making

3.3.1. Differential delay

After recovery and retraining, on the test day, the animals were administrated withSB334867 (3, 30 and 300 µM/0.5 µl DMSO) or DMSO 20%and were tested for differential delay task. Two-way repeated measure ANOVA followed by Bonferroni test [Treatment effect: $F(2,22) = 10.57$, $P < .0002$; Day effect: $F(3,66) = 43.39$, $P < .0001$; Treatment × Day: $F(9,66) = 5.85$, $P < .0001$)] revealed that blockade of OFC OX1 receptors significantly attenuated HRC percentage and in these animals, HRC percent significantly decreased compared to vehicle-control group (Fig. 5A).In addition, there is no significant difference between the high dose of antagonist (300 nM) with the vehicle group (Fig. 5A).

3.3.2. Equal delay

In this experiment introducing 15 s delay for both HR and LR caused animals to shift to HRA after OX1r blocked by SB334867(30 µM/0.5 µl DMSO). Two-way repeated measure ANOVA followed by Bonferroni test [Treatment effect: $F(1,9) = 3.74$, $P = .0852$; Day effect: $F(3,27) = 1.64$, $P = .2034$; Treatment × Day: $F(3,27) = 0.29$, $P = .83$)] revealed that in equal control task, SB334867-treated animals preferred high/delay reward and there was not a significant reduction in HRC percentage compared to vehicle-control group (Fig. 5B).

3.4. Effect of OFC OX1 receptors on transient and decision-making time in delay-based decision-making

The results showed [Treatment effect: $F(3,18) = 0.48$, $P = .70$; Day effect: $F(3,54) = 4.28$, $P = .0088$; Treatment × Day: $F(9,45) = 0.83$, $P = .5$)] showed that administration of different doses of SB334867 into the OFC had no effect on transient time (latency of passing entrance arm)compared to vehicle-control group (Fig. 6A), and also in equal control task, administration of SB334867 (30 µM) [Treatment × Day: $F(3,27) = 0.61$, $P = .61$); Day effect: $F(3,27) = 1.08$, $P = .3731$, Treatment: $F(1,9) = 4.86$, $P = .055$] (Fig. 6B).

Two-way repeated measure ANOVA followed by Bonferroni test showed that administration of different doses of SB334867 led to increase of decision time in the rat [Day effect: $F(3,63) = 6.99$, $P < .0004$; Treatment × Day: $F(9,63) = 3.20$, $P = .003$)] but there is no difference between treatments [Treatment effect: $F(3, 21) = 1.38$, $P = .275$].The results showed that intra- OFC administration of SB334867 (30 µM) had a significant effect on decision time($P < .01$) group(Fig. 7A). On the other hand, in equal control task, administration of SB334867 (30 µM) led to increase of decision time for reward achievement [Treatment effect: $F(1,10) = 11.05$, $P = .0077$; Day effect: $F(3,30) = 3.96$, $P = .0173$; Treatment × Day: $F(3,30) = 2.99$, $P = .0046$)] compared to DMSO-control group (Fig. 7B).

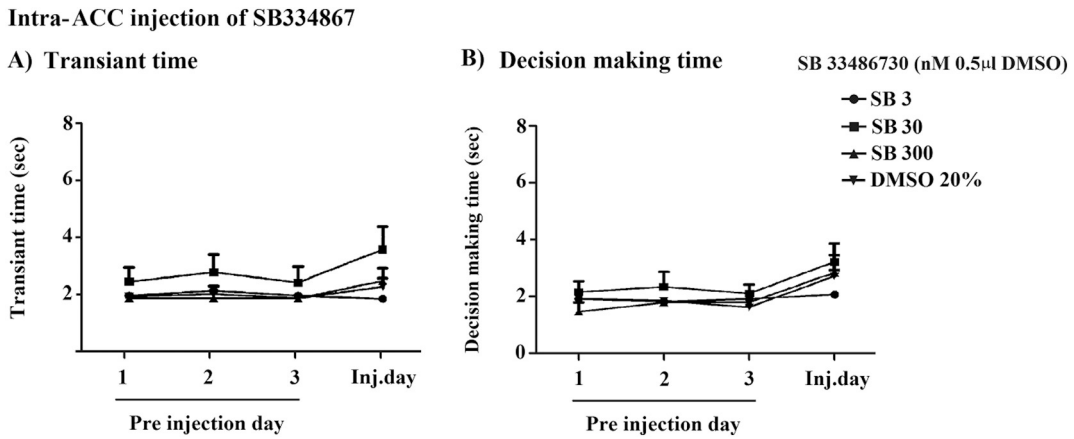

Fig. 4. *(A)* Administration of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) into the ACC on the test day had no effect on transient time for reward achievement. *(B)* Administration of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) into the ACC on the test day had no effect on decision time of choosing an arm. Data show as mean ± SEM for 5–6 rats.

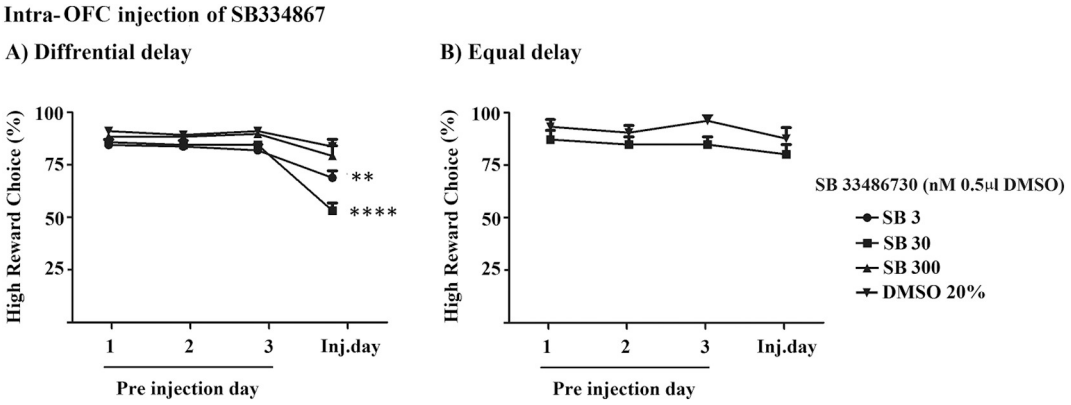

Fig. 5. *(A)* Microinjection of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) into the orbitofrontal cortex on test day decreased percent of high reward choice. *(B)* The performance on a control task in which the same group of rats had to wait (15 s) to receive reward in both goal arms. Data show as mean ± SEM for 5–7 rats. ** $P < .01$; **** $P < .0001$ different from the vehicle-control group.

3.5. Effect of ACC/OFC OX1 receptors on locomotor activity

The distance traveled following SB334867 treatments in the ACC/OFC areas was calculated during 10 min, and it suggested that locomotor activity has not changed by OX1 receptors antagonist, as shown in Supplementary Fig. 1. This was confirmed by paired *t*-test, t (6) = 0.9437, $P = .3817$ (OFC) and, t(5) = 0.9151, $P = .4021$ (ACC).

4. Discussion

This study aimed to define the possible effect of OX1r on the two most important regions of the prefrontal cortex (ACC and OFC) on cost-benefit decision-making. The main finding of the present study was that the OX1r inactivation in the OFC substantially decreases rat's preference for high reward in delay-based decision- making task. In addition, the blockade of OX1r could increase the decision-making time during the task but it had no effect on transient time. Also, the present effort-based task results revealed that the OX1 receptor antagonist in the ACC reduced the effort required to receive a high reward, but it had no effect on the decision-making time during the task and also on the transient time. On the other hand, locomotor activity was tested and the results showed that OX1r inactivation had no effect on it.

The shift from a high reward to a low reward may be result of 1) a change in value encoding, 2) a difference in cost encoding or 3) a change in processing cost-benefit computations. To distinguish between these possibilities, we designed a task with an equal cost for both arms (HRA or LRA). The SB334867 administration in the mPFC areas in the equal task was due to select HRA by animals, this result supports the hypothesis that in the differential task the results do not purely depend on reward value choice. Also, the equal tasks have indicated that the presentation of the cost may not be a certain cause for selecting the LR arm in the cost differed tasks. A proper hypothesis is that OX1 receptors in these areas are required not only for value or cost encoding but also for cost-benefit computations. These experiments emphasized that orexin 1 mediates pathways which are important for allowing animals to overcome costs such as delay or effort –related cost to obtain high rewards.

These effects were specific to a reduction in motivation to achieve a high reward, and not due to the effect of SB334867 on locomotors activities. SB334867 had no effect during equal cost test, showing that it did not affect the animals' power to move towards the high rewards. Therefore, the current data demonstrated that SB334867 specifically reduced HRA selection in both forms of decision-making task, in agreement with the proposed role of orexin in controlling behavior under a motivational state. It appears that blocking OX1r in this region decreases motivation for performing an action (Mahler et al., 2014; Saper, 2006; Sakurai, 2014).

This is the first demonstration that orexin signaling via OX1r in the mPFC (ACC and/or OFC) may play a critical role in mediating cost and benefit decision-making tasks. However, these findings match other recent studies implicating orexin as important for decision-making (Borgland et al., 2009; Karimi et al., 2017; Thompson and Borgland, 2011) and motivational behavior.

This result of a reduction in motivation for a high reward by SB334867 could be achieved by either direct or indirect effect via glutamatergic neurons or GABAergic neurons. Notably, cell bodies of

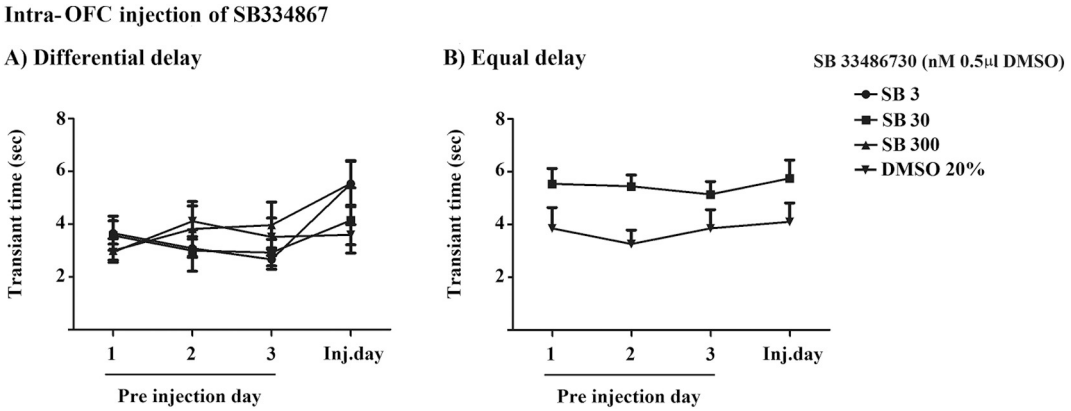

Fig. 6. *(A)* Administration of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) into the OFC on the test day had no effect on transient time. *(B)* The performance on a control task in which the same group of rats had to wait (15 s) to receive reward in both goal arms. Data show as mean ± SEM for 5–6 rats.

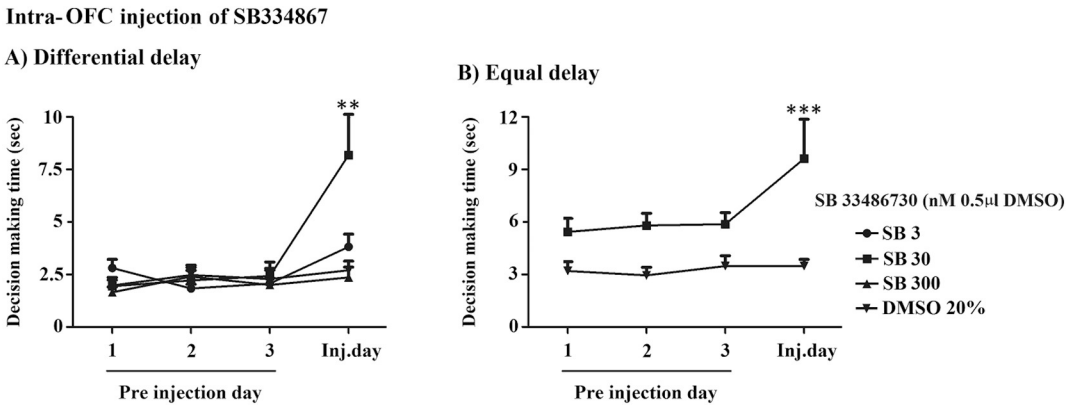

Fig. 7. *(A)* Administration of SB334867 (3, 30 and 300 µM/0.5 µl DMSO) into the OFCon test day increased decision time for reward achievement.*(B)* The performance on a control task in which the same group of rats had to wait (15 s) to receive reward in both goal arms. Data show as mean ± SEM for 6 rats. ** $P < .01$; *** $P < .001$ different from the vehicle-control group.

the glutamatergic neuron project to the NAc shell, including the prefrontal cortex and basolateral amygdala, which express OX1r (Trivedi et al., 1998; Marcus et al., 2001). Furthermore, the OX1r promotion of glutamate release has been demonstrated in other brain regions such as the VTA (Borgland et al., 2009; Borgland et al., 2006; Wang et al., 2009) amygdala (John et al., 2003), nucleus accumbens (Patyal et al., 2012) and hippocampus (Stanley and Fadel, 2011). In previous studies, Aracri et al. demonstrate that in the PFC, the application of orexin excited fast-spike interneurons, causing the release of GABA onto pyramidal cells and this effect was mediated by OX1rs (Aracri et al., 2013; Burdakov et al., 2003).

Therefore, it is feasible that activation of OX1r on glutamatergic neurons may affect the release output to other brain regions. Further studies are required to investigate this possibility.

On the other hand, the reduction of interest for having the high reward may be due to the effect of orexin on glutamatergic, dopaminergic and also GABAergic neurons indirectly via the cannabinoid receptor 1 (CB1) or OX1r heteromultimers. OX_1 receptors seem to form a homomeric and heteromeric complex (Xu et al., 2011). The previous research confirmed the capacity of the CB1 and OX 1 receptors to interact directly and showed that this complex could regulate with orexin A. orexin A has a higher potency to CB1-OX1 heteromer compared with OX1-OX1 homomer. CB1 antagonist could affect the function of orexin A and this disruption could suggest interplay between these two systems that may modulate appetite, feeding, and wakefulness (Xu et al., 2011; Ward et al., 2011). Our results may be affected by crosstalk between these neurotransmitter via the OX1-CB1 heteromer.

Endocannabinoid and orexinergic systems are also involved in the regulation of the mesocorticolimbic reward system, a circuit responsible for the pleasurable feelings associated with natural rewards and the consumption of drugs of abuse (Flores et al., 2013). Glutamate synaptic transmission in the NAc and VTA, mainly from neurons of the PFC, is similarly modulated by the activation of CB1 receptors (Melis et al., 2004). The final effect of endocannabinoids on the modulation of dopaminergic activity, which depends on the functional balance between these GABAergic and glutamatergic inputs, is predominantly excitatory (Maldonado et al., 2006). The enhancement in dopamine extracellular levels in the NAc induced by Δ(9)-tetrahydrocannabinol was blocked in mice lacking the OX1 (Flores et al., 2014). Tung and his colleges found that orexin A inhibited GABAergic transmission onto dopaminergic neurons in VTA slices via a presynaptic mechanism. This effect was antagonized by OX1r and CB1r antagonists (Tung et al., 2016). They have described that during stress, orexin release in the VTA and activate postsynaptic OX1 receptors on dopaminergic neurons. Activation of the OX1 receptor leads to generate an endocannabinoid, then this endocannabinoid travels across the synapse to inhibit GABA release by activating presynaptic CB1 receptors on the GABAergic terminal (Tung et al., 2016).These data show the interaction between orexin and cannabinoid and may some similar mechanism occurs during decision-making in the mPFC and OX1 receptors antagonist could inhibit the release of dopamine in the same region in other region by the mPFC projection.

On the other hand, our pervious result showed that the cannabinoid agonist changes the preference of rat to choose the HRA and the agonist disrupt decision-making (Khani et al., 2015). Perhaps the effect of cannabinoid receptor agonist and orexin 1 receptor antagonist on the decision-making correlates with the crosstalk between these neurotransmitters and the contribution between these two neurotransmitters for regulating others like glutamate, dopamine and GABA in other brain region. The previous study has been shown that cannabinoids reduced the activity of orexin neurons in the lateral hypothalamus by presynaptic attenuation of glutamate release (Huang et al., 2007). Perhaps the same mechanism occurs in the mPFC and it should be investigated more.

Interestingly, here it was found that the microinjection of SB334867 in the OFC affect decision- making in a dose dependent manner. It was found that the microinjection of SB334867 (30 nM/0.5 µl) had a highly significant disruption in decision-making and the high dose of the OX1r antagonist (300 nM/0.5 µl) had no effect on delay-based decision-making. Some evidence showed the dose dependent effect of SB334867 in other research like, anxiety (Staples and Cornish, 2014), food intake (Rodgers et al., 2001) and also in the release of dopamine in the NAc before administration of risperidone (Rasmussen et al., 2007). It has been suggested that Orexin could be both anxiogenic and anxiolytic in the investigation of rodent models (Kukkonen and Leonard, 2014).

The roles of OFC and ACC's OX1rs on the decision-making time and transient time were studied. There was no observed significant difference in transient time in both tasks, which suggests that orexin 1 in these regions, did not act while passing the entrance arms. Conversely, even though SB334867 treatment in the OFC increased decision-making time, it had no effect on decision-making time when it was administrated in the ACC. Furthermore, the examination of the locomotor activity has demonstrated that OX1 has not been able to affect locomotor activity. So this increase of decision-making time is not result of the locomotor activity disruption. This strongly suggests that SB334867 in the OFC increased decision-making time and it appears that OX1r could accelerate process which leads to decision. Perhaps orexin has a role in time of calculation of the cost and benefit in the delay-based decision- making but not in effort-based decision- making. Early experiments indicated that the OFC plays a crucial role in decision-making and planning, judging whether the decisions made had failed and had long term severe consequences and also, in the ability to identify and recall the implied meaning or importance of events/situations (Steiner, 2014). The previous study has provided evidence that

OFC is causally required for confidence reporting independent of perceptual decision-making (Lak et al., 2014). Previously, Kepecs found that rat OFC contains an explicit representation of decision confidence (Kepecs et al., 2008). Also, OFC has been implicated in goal-directed or intentional decisions requiring the evaluation of predicted outcomes (Wallis, 2007; Padoa-Schioppa and Assad, 2006; Rolls and Grabenhorst, 2008; Schoenbaum et al., 2009; Kennerley et al., 2011).

The main part of the neurobiology of decision-making focuses on "what is it?" Before making a decision neurons accumulate information about the stimulus in the form of slowly increasing firing rates and reach a decision when those firing rates reach a threshold (Sadacca et al., 2016). The blockade of the OX1 receptor may affect the accumulation of the stimuli in the OFC and it has increased decision-making time. So, the preference of lesser reward observed although the time increase in the different cost task.

In summary, this study showed that pharmacological OX1r blockade in the anterior cingulate cortex and orbitofrontal cortex, especially decreased high reward choices in the cost and benefit decision-making tasks. This disruption in decision- making by OX1r antagonism also increased decision-making time in the delay-based task. This finding clearly revealed that orexin signaling via OX1r at the OFC and ACC is critical for having decisions that optimize the reward magnitude in cost and benefit decision-making tasks. Also, the OFC region played a crucial role in a decision time.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2018.09.006.

Ethical statement

All experiments were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti Universities of Medical Sciences, Tehran, Iran.

The authors declare that they do not have any conflict of interest.

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