

Research Paper: The Modulatory Role of Orexin 1 Receptor in CA1 on Orofacial Pain-induced Learning and Memory Deficits in Rats



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ABSTRACT

Introduction: Cognitive impairment is commonly associated with pain. The modulatory role of *orexin 1 receptor* (OX1R) in pain pathways as well as learning and memory processes is reported in several studies. The current study was designed to investigate the possible role of CA1-hippocampal OX1R on spatial learning and memory of rats following capsaicin-induced orofacial pain.

Methods: Orofacial pain was induced by subcutaneous intra lip injection of capsaicin (100 µg). CA1 administration of orexin A and its selective antagonist (SB-334867-A) were performed 20 minutes prior to capsaicin injection. Learning and spatial memory performances were assessed by Morris Water Maze (MWM) task.

Results: Capsaicin treated rats showed impairment in spatial learning and memory. In addition, pretreatment with orexin A (20 and 40 nM/rat) significantly attenuated learning and memory impairment in capsaicin-treated rats. Conversely, blockage of OX1R via SB-334867-A (40 and 80 nM/rat) significantly exaggerated learning and memory loss in capsaicin-treated rats.

Conclusion: The obtained results indicated that CA1 OX1R may be involved in modulation of capsaicin-induced spatial learning and memory impairment.

Key Words:

Orofacial pain, Orexin 1 Receptor, CA1, Learning and memory, Capsaicin

1. Introduction

Cognitive impairment is observed in patients with pain, especially chronic pain (Hart, Martelli, & Zasler, 2000; Moriarty,

McGuire, & Finn, 2011). Several studies attempted to characterize anatomical and functional brain changes in patients with pain. Orofacial pain entities, covering any pain which is felt in facial and mouth structures, are often very common and complex (Macfarlane et al.,

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2002; Sessle, 1999). The trigeminal nerve conveys most pain impulses from the orofacial region (Capra & Dessem, 1992). Although several researches support the association between chronic pain and impaired cognitive function, a small portion of them are focused on orofacial pain. Two studies recently showed that capsaicin-induced pulpal pain is associated with impairment of learning and memory, and neuronal apoptosis in hippocampus of rats (Raouf et al., 2015a; Raouf et al., 2015b).

Orexin neuropeptides (A and B), produced by lateral hypothalamus neurons, are agonists for 2 Gq-protein coupled receptors, OX1R and OX2R (Sakurai et al., 1998). Orexin receptors (OXRs) are diffusely distributed in various areas of the brain and implicated in the regulation of multiple physiological functions (Marcus et al., 2001; Peyron et al., 1998; Van Den Pol, 1999). Hippocampus formation is an important target receiving strong orexinergic fibers' inputs, suggesting that orexin may contribute to processing of memory (Jaeger et al., 2002; Peyron et al., 1998; Trivedi et al., 1998). Blockade of CA1 OX1Rs leads to impairment in spatial memory in rats (Akbari, Naghdi, & Motamedi, 2006). Moreover, blockade of dentate gyrus (DG) OX1Rs is associated with decreased occurrence of long-term potentiation (LTP) in the DG granular cells (Akbari, Motamedi, Davoodi, Noorbakhshnia, & Ghanbarian, 2011).

Aou et al. (2003) reported that intracerebroventricular (i.c.v.) administration of orexin A induced spatial memory deficient in Morris Water Maze (MWM) and resulted in a significant impairment of LTP in the hippocampal CA1 area. In addition, orexin fibers densely project to pain transmission pathways in the brainstem, spinal cord, periaqueductal gray, thalamus, and hypothalamus (Peyron et al., 1998; Van Den Pol, 1999). It is shown that systemic and central administration of orexin A can suppress nociceptive inputs at either spinal or supraspinal levels (Bingham et al., 2001; Holland, Akerman, & Goadsby, 2006; Yamamoto, Saito, Shono, Aoe, & Chiba, 2003). Hippocampal formation indirectly receives trigeminal inputs from preoptic area, orbitofrontal cortex, and amygdale (Burstein & Giesler, 1989).

Especially, it is shown that orexin A microinjection into trigeminal nucleus caudalis can attenuate capsaicin-induced orofacial pain as well as pain-induced learning and memory deficiency on MWM performance (Kooshki, Abbasnejad, Esmaeili-Mahani, & Raouf, 2016). However, the role of hippocampal OX1Rs in orofacial pain-induced learning and memory impairment is not fully clarified. Therefore, the current study aimed at finding the effect of OX1Rs agonist (orexin A)

and antagonist (SB-334867-A) microinjection into hippocampal CA1 on spatial learning and memory in the rat model of orofacial pain.

2. Methods

2.1. Animals

Adult male Wistar rats, weighed 230 to 270 g, purchased from animal house of Shahid Bahonar University of Kerman were used in the current study. The rats were housed under controlled the conditions ($23\pm 1^{\circ}\text{C}$; 12:12 hours light/dark cycle). Rats had free access to food and water ad libitum. The Ethical Committee of Kerman University of Medical Sciences approved the protocols used in the current study.

2.2. Surgery

Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg). Afterwards, animals were placed in a stereotaxic apparatus and two 22-gauge stainless steel guide cannulae were bilaterally implanted in the CA1 region according to the Paxinos and Watson Atlas of the rat brain (3.8 mm posterior to the bregma, 2.2 mm lateral from the midline and 3.2 mm depth to the cortical surface). Cannulas were fixed to the skull surface with 2 small screws and dental cement. The cannulas were, then, closed with a stylet. After surgery, animals were housed individually and allowed at least 1 week to recover from surgery prior to drug administration and behavioral experiments.

2.3. Drugs

Capsaicin was purchased from Sigma-Aldrich, USA, and dissolved in ethanol/ Tween 80 /distilled water (1:1:8). Orexin A and SB-334867-A were purchased from Tocris Co. (USA). Orexin A was dissolved in distilled water. SB-334867-A was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO was less than 0.1%.

2.4. Microinjection

Intra-CA1 microinjection of orexin A and SB-334867-A were performed using 27-gauge needle attached to a 1 μL Hamilton micro-syringe. The injection needle was inserted 1 mm beyond the tip of the guide cannula and infusions were delivered in a total injection volume of 2 μL (1 μL each side) during 1 minute. After each infusion, the needle was remained in the place for 30 seconds before it was slowly retracted.

2.5. Experimental design

Rats were divided into 6 groups (n=6) as follows: capsaicin vehicle group (Caps vehicle), which received vehicle of capsaicin; capsaicin-treated group (Caps), which received subcutaneous injections of capsaicin (100 µg) into the upper lip; capsaicin+orexin A (Caps+OXA) treated group, which received intra-CA1 microinjections of orexin A (20 and 40 pM) 20 minutes prior to injection of capsaicin; and capsaicin+SB-334867-A (Caps+SB) treated group, which received intra-CA1 microinjections of SB-334867-A (40 and 80 nM) 20 minutes prior to capsaicin injection, capsaicin plus orexin A vehicle (Caps+OXA vehicle) group, which received distilled water as orexin A vehicle (Shu et al., 2014), and capsaicin plus SB-334867-A vehicle-treated (Caps+SB vehicle) group, which received DMSO as SB-334867-A vehicle.

On the examination day, animals were taken to the testing room at least one hour prior to the experiments. Orofacial pain was induced by subcutaneous injection of capsaicin into the left upper lip in a volume of 10 µL just lateral to the midline and the nose of the rat with a 30 gauge hypodermic needle. After capsaicin injection, the rats were placed in Plexiglass observation chambers, with a mirror located at a 45° angle beneath the floor allowing unhindered observation of the rats. Time of typical pattern of face rubbing was used to assess nociceptive behaviors as previously described (Kooshki et al., 2016). Twenty minutes after capsaicin injection, learning and memory performances were considered by MWM test (Morris, 1984).

2.6. Morris Water Maze Test

The water maze apparatus comprised of a black circular tank. The pool was divided into 4 equal quadrants defined by the cardinal directions (N, E, S, and W). The pool was filled with water (20±1°C) to a depth of 25 cm. A hidden circular platform (11 cm diameter) was located in the center of one of the quadrants 2 cm beneath the water surface. Cues, consisted of geometric images, were hung on the walls of the test room and were visible to the rats. The position of each rat was monitored by a video camera and analyzed using computerized tracking system. A day before the experiment, rats were allowed to swim for 60 seconds without the platform for habituation.

2.6.1. Acquisition Test

The acquisition test protocol consisted of 4 blocks (4 trials per block). In each trial, the rats were allowed to swim for 60 seconds to find the hidden platform. If a rat could find the platform, the animal was allowed to stay

30 seconds on it. However, if a rat failed to find the platform within 60 seconds, the animal was manually guided to the platform. There was a 5-minute interval between the trials in a block and a 20-minute rest between the blocks. The rats' escape latency, traveled distance, and swimming speed to reach the hidden platform in each block were recorded (average of 4 trials). In addition, the main latency, travelled distance, and swimming speed in all blocks (including 16 trials) were considered.

2.6.2. Probe Test

A single probe trial was performed 2 hours after the last training trial to test the spatial memory and retrieval capabilities of platform position. In this trial, the platform was removed from the maze. Animals were placed in the quadrant opposite the target quadrant and were allowed to swim freely for 60 seconds. The total time spent in the goal quadrant and swimming paths during the probe trial were recorded.

2.7. Statistical analysis

Data related to the 4 training blocks from acquisition test were analyzed by repeated-measures ANOVA. In addition, data from each block of hidden platform tests and probe training sessions were analyzed by one-way ANOVA. Post hoc analysis was performed using the Tukey test and the significance level was set at $P < 0.05$.

3. Results

3.1. Acquisition Test

3.1.1. Latency time

Figure 1 A shows the effects of orexin A administration on escape latency of capsaicin-treated rats. The results of repeated-measures ANOVA showed significant effects of treatment [$F(4, 115)=8.023$; $P=0.001$] and blocks [$F(3, 345)=15.7$; $P=0.001$]. In the capsaicin-treated group, the escape latency significantly increased in the second ($P < 0.05$), third ($P < 0.001$), and fourth ($P < 0.01$) blocks of acquisition test as compared to that of the vehicle group. However, the effect of capsaicin on escape latency in the third block of the acquisition test significantly attenuated by microinjection of orexin A 20 and 40 pM ($P < 0.01$ and < 0.05 , respectively) (Figure 1A, upper graph). In addition, capsaicin-treated rats showed a significant increase in the mean escape latency compared with the vehicle group ($P < 0.001$) (Figure 1A, lower graph). Moreover, administration of orexin A at 20 pM ($P < 0.01$) and 40 pM ($P < 0.05$) significantly decreased the escape latency in

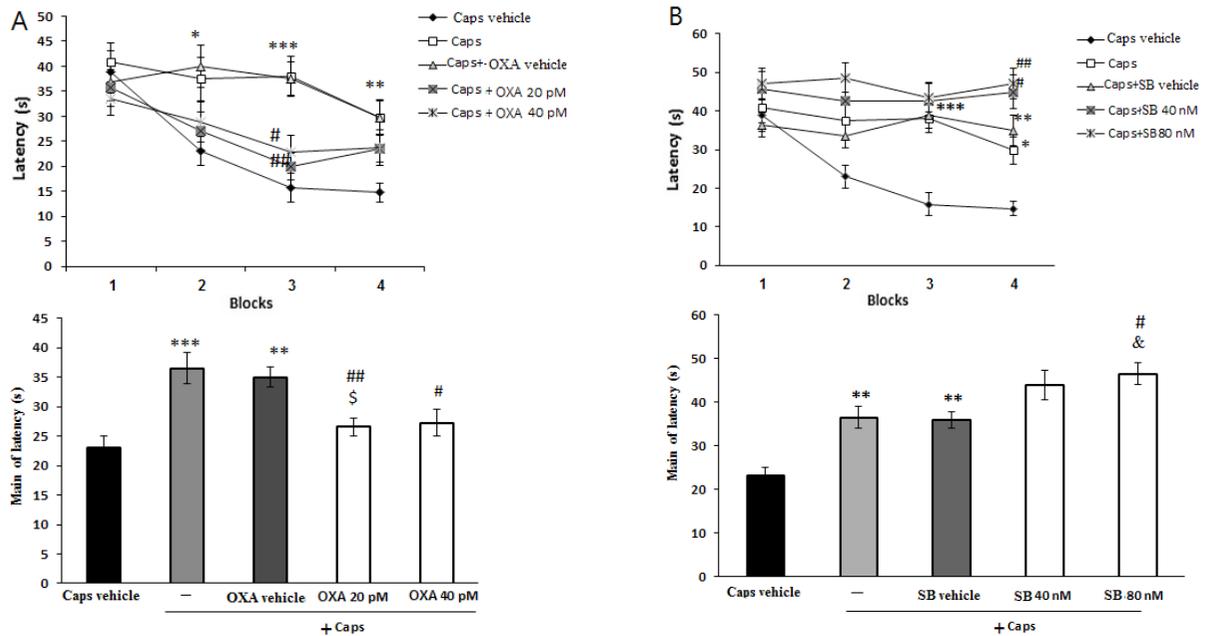


Figure 1. Effects of pre-training intra-CA1 administration of orexin-A (A) and SB-334867-A (B) on escape latency in capsaicin-treated rats. Mean escape latency in each block (upper graph) and in 4 blocks (lower graph) are shown. Values correspond to mean±SEM (n=6), ***P<0.001, **P<0.01, *P<0.05 versus Caps vehicle group, ##P<0.01, #P<0.05 versus capsaicin-treated group (Caps), \$P<0.05 versus Caps+OX-A vehicle group, &P<0.05 versus Caps+SB vehicle.

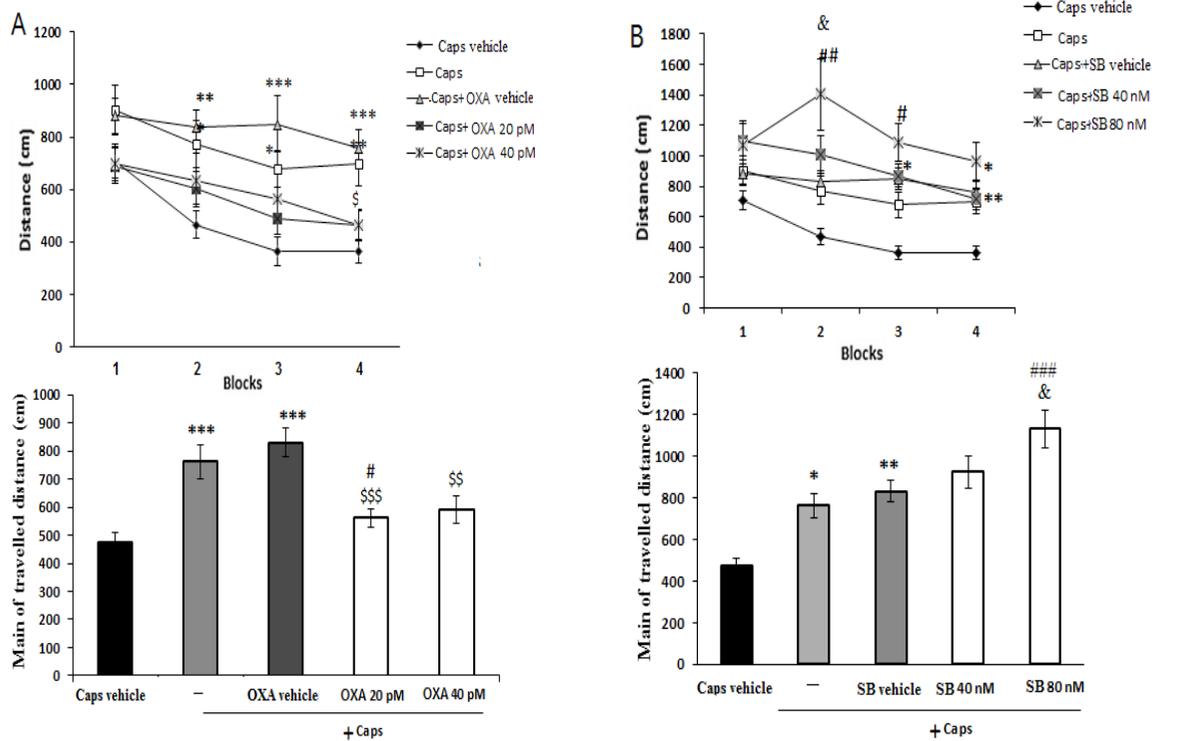
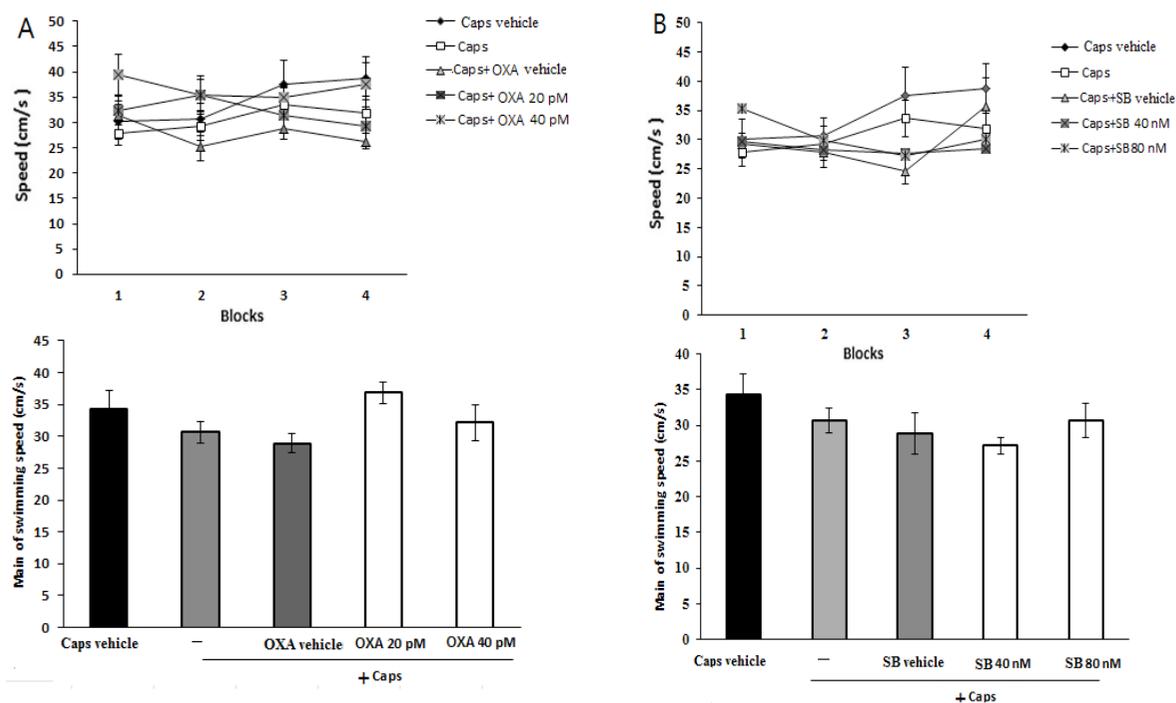


Figure 2. Effects of pre-training intra-CA1 administration of orexin-A (A) and SB-334867-A (B) on traveled distance in capsaicin-treated rats. Mean traveled distance in each block (upper graph) and in 4 blocks (lower graph) are shown. Values correspond to mean±SEM (n=6), ***P<0.001, **P<0.01, *P<0.05 versus Caps vehicle; ###P<0.001, ##P<0.01, #P<0.05 versus capsaicin-treated group (Caps), \$\$\$P<0.001, \$\$P<0.01, \$P<0.05 versus Caps+OX-A vehicle, &P<0.05 versus Caps+SB vehicle.



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Figure 3. Effects of pre-training intra-CA1 administration of orexin A (A), and SB-334867-A (B) on swimming speed in capsaicin-treated rats; Mean of swimming speed in each block (upper graph) and in 4 blocks (lower graph) are shown. Values correspond to mean±SEM (n=6).

capsaicin-treated rats (Figure 1A, lower graph). Figure 1B illustrates the effect of SB-334768-A administration on escape latency during acquisition test in capsaicin-treated rats.

There were significant differences in treatment [(F(4, 115)=13.549; P=0.001)] and blocks [(F(3, 345)=4.647; P=0.003)]. In capsaicin-treated rats, SB-334867-A at doses of 40 nM (P<0.05) and 80 nM (P<0.01) enhanced the escape latency to find the platform during the block 4 of acquisition testing (Figure 1B, upper graph). Moreover, pretreatment with SB-334867-A (80 nM) significantly increased the mean escape latency in capsaicin-treated rats (P<0.05) (Figure 1B, lower graph).

3.1.2. Traveled distance

Figure 2 A shows the effects of orexin A microinjection on the distance traveled by rats to find the hidden platform. Significant effects of treatment [F(4, 115)=9.912; P=0.001] and blocks [F(3, 345)=11.688; P=0.001] were observed. Capsaicin-treated rats traveled significantly greater distances to find the hidden platform than the ones in the vehicle group (P<0.001) (Figure 2A, lower graph). During block 4 of the acquisition trial, the administration of orexin A (20 pM) significantly decreased

the traveled distance as compared with that of the capsaicin group (P<0.05) (Figure 2A, upper graph).

In addition, the overall distance traveled by rats with pain pretreated with 20 pM orexin A significantly decreased as compared to that of capsaicin-treated group (P<0.05) (Figure 2A, lower graph). Pre-treatment with SB-334867-A (80 nM) significantly increased traveled distance to find the hidden platform in blocks 2 and 3 as compared to that of capsaicin-treated rats (P<0.01 and <0.05, respectively) (Figure 2B, upper graph). Furthermore, an increase in total traveled distance was observed following capsaicin-plus SB-334867-A (80 nM) administration (P<0.001) (Figure 2B, lower graph).

3.1.3. Swimming speed

As shown in Figure 3A, administration of orexin A (20 and 40 pM) did not significantly change swimming speed within acquisition trial blocks [F(3, 345)=0.838; P=0.474] (Figure 3A, upper graph) and the mean swimming speed [F(4, 115)=1.998; P=0.099] in capsaicin-treated rats (Figure 3A, lower graph). Likewise, pretreatment with SB-334867-A (40 and 80 nM) had no significant effect on the mean of swimming speed [F(4, 115)=1.558; P=0.19] (Figure 3B, lower graph), and

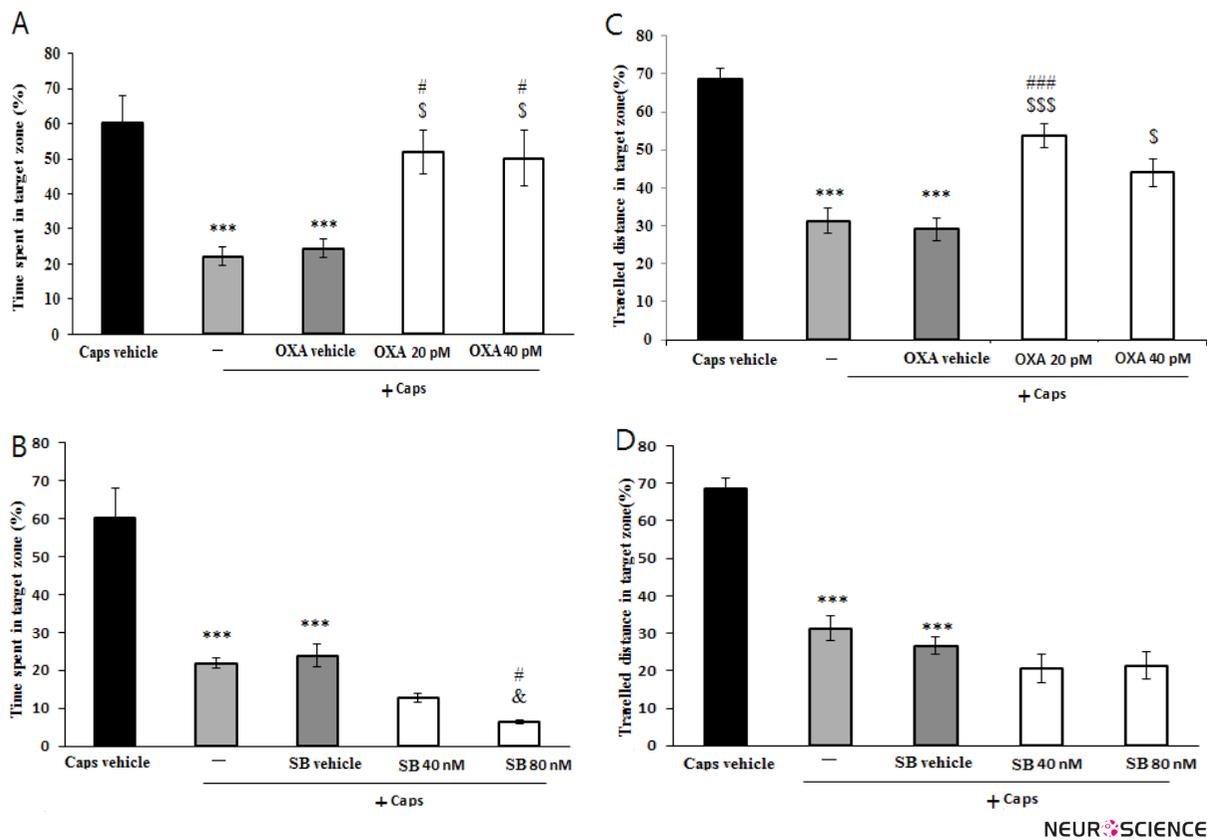


Figure 4. Retention performance was assessed in probe trial. Effects of pre-training intra-CA1 administration of orexin A and SB-334867-A on mean of time spent (A and B) and traveled distance (C and D) in capsaicin-treated rats (Caps) in the target region. Values correspond to mean±SEM (n=6), ***P<0.001 versus Caps vehicle group; ###P<0.001, #P<0.05 versus Caps group, \$\$\$P<0.001, \$\$P<0.0105 versus Caps+OXA vehicle; &P<0.05 versus Caps+SB vehicle.

swimming speed during each block of acquisition test [$F(3, 345)=0.674$; $P=0.579$] (Figure 3B, upper graph).

3.2. Probe Test

The results of probe trial are presented in Figure 4. The data showed that time spent ($P<0.001$) and the path length traveled ($P<0.001$) in the target region significantly reduced in rats treated with capsaicin as compared with that of vehicle group (Figure 4A and 4B). In addition, pretreatment with orexin A (20 and 40 pM) attenuated the effects of capsaicin on time spent in the target region ($P<0.05$) (Figure 4A). Likewise, orexin A (40 pM) significantly increased distance traveled in the target zone ($P<0.01$) (Figure 4B). As shown in Figure 4C, rats pretreated with SB-334867-A (80 nM/rat) prior to capsaicin administration, spent significantly less time in the target quadrat than capsaicin treatment group ($P<0.05$). Moreover, traveled distance decreased slightly, but not significantly in SB-334867-A (40 and 80 nM)-treated rats (Figure 4D).

4. Discussion

According to the current study, the capsaicin-treated animals that received intra-CA1 microinjection of orexin A showed a better performance in learning and memory tasks. Conversely, in rats pretreated with a selective OX1R antagonist SB-334867-A, learning, and memory efficiency were worse than those of the capsaicin treated rats. Pain-related cognitive dysfunction is examined in numerous rodent models of pain (Apkarian et al., 2004; Biessels et al., 1996; Cain, Francis, Plone, Emerich, & Lindner, 1997; Leite-Almeida et al., 2009; Raof et al., 2016). Particularly, orofacial pain related learning, and memory dysfunction are recently noted. Rats' MWM performance deficiency was reported following the tooth pulpal and intra-lip injection of capsaicin (Kooshki et al., 2016; Raof et al., 2015b). Such phenomenon was related to hippocampal neuronal apoptosis and an increased Bax/Bcl2 ratio and elevated caspase 3 activity (Raof et al., 2015a). However, the precise mechanism(s) of pain-induced memory deficiency is not fully clarified.

In the current study, CA1 administration of orexin A could attenuate spatial learning and memory deficiency in capsaicin treated rats. Orexin A-containing neurons are broadly distributed in several brain regions involved in the relay and modulation of nociceptive inputs (Peyron et al., 1998; Van Den Pol, 1999). It is extensively reported that orexin A has a modulatory effect on pain (Bingham et al., 2001; Ho et al., 2011). Surprisingly, previous studies showed that orexin A can modulate nociceptive transmission in the trigeminal complex mainly in Trigeminal Nucleus Caudalis (TNC) (Holland et al., 2006; Kooshki et al., 2016). In the current study, it is possible that the enhancing effect of orexin A on learning and memory is performed by modulation of hippocampal trigeminal nociceptive inputs (Burstein & Giesler, 1989).

It is shown that orexin neurons are innervated by neuropeptides releasing terminals including glutamate, gamma-aminobutyric acid (GABA), acetylcholine, nor-adrenaline, and serotonergic (Rosin et al., 2003; Tors realba, Yanagisawa, & Saper, 2003; Yamanaka, Muraki, Tsujino, Goto, & Sakurai, 2003). Actually, OXRs activation might modify the secretion of other neurotransmitters co-localized with orexin terminals. Especially, orexin neurons coexist with glutamatergic neurons in various brain regions (Torrealba et al., 2003). It is shown that orexin modulates neural plasticity and potentiates N-methyl-D-aspartate (NMDA) receptor currents in the hippocampus through the release of norepinephrine, acetylcholine, and glutamate (Xia et al., 2009). Therefore, it could be suggested that orexin A plays a positive role in learning and memory function, partially mediated by increased glutamatergic currents. However, finding the detailed mechanisms of orexin on glutamate receptors signaling needs more and further investigations.

Chronic pain is commonly associated with overexpression of inflammatory and pro-apoptotic agents, which may increase the risk of neural inefficiency (Khairova, Machado-Vieira, Du, & Manji, 2009; Kozlovsky et al., 2007; Lucas, Rothwell, & Gibson, 2006). Particularly, inflammatory agents can suppress or disrupt the activity of orexinergic neurons (Gaykema and Goehler, 2009). It is shown that tumor necrosis factor has inhibitory effects on orexin neurons, predominately via degradation of orexin precursor mRNA (Zhan et al., 2011). Moreover, decrease in hippocampal expression of OX1Rs in orofacial pain situation is reported (Raouf et al., 2015b). In addition, orexin A anti-apoptotic and neuroprotective properties are reported in various cellular and molecular studies (Butterick, Nixon, Billington, 2012; Esmaeili-Mahani, Vazifekhhah, Pasban-Aliabadi, Abbasnejad, & Sheibani, 2013; Kitamura et al., 2010). Therefore, it is

plausible to assume that the possible neuroprotective features of orexin A may play an effective role to prevent learning and memory impairment in the current study. In conclusion, the results of the current study demonstrated that CA1-hippocampal OX1R may play a crucial role in pain-induced memory dysfunction.

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Conflict of Interest

The authors declared no conflicts of interest.

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