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Experiencing neonatal maternal separation increased pain sensitivity in adult male mice: Involvement of oxytocinergic system

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ABSTRACT

Early-life stress adversely affects the development of the brain, and alters a variety of behaviors such as pain in later life. In present study, we investigated how early-life stress (maternal separation or MS) can affect the nociceptive response later in life. We particularly focused on the role of oxytocin (OT) in regulating nociception in previously exposed (MS during early postnatal development) mice that were subjected to acute stress (restraint stress or RS). Further, we evaluated whether such modulation of pain sensation in MS mice are regulated by shared mechanisms of the OTergic and opioidergic systems. To do this, we assessed the underlying systems mediating the nociceptive response by administrating different antagonists (for both opioid and OTergic systems) under the different experimental conditions (control vs MS, and control plus RS vs MS plus RS). Our results showed that MS increased pain sensitivity in both tail-flick and hot-plate tests while after administration of OT (1 μg/μl/mouse, i.c.v) pain threshold was increased. Atosiban, an OT antagonist (10 μg/μl/mouse, i.c.v) abolished the effects of OT. While acute RS increased the pain threshold in control (and not MS) mice, treating MS mice with OT normalized the pain response to RS. This latter effect was reversed by atosiban and/or naltrexone, an opioid antagonist (0.5 μg/μl/mouse, i.c.v) suggesting that OT enhances the effect of endogenous opioids. OTergic system is involved in mediating the nociception under acute stress in mice subjected to early-life stress and OTergic and opioidergic systems interact to modulate pain sensitivity in MS mice.

1. Introduction

Social environment plays a critical role in shaping the brain and behavior during lifespan (Lupien et al., 2009). Emerging lines of evidence indicates that experiencing early-life stressful events negatively affects the development of neural transmission system such as circuits, which are involved in response to painful stimuli (Jennings et al., 2014; Uhelski and Fuchs, 2010; Amini-Khoei et al., 2015). Neonatal maternal separation (MS), as an animal model of early-life stress, has been shown to induce significant changes in animal behaviors such as abnormal response to stress as well as painful stimuli (Kwok et al., 2014; Bernardi et al., 1986). In this regard, it has been well-evident that early-life stressors (including MS) alter the development of opioid system, which ideally regulates behaviors such as nociception. Decrease in pain threshold and the activity of the opioid system have been reported to play a role in the effects of early-life stress on nociception (Amini-Khoei et al., 2015; Ploj et al., 2003; Taylor et al., 2015).

On the other hand, endogenous hormone/neurotransmitter oxytocin (OT) is potently involved in the regulation of several physiological functions (Goodin et al., 2015). Environmental factors affect the

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development of OT system through altering oxytocin levels and responsiveness. Clinical and preclinical evidence suggests that early-life stressors, mainly those associated with poor maternal care, have long-lasting effects on development of OT system (Buisman-Pijlman et al., 2014). In case of animal studies, MS stress is able to induce significant alterations in the OTergic system activity by reducing the level of OT and number of oxytocin-positive neurons in central nervous system (CNS) (Veenema, 2012; Oreland et al., 2010; Lukas et al., 2010). OTergic and opioidergic systems have several biological interactions, which regulate a variety of behaviors such as addictive behaviors, reward, attachment and nociception (Carter, 2014; Russo et al., 2012; Georgiou et al., 2016). In this regard, previous studies demonstrated that OT not only induces a reduction in nociception, but enhances the antinociceptive effects of morphine (Arletti et al., 1993). Further, OT null mice showed reduced stress-induced antinociception suggesting that OT plays a role in mediating the pain responses in response to stress (Robinson et al., 2002).

Under acute stressful conditions, such as restraint stress (RS) in rodents, activation of opioid and non-opioid pathways in the CNS is able to suppress the pain (Robinson et al., 2002; Hohmann et al., 2005; Madden et al., 1977). In addition, OT is known to mediate the analgesic effects of acute restraint stress in mice, and endogenous opioids contribute to release of OT in the brain and periphery (Kwok et al., 2014; Han et al., 2014; Condés-Lara et al., 2009; Ge et al., 2002). We recently showed that MS stress changed mice nociceptive responses to acute RS, and changes in opioid response were associated with altered seizure susceptibility in animals (Amini-Khoei et al., 2015). Since both opioid and OTergic system contribute to regulation of nociceptive behaviors, and also both of them are negatively affected by early life stress, in this study, we aimed to investigate; 1) role of OTergic system in mediating the nociception under acute stress in mice subjected to early-life stress and, 2) whether OTergic and opioidergic systems have possible interactions in modulation of pain in MS mice.

2. Materials and methods

2.1. Animals and housing conditions:

Pregnant (gestation day 1) NMRI mice were purchased from the Pasteur Institute of Iran (Tehran, Iran), and housed individually under standard laboratory conditions (temperature: 22 ± 2 °C, humidity: 50 ± 10%, 12-h light–dark cycle, and free access to food and water), in the animal facility center of the Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences. Neonatal MS paradigm was carried out based on previous studies (Amini-Khoei et al., 2015; Desbonnet et al., 2010; Amiri et al., 2016). Briefly, the day of birth was considered as postnatal day (PND) 0 and pups were subsequently subjected to MS paradigm. Then, litters were briefly handled and separated from their mothers for 180 min daily during PND 2 to PND 14, beginning at 09:00 a.m. At the PND 21, male offspring's were housed in groups (4 mice per cage) until the PND 60. All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80-23). Institutional guidelines were strictly followed for animal care and use (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences).

2.2. Study design

This study was consisted of 2 different experiments. In experiment 1 we aimed to investigate the role of OTergic and opioidergic systems and their interaction in modulation of nociception in control and MS mice in adulthood. To do this, we firstly applied OT at different doses (0.5, 0.75, 1 and 1.25 μg/μl/mouse) to treated both control and MS mice to determine the effective and subthreshold doses of oxytocin in animals. On the next step, both MS and control mice were subjected to tail-flick and hot-plate tests. Each mouse only used for one test. The treatment as primary factor

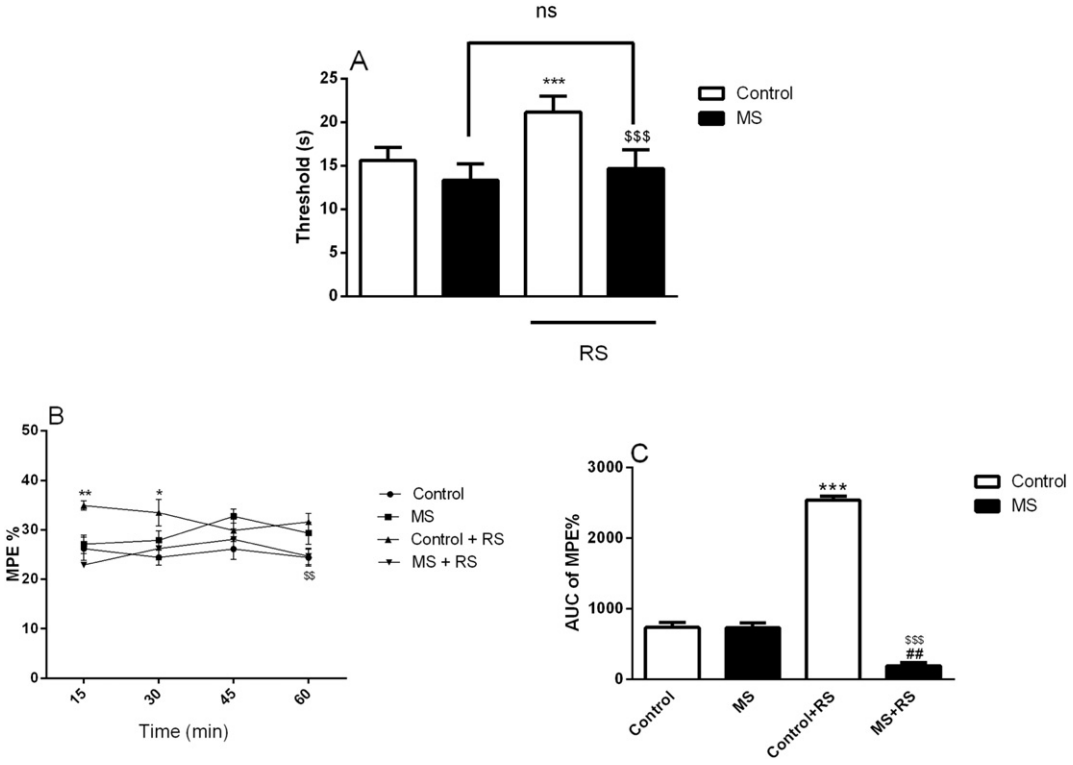

Fig. 1. Effects of MS and RS on nociceptive responses in the hot-plate and tail- flick tests. Data presented as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with control group, $^{\$}P < 0.05$ and $^{\$\$\$}P < 0.001$ compared to control + RS group, $^{\#\#}P < 0.01$ compared to MS group.

is assigned randomly to the each groups of control and MS mice as follows 1) saline, 2) OT (1 μg/μl/mouse, 15 min before test), 3) atosiban, a specific antagonist of OT receptor, (10 μg/μl/mouse, 10 min before test), and 4) atosiban + OT (atosiban 10 min prior to oxytocin injection and 25 prior test).

In the experiment 2, we used RS, as an acute stress-induced antinociception, to evaluate the involvement of OTergic and opioidergic systems in mediating the nociception under acute stress in mice subjected to early-life stress. To do this, both control and MS mice were randomly divided into following experimental groups; 1) RS (30 min prior test), 2) RS + OT (1 μg/μl/mouse, immediately before RS and 30 min prior test), 3) RS + naltrexone (an opioid antagonist, 0.5 μg/μl/mouse, 15 min before RS and 45 min prior test), 4) RS + atosiban (10 μg/μl/mouse, 10 min before RS and 40 min prior trial), 5) RS + atosiban and OT (atosiban 10 min prior to oxytocin injection and 40 min before examination), and 6) RS + naltrexone and OT (naltrexone 15 min before oxytocin and 45 prior test) (Amini-Khoei et al., 2015; Robinson et al., 2002). We used 30 min RS instead pharmacological manipulations such as injection of morphine in order to evaluate the opioidergic system. In this regards, it has been shown that applying 30 min RS to rodents enhanced the level of endogenous opioids (Amini-Khoei et al., 2015; Parikh et al., 2011). Considering, RS was used to enhance the level of opioids and naltrexone was used to antagonize this effect.

All behavioral tests were carried out in adulthood (PND 60-62) in both MS and control mice. Aforementioned drugs were injected intracerebroventricularly (i.c.v) based on the method previously described by Haley and McCormick (Haley and McCormick, 1957). Doses and time of drug administrations were chosen according to previous studies as well as our pilot studies (Han et al., 2014; Qi et al., 2012). The sample size was calculated by power calculations using G power software (ver.3.1.7, Franz Faul, Universitat Kiel, Germany). We set α error at 0.05 and power (1-β) at 0.8 and the required total sample size per group was calculated as 6–8 in behavioral tests. We also calculated the power value in each experimental group and analyses have shown that the power values were larger than 0.8 in all ANOVA analyses.

2.3. Evaluation of nociception

In order to investigate the involvement of OTergic and opioidergic systems in the regulation of the effects of different forms of stress on nociceptive response, hot-plate and tail-flick tests were used. Hot plate and tail flick tests are validate models for assessment of pain. The difference between these two tests is that hot plate test show the supraspinally response while tail-flick test assess the nociceptive response predominantly in the spinal level (Bannon and Malmberg, 2007; Langerman et al., 1995). For the hot-plate test, mice were placed on a 52 ± 0.2 °C heated plate (Socrel Hot-plate Model DS37; Ugo Basile, Comerio VA, Italy) and the time last to lick the forepaw, hind paw or jump were measured (Aloe et al., 2000). We considered cut- off time of 60 s to avoid any tissue injury.

Tail-flick test was done according to the criteria described by D'amour and Smith (1941). Radiant heat was applied to the tail (5–8 cm from the tip) using a tail-flick apparatus (type 812, Hugo Sachs Electronics, Germany). Tail-flick latency was measured as the time interval between the application of a standardized beam focused on the tail and the abrupt removal of the tail from the nociceptive stimulus. Multiple measures collected from the same animals at different time points (mice re-tested every 15 min). The cut-off time of 10 s was employed to prevent damage to the tail. Antinociception was measured using the percent maximal possible effect (%MPE) according criteria

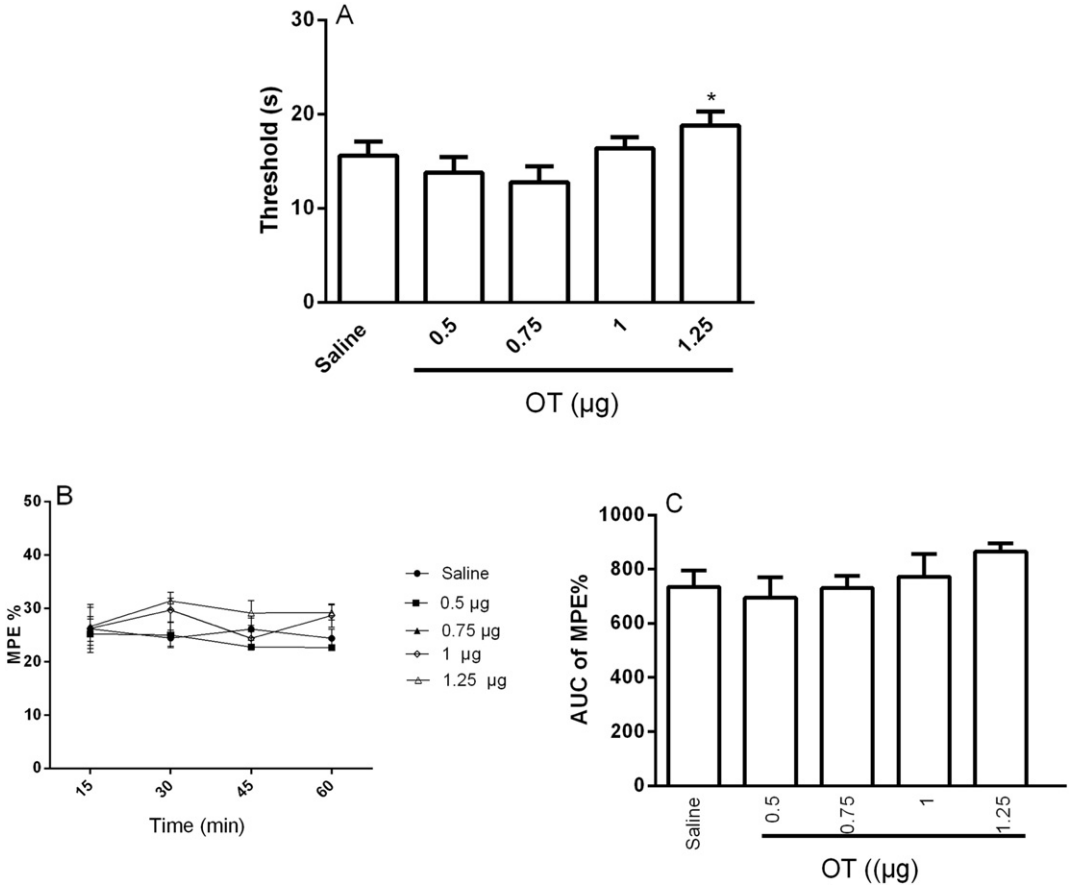

Fig. 2. Effects of different doses of oxytocin (0.5, 0.75, 1 and 1.25 μg/μl/mouse) on the nociceptive behavior of control mice in the hot-plate and tail- flick tests. Data expressed as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). *$P < 0.05$ compared to saline treated group. OT: oxytocin.

described by Harris and Pierson (1964) as follows: %MPE = [(test − baseline) / (10 − baseline)] × 100. The area under the time response curve (AUC) for each mouse was calculated by the trapezoidal method. It should be considered that we used different groups of animals for study of nociceptive effects of drugs in the tail-flick and hot plate tests and each mouse only used for one test.

2.4. Restraint stress procedure

In order to assess the function of the opioidergic system, acute stress (30 min RS) was applied to both experimental groups. It has been known that applying 30-min RS to rodents induced a strong analgesic effect through enhancement of the endogenous opioid system (Parikh et al., 2011; Drolet et al., 2001; Gamaro et al., 1998). Restraint stress paradigm was performed according to the method described by Bonneau et al. (1993). 50 ml falcon tubes with suitable ventilation holes (0.4 cm in diameter) were used. Adult mice were restrained for 30 min in the tube without giving any food and water. The control mice were also deprived from food at the same time, however they remained in cages for that specific time.

2.5. Statistical analysis

Comparison between groups was analyzed using one-way ANOVA and two-way ANOVA followed by Tukey's post hoc test. Tail-flick data were analyzed using two-way ANOVA with repeated measures. In two-way ANOVA analyses, row factor (RF) was considered as time interval and column factor (CF) was defined as treatments. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of MS and RS on pain threshold in the hot-plate and tail-flick tests

In the hot-plate test, two-way ANOVA revealed that there are significant differences among groups (F_{int} (9, 22) = 7.84, $P < 0.05$; F_{RF} (3, 22) = 11.28, $P < 0.001$; F_{CF} (3, 22) = 12.36, $P < 0.001$, Fig. 1A). Tukey's post-test analysis revealed that applying RS (30 min before the test) to control mice significantly enhanced hot-plate latency compared with non-stressed controls ($P < 0.001$). However, applying RS to MS mice, did not change the hot-plate latency when compared to non-stressed MS animals ($P > 0.05$). Additionally, control + RS mice showed an increase in the hot-plate threshold when compared with MS + RS mice ($P < 0.001$).

Two-way ANOVA analysis for MPE% of tail-flick test showed that there are significant differences in pain threshold among experimental groups (F_{int} (9, 80) = 1.831, $P < 0.05$; F_{RF} (3, 80) = 1.619, $P < 0.05$; F_{CF} (3, 80) = 12.81, $P < 0.001$, Fig. 1B). Repeated measure evaluation reveals that RS (30 min before test) significantly enhanced MPE% of the tail-flick latency in control mice in comparison with saline-treated mice ($P < 0.05$). Comparison between underwent RS groups demonstrated that pain threshold in control + RS mice is more than MS + RS mice ($P < 0.01$).

One-way analysis for the AUC of MPE% showed that there are significant differences among different sets of animals (F_{int} (9, 22) = 9.28, $P < 0.05$; F_{RF} (3, 22) = 18.15, $P < 0.001$; F_{CF} (3, 22) = 10.42, $P < 0.001$, Fig. 1C). Tukey's analysis demonstrated that applying RS in control mice significantly increased pain threshold when compared with

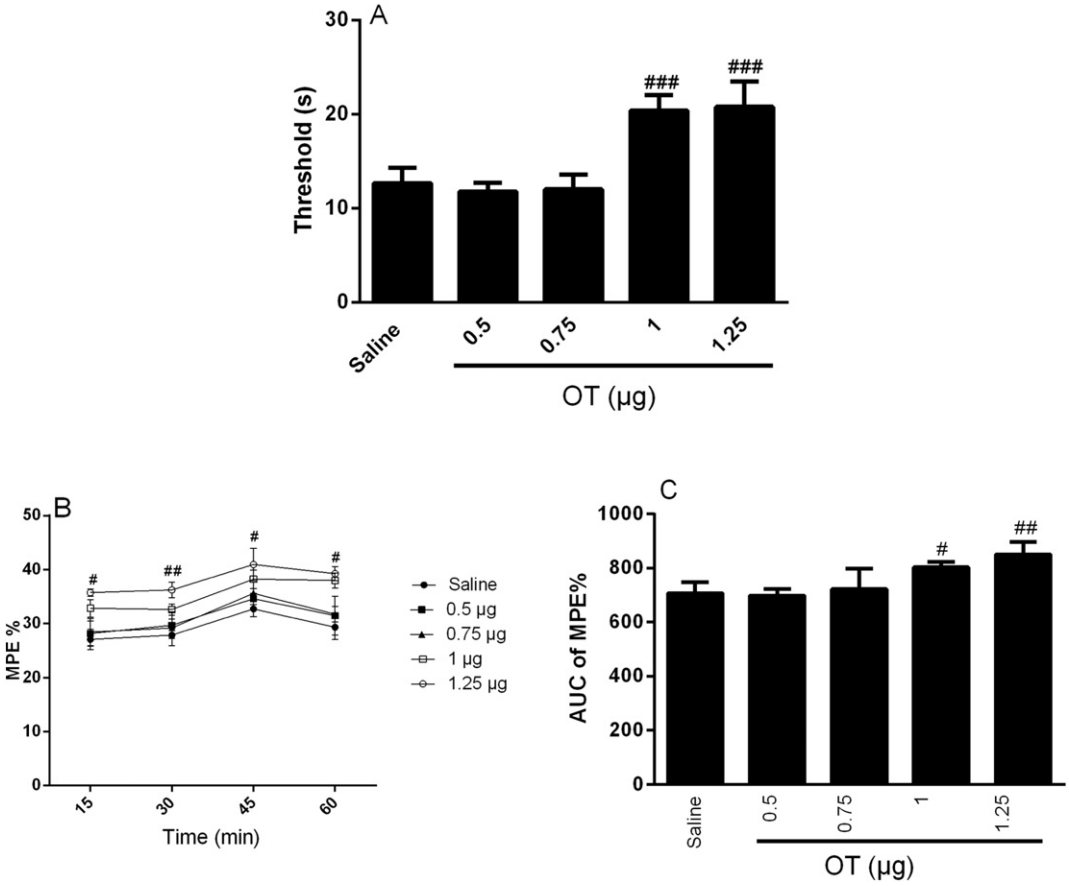

Fig. 3. Effects of different doses of oxytocin (0.5, 0.75, 1 and 1.25 µg/µl/mouse) on the nociceptive behavior of MS mice in the hot-plate and tail- flick tests Data expressed as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to saline-treated group. OT: oxytocin.

non-stressed control mice ($P < 0.001$). Conversely, RS significantly decreased AUC of MS mice when compared with non-stressed MS mice ($P < 0.01$). Moreover, RS significantly decreased the AUC of MPE% of MS mice in comparison with stress control mice ($P < 0.001$).

3.2. Various doses of OT were injected in order to find optimum dose on pain threshold

In the hot-plate test, one-way ANOVA analysis showed that treatment with different doses of oxytocin produced significant changes in the pain response in control ($F(4, 32) = 11.11$, $P < 0.01$, Fig. 2A) and MS mice ($F(4, 30) = 29.71$, $P < 0.001$, Fig. 3A) when compared to their saline-treated counterparts. Tukey's analysis showed that only administration of OT (1.25 but not 0.5, 0.75 and 1 μg/μl/mouse) to control mice significantly increased pain threshold in comparison with saline-treated control ($P < 0.05$). In MS mice, administration of OT (1 and 1.25 but not 0.5 and 0.75 μg/μl/mouse) increased pain threshold in comparison with saline-treated group ($P < 0.001$).

Two-way ANOVA analysis for MPE% of tail-flick test demonstrated that there are no significant differences following administration of various doses of OT among control mice ($F_{int}(12, 76) = 0.5103$, $P > 0.5$; $F_{RF}(3, 76) = 0.6829$, $P > 0.05$; and $F_{CF}(4, 76) = 3.259$, $P < 0.05$, Fig. 2B). In contrast, same treatments in MS mice produced significant changes in MPE% ($F_{int}(12, 80) = 2.199$, $P < 0.05$; $F_{RF}(3, 80) = 10.18$, $P < 0.001$; $F_{CF}(4, 80) = 14.58$, $P < 0.001$, Fig. 3B). Post-hoc analysis for control mice showed that different doses of OT failed to increase pain threshold in comparison with saline-treated group ($P > 0.05$). Results indicated that treatment with OT (1 and 1.25 but not 0.5 and 0.75 μg/μl/mouse) increased MPE% of MS mice when compared to saline-treated counterpart ($P < 0.05$).

One-way ANOVA analysis for AUC of MPE% showed that there are no significant differences among control mice treated with OT ($F(4, 25) = 4.322$, $P > 0.05$, Fig. 2C). Our finding showed that there are significant differences between MS mice following OT treatment in different doses ($F(4, 26) = 8.466$, $P < 0.001$, Fig. 3C). Post-hoc test showed that OT (all doses) did not increase pain threshold in comparison with saline-treated group in control animals ($P > 0.05$). In contrast, OT (1 and 1.25 but not 0.5 and 0.75 μg/μl/mouse) significantly decreased pain sensitivity in MS mice when compared to saline-treated group ($P < 0.05$).

3.3. Oxytocin possessed analgesic effect in MS mice in the hot-plate and tail-flick tests

One-way ANOVA analysis for hot-plate test showed that either oxytocin or atosiban failed to produce significant alteration in latencies of control mice ($F(3, 27) = 2.250$, $P > 0.05$, Fig. 4A). In contrast, same treatments significantly changed hot-plate latencies in MS mice ($F(3, 26) = 23.21$, $P < 0.001$, Fig. 5A). Tukey's analysis showed that there are no significant differences among treatment groups in comparison to saline-treated mice ($P > 0.05$). Our results showed that OT (1 μg/μl/mouse) significantly increases pain threshold of MS mice when compared to saline-treated counterparts ($P < 0.001$). Moreover, pretreatment with atosiban significantly reversed the effect of OT in MS mice ($P < 0.001$).

Two-way ANOVA analysis for the MPE% of tail-flick test revealed that aforementioned treatments produced significant alteration in MPE% of MS mice ($F_{int}(9, 84) = 2.759$, $P < 0.05$; $F_{RF}(3, 80) = 1.212$, $P > 0.05$; $F_{CF}(3, 84) = 16.48$, $P < 0.001$, Fig. 5B), but not in control mice ($F_{int}(9, 80) = 0.4452$, $P > 0.05$; $F_{RF}(3, 80) = 0.2122$, $P > 0.05$; $F_{CF}(3, 80) = 1.282$, $P > 0.05$, Fig. 4B). Repeated measure analysis showed that unlike

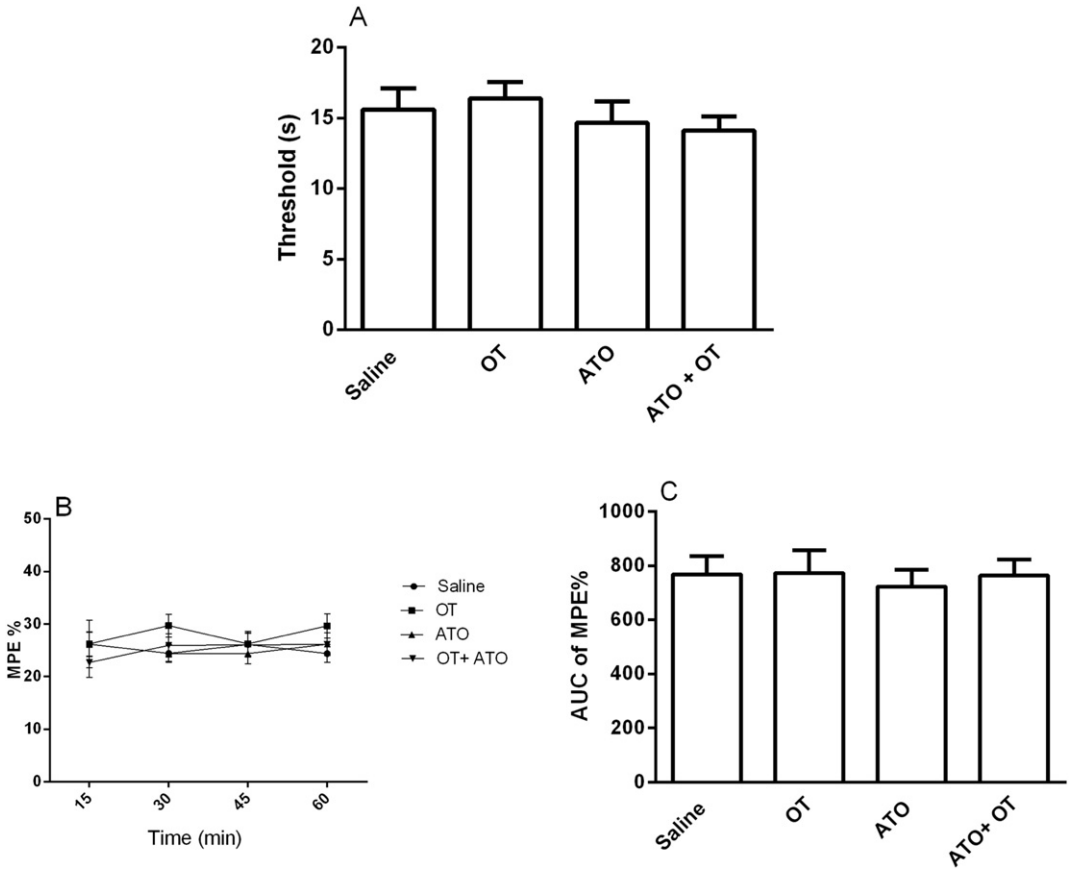

Fig. 4. Effect of oxytocin (1 μg/μl/mouse) on the pain threshold of control mice in the hot-plate and tail- flick tests. Data presented as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). OT: oxytocin, ATO: atosiban.

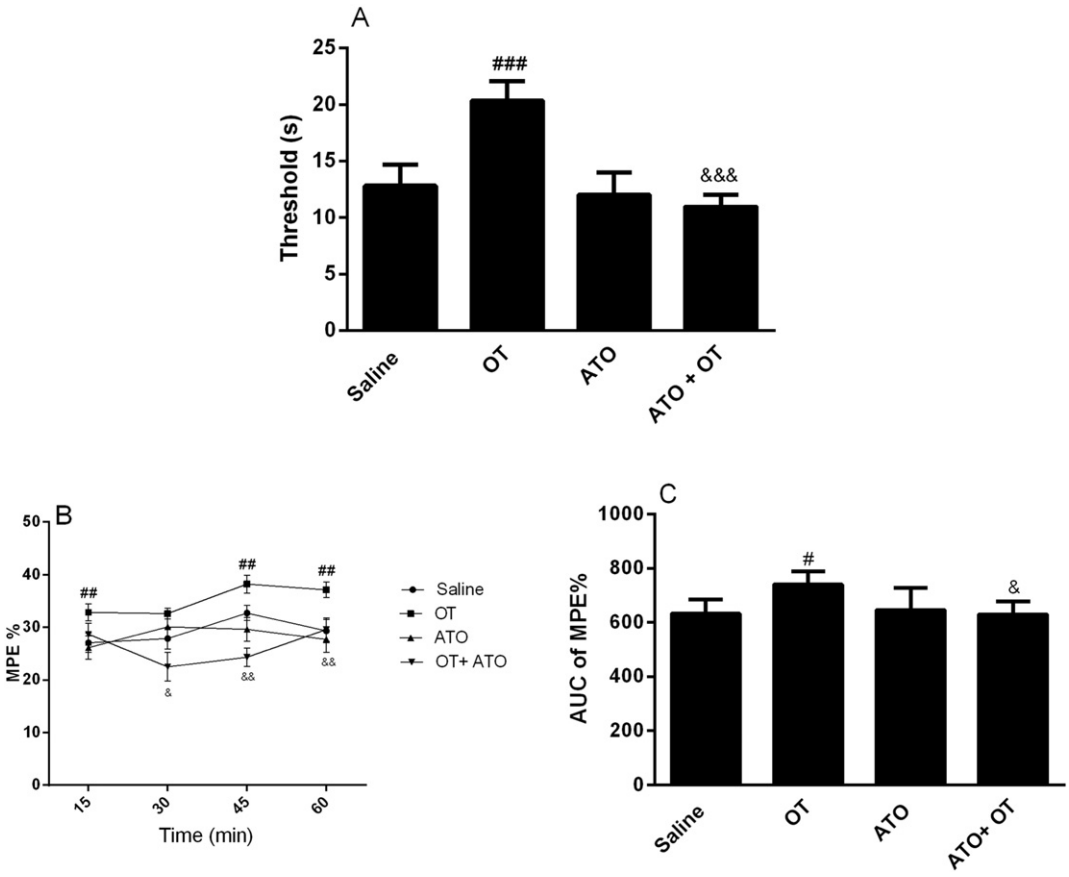

Fig. 5. Effect of oxytocin (1 μg/μl/mouse) on nociceptive behavior of MS mice in the hot-plate and tail- flick tests. Data presented as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to saline treated group. $^{\&}P < 0.05$ and $^{\&\&\&}P < 0.001$ compared to OT group. OT: oxytocin, ATO: atosiban.

the control mice, administration of OT (1 μg/μl/mouse) significantly increased MPE% of the tail-flick in MS mice in comparison with saline-treated counterparts ($P < 0.01$). Pretreatment with atosiban effectively reversed the analgesic effect of OT in MS mice ($P < 0.01$).

One-way ANOVA analysis for the AUC of MPE% in the tail-flick test showed that unlike the control mice ($F(3, 27) = 0.4456$, $P > 0.05$, Fig. 4C), treatment with OT, atosiban and OT + atosiban significantly changed the AUC value of MS mice ($F(3, 26) = 3.064$, $P < 0.05$, Fig. 5C). Tukey's pst-test analysis revealed that administration of OT significantly increased the AUC of MPE% of MS mice when compared with MS saline-treated group ($P < 0.05$). Moreover, pretreatment with atosiban significantly decreased the analgesic effect of OT in AUC of MPE% of MS mice ($P < 0.05$).

3.4. Analgesic effect of OT is partially modulated via opioid system

One-way ANOVA for hot-plate test revealed that OTergic and opioidergic manipulations significantly altered pain threshold in control ($F(5, 36) = 31.61$, $P < 0.001$, Fig. 6A) as well as MS ($F(5, 38) = 58.19$, $P < 0.001$, Fig. 7A) mice. Tukey's post-hoc analysis demonstrated that OT significantly increased hot-plate threshold in control + RS ($P < 0.05$) and MS + RS ($P < 0.001$) mice when compared to their control counterparts. Further, unlike MS, injection of atosiban significantly reduced the pain threshold in control + RS mice in comparison with saline-treated group ($P < 0.05$). Furthermore, co-administration of atosiban and naltrexone with OT as well as naltrexone alone in both stressed MS and control mice significantly decreased the pain threshold in comparison with saline-treated counterparts ($P < 0.01$). In addition, pretreatment with atosiban and naltrexone significantly reversed the analgesic effect of OT in both stressed MS and control mice ($P < 0.001$ for all).

Two-way ANOVA analysis for the MPE% of tail-flick revealed that there are significant differences among experimental groups following OTergic and opioidergic interventions in control ($F_{int}(15, 119) = 1.542$, $P < 0.05$; $F_{RF}(3, 119) = 0.3140$, $P > 0.05$; $F_{CF}(5, 119) = 4.820$, $P < 0.001$, Fig. 6B) and MS mice ($F_{int}(15, 124) = 1.930$, $P < 0.05$; $F_{RF}(3, 124) = 1.351$; $P < 0.05$, $F_{CF}(5, 124) = 5.815$, $P < 0.001$, Fig. 7B). Post-hoc analysis showed that MPE% following OT treatment significantly increased in comparison with RS + control group ($P < 0.01$), which significantly reversed with co-administration of naltrexone and/or atosiban ($P < 0.01$, for both).

One-way ANOVA analysis for the AUC of MPE% in the tail-flick test showed that OTergic as well as opioidergic interventions produce significant differences among animal sets in control ($F(5, 36) = 17.22$, $P < 0.001$, Fig. 6C) and MS ($F(5, 38) = 27.13$, $P < 0.001$, Fig. 7C). Post-hoc test analysis showed that the administration of OT in both control ($P < 0.05$) and MS ($P < 0.01$) mice increased AUC value of tail-flick in comparison with RS counterparts. Tukey's analysis revealed that co-administration of atosiban as well as naltrexone significantly reversed the effect of OT in AUC of stressed mice in both control and MS animals ($P < 0.01$ and $P < 0.001$, respectively).

4. Discussion

The results of the current study showed that MS stress is able to reduce the pain threshold in adult male mice. Further, results showed that icv administration of OT increased the pain threshold in MS mice and this effect was blocked by administration of atosiban suggesting the involvement of OTergic system in abnormal nociception in MS mice. Further, our results demonstrated that acute RS increased the pain threshold in control (and not MS) mice suggesting that early

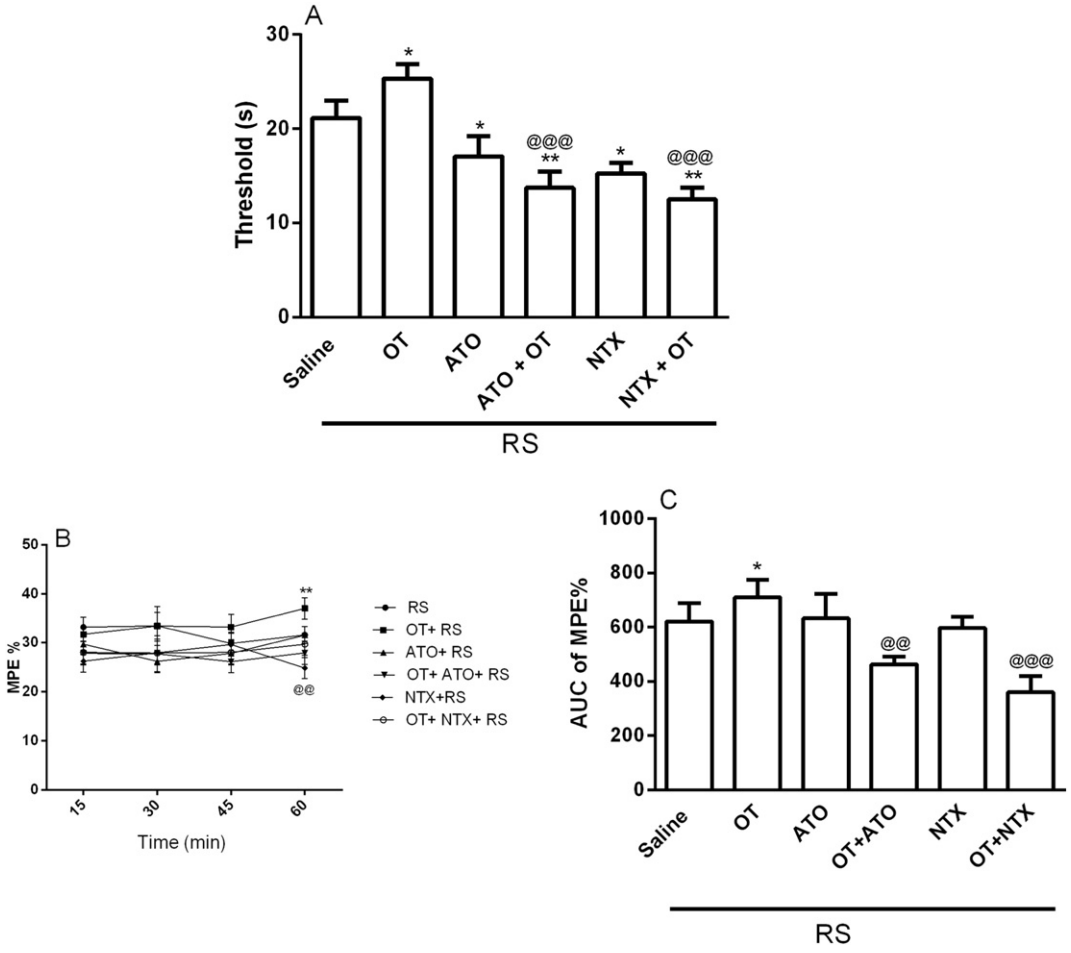

Fig. 6. Interaction of oxytocinergic and opioidergic on the nociceptive behavior of control mice in the hot-plate and tail flick tests. Data presented as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). $^{*}P < 0.05$, and $^{**}P < 0.01$ compared to saline treated group, $^{@@}P < 0.01$ and $^{@@@}P < 0.001$ compared to oxytocin treated group. OT: oxytocin, ATO: atosiban, NTX: naltrexone, RS: restraint stress.

life stress is able to alter nociceptive behaviors under acute stress conditions. Finally, administration of OT to MS mice normalized the pain responses to the RS. These effects were blocked by administration of OT and opioid antagonists suggesting the involvement of OTergic and opioidergic system in the regulation of pain responses to acute stress following MS.

It is now well-known that experiencing early-life stress has a profound and long-lasting effect on the development of the brain and behavior in later life (Lupien et al., 2009). Our results revealed that MS significantly altered the pain responses in animals. Applying 30 min of RS to rodents has been reported to have analgesic effects by increasing the levels of endogenous opioids (Amini-Khoei et al., 2015; Parikh et al., 2011). In our study, MS mice did not exhibit increased pain threshold following RS. This result is in agreement with previous studies that MS disrupts the pain response under acute stress conditions (Amini-Khoei et al., 2015; Kwok et al., 2014). In addition, early-life stress has been shown to disrupt the development of opioid system and alter the analgesic response to morphine treatment by reducing the number opioid receptors (Kwok et al., 2014; Bernardi et al., 1986; Weaver et al., 2007; Kalinichev et al., 2001). On the other hand, RS stress in known to produce strong antinociception effects by increasing the opioid tone. Thus, MS mice did not exhibit increased pain threshold following RS because it seems that MS and RS neutralize effects of each other. It is well-established that opioids have a pivotal role in CNS development (Fleming et al., 1999). Endogenous opioid system and opioid receptors are extensively expressed in various areas of the CNS (Erbs et al., 2014). Experiencing stressful conditions leads to induction of significant changes in the opioid system, which contributes to the regulation of several behaviors like nociception and addiction (Sinha, 2008; Martenson et al., 2009). In this regard, evidence indicates that experiencing MS induces long-lasting alterations in the endogenous opioid system such as reduction in the number of opioid receptors in the brain (Kwok et al., 2014; Bernardi et al., 1986; Preter and Klein, 2014; Troisi et al., 2012). Previous investigations reported that MS stress decreased the pain threshold in rodents, and this effect was due to hypo-function of the opioid system in the CNS, and MS mice were more susceptible to the pain stimuli in comparison with control mice (Bernardi et al., 1986; Kehoe and Blass, 1986). In line with these findings, we recently reported that abnormal nociceptive behaviors in MS mice are associated with impairment in opioid system (Amini-Khoei et al., 2015).

Interestingly, unlike control mice, the administration of subthreshold dose of OT (1 μg/μl/mouse) increased the pain threshold in MS mice, while pretreatment with atosiban significantly decreased the analgesic effect of OT. These results suggest that MS-induced decrease in pain threshold may be associated with reduced activity of OTergic system. In this regard, previous research has shown that MS is able to decrease the levels of OT and oxytocin-positive neurons in different regions of the brain (Veenema, 2012; Oreland et al., 2010; Lukas et al., 2010). Also, development and functioning of the OT system is sensitive towards peripheral stimuli such as social environment and stress (Buisman-Pijlman et al., 2014). Development of endogenous OT system

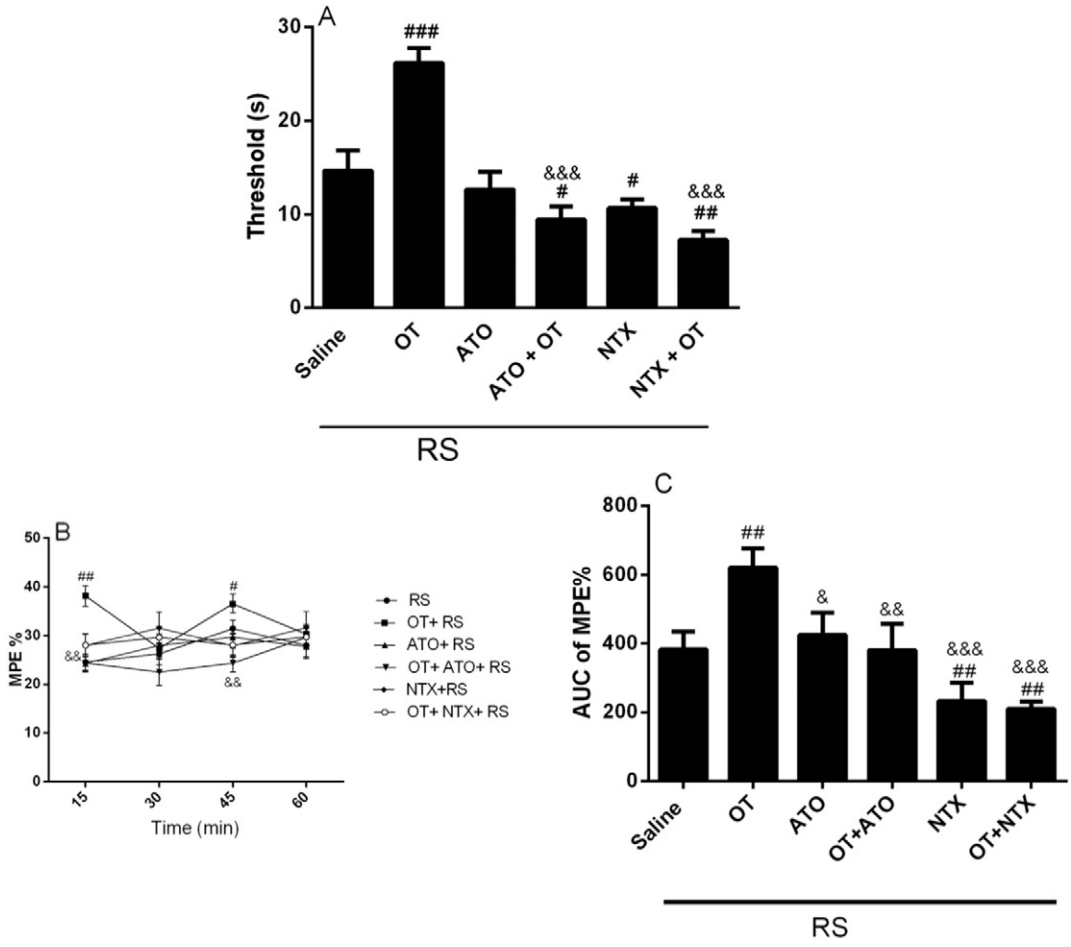

Fig. 7. Interaction of oxytocinergic and opioidergic on the nociceptive behavior of MS mice in the hot-plate and tail flick tests. Data presented as mean ± S.E.M. ($n = 6–8$). One-way ANOVA followed by Tukey's post-hoc test was used for hot-plate threshold (A) and AUC for MPE% (B) analysis. Two-way ANOVA followed by repeated measure was used for analysis of MPE% of tail-flick test (C). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to saline treated group, $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ and $^{\&\&\&}P < 0.001$ compared to oxytocin treated group. OT: oxytocin, ATO: atosiban, NTX: naltrexone, RS: restraint stress.

starts in utero and continues through lifespan (Nylander and Roman, 2012). A recent study by Lukas et al. showed that the density of OT receptors changes during the lifespan and is profoundly influenced by MS (Lukas et al., 2010). Also, it has been demonstrated that maternal care (grooming) positively affects the expression of OT receptors in the brain (Champagne, 2008). Thus, it is possible that abnormal nociceptive behavior in MS mice is related to the abnormal OTergic system activity. Also, our results indicate that OT plays an important role in mediating the nociceptive behaviors under acute stress conditions. Preclinical and clinical evidence demonstrated that OT has analgesic properties (Condés-Lara et al., 2009; Li et al., 2015; Juif and Poisbeau, 2013; Rash et al., 2014). In this regard, previous studies showed that direct administration of OT into the brain, spinal cord or systematically possessed analgesic effects (Gao and Yu, 2004; Miranda-Cardenas et al., 2006; Reeta et al., 2006). Moreover, it has been shown that atosiban significantly attenuated the analgesic effects of oxytocin (Li et al., 2015). In this work, we also observed that treating animals with naltrexone abolished the effects of OT suggesting the interaction between opioid and OTergic system in mediating the nociception under acute stress conditions. It has been reported that OTergic system activity is closely related to the opioidergic system. Also, it has been shown that opioids are linked with central oxytocin-related analgesic effects (Fleming et al., 1999; Reeta et al., 2006). Animal studies revealed that icv administration of OT has strong analgesic effect, which significantly reversed by icv injection of opioid antagonists (Ge et al., 2002; Reeta et al., 2006). Therefore, our findings suggest that abnormal interaction between OTergisc and opioidergic systems at least partly underlies the altered responses of MS male mice to analgesic effects of acute RS. We also investigated the effects of MS on nociceptive responses of female mice. We found that abnormal responses to painful stimuli in adult female mice were associated with hormonal level and status of the estrus cycle of animals (data not shown). This study has certain limitations. This study showed that OT (and to some extent opiodergic system) is involved in abnormal nociceptive alterations following MS, and in response to acute stress. However, we did not show which brain structures are involved and further molecular assessments should be done in order to measure the alterations of OT or opioid system activity after MS or RS in the animals.

5. Conclusion

In conclusion, the results of the current study showed that 1) MS induced abnormal nociceptive responses in the form of thermos-sensation in the adult male mice, 2) both opioidergic and OTergic systems are involved in altered nociception 3) OTergic and opioidergic systems mediate the analgesic effects of RS, and 4) MS disrupts the analgesic response of the RS at least partly through decreasing the OTergic system activity in the CNS.

Conflicts of interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

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