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Intra-LPGi microinjection of glutamate receptors antagonists abolish 17β-estradiol-induced analgesic effect in the ovariectomized rats

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This study was designed to investigate a possible interaction between 17β-estradiol and glutamate receptors of the paragigantocellularis lateralis (LPGi) nucleus on pain coping behavior using the formalin test in ovariectomized (OVX) rats. The results showed that intra-LPGi injection of 17β-estradiol declined flexing behavior in both phases of the formalin test. Still, it only diminished the late phase of licking behavior in the OVX rats. NMDA receptor antagonist, AP5, reversed the analgesic effect of 17β-estradiol on flexing behavior in both phases of the formalin test in the OVX rats. The 17β-estradiol-induced anti-nociceptive effect on the flexing duration was prevented by CNQX (AMPA receptor antagonist) only in the early phase of the formalin test in the OVX rats. AP5 and CNQX reduced the anti-nociceptive effect of 17β-estradiol in the late phase, but not the early phase of licking response in the OVX rats. These results suggested: (i) The intra-LPGi injection of 17β-estradiol is satisfactory in producing modest analgesia on the formalin-induced inflammatory pain in the OVX rats; (ii) Co-treatment of glutamate receptors (NMDA and AMPA) antagonists and 17β-estradiol in the LPGi nucleus decrease the analgesic effect of 17β-estradiol in the OVX rats; (iii) There is a possible association between 17β-estradiol and glutamate receptors of the LPGi nucleus on pain coping behavior in the OVX rats.

Key words: 17β-estradiol, NMDA and AMPA receptors, pain, analgesia

INTRODUCTION

Female rats indicate higher pain sensitivity than males in several nociceptive assessments of inflammation (Kuba et al., 2005). The greater sensitivity to pain perception is adjusted by gonadal steroid hormones, principally testosterone, and 17β-estradiol (Hernandez-Leon et al., 2018). Estradiol is a neuroactive steroid that regulates the brain activity, plasticity required for controlling reproduction, and has general functions in the central nervous system (CNS) (Azcoitia et al., 2017). The local synthesis of estradiol in the CNS modulates behavior and cognition in male and female animals (Bailey et al., 2013). It exerts neuroprotective effects (Azcoitia et al., 2001; Saldanha et al., 2005; Zhang et al., 2014) and modulates pain sensitivity (Mensah-Nyagan et al., 2008; 2009; De Nicola et al., 2013). Neuroactive steroids such as 17β-estradiol are naturally found at nanomolar levels in the CNS. Though, the central concentrations of these neurosteroids can greatly increase following stress and pain. The expression of the ERα isoform of estrogen receptors in the LPGi neurons of both sexes is demonstrated by the immunohistochemical technique (Normandin and Murphy, 2008). Moreover, 17β-estradiol interacts with glutamate and GABA neurotransmitter receptors in many brain areas. Hence, in the LPGi nucleus,

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17β-estradiol modulates nociception by coupling to its receptors and allosteric interaction with other membrane-bound receptors such as glutamate and $GABA_A$ receptors (Potes et al., 2006; Khakpay et al., 2010).

Glutamate and its receptors (i.e., NMDA and AMPA receptors) in the brainstem have a crucial role in both inhibitory and facilitatory pain control pathways (Da Silva et al., 2010). The NMDA receptor activation is necessary for AMPA receptor action during synaptic plasticity (Guan et al., 2004). The AMPA receptor activation triggers the rapid excitatory synaptic transmission associated with the glutamatergic pain signaling in the ascending nociceptive pathways. These rapid excitatory synaptic transmissions are found in the primary sensory, spinal cord, and thalamic projections (Bleakman et al., 2006). An evidence illustrated the vital role of AMPA receptors in the descending pain pathways (Guan et al., 2004). The NMDA receptors are also distributed in the nociceptive pathways of the CNS (Bleakman et al., 2006). Formalin-produced inflammatory pain enhanced the GLUN2A subunit of NMDA receptor expression in the rostral ventromedial medulla (RVM), but it decreased the GLUN2C in the RVM (Gaunitz et al., 2002) that demonstrated a role of the RVM NMDA receptors in the facilitation of nociception (Da Silva et al., 2010).

In the rat brain, the RVM includes the raphe nucleus, reticularis gigantocellularis pars α, and paragigantocellularis lateralis (LPGi) (Fields et al., 1991; Mason, 1999; Yang et al., 2002; Willis and Coggeshall, 2004). The periaqueductal gray (PAG) participates in numerous gonadal steroid-sensitive behaviors such as responsiveness to pain. The PAG projects to the RVM, which includes the primary circuit driving pain inhibition. The expression of steroid receptors in the PAG and the descending pathway involved in pain inhibition may suggest a mechanism whereby the gonadal steroids regulate pain (Loyd and Murphy, 2008). Furthermore, the LPGi is a reticular nucleus in the medulla oblongata, which plays an important role in the descending pain-coping behavior *via* the spinal cord (Erami et al., 2012; Shamsizadeh et al., 2014; Soleimani et al., 2013; Azhdari-Zarmehri et al., 2014; 2015). The LPGi nucleus receives inputs from the vestibular nucleus, tractus solitarus, and lateral hypothalamus (Azhdari-Zarmehri et al., 2013). The projections of LPGi neurons are sent to the locus coeruleus (LC) (Andrezik et al., 1981). The LPGi nucleus and its glutamatergic projection to the LC nucleus are the main mechanisms in the descending pain modulatory system (Aston-Jones et al., 1991).

Moreover, our previous study revealed that intra-LPGi injection of 17β-estradiol elicited potent analgesia in the formalin-induced pain in male rats (Khakpay et al., 2020). This pain-relieving effect of 17β-estradiol is possibly mediated by both NMDA and AMPA receptors of the LPGi in male rats (Khakpay and Azaddar, 2017; Khakpay et al., 2020). Thus, the present study was planned to evaluate a possible NMDA and AMPA interaction between 17β-estradiol and glutamate receptors of the LPGi on pain coping behavior using the formalin-test in the OVX female rats.

EXPERIMENTAL PROCEDURE

Animals

Adult female Wistar rats (200-270g) were obtained from the Tabriz University of Medical Sciences (Pharmacy Faculty, Tabriz, Iran). The rats were maintained in a 12-h light/dark cycle at 22-24°C and were allowed free access to food and water. The experiments were done in the light phase. All researches and animal care procedures were accomplished according to the guidelines on the use of laboratory animals and approved by Tabriz University's ethical committee for animal research.

Surgery

Adult female rats with normal estrus cycles were ovariectomized (OVX) under ketamine (60 mg/kg) and xylazine (7.5 mg/kg) anesthesia. Ovariectomy was performed according to the technique described by Chakraborty and Gore (2004). Briefly, both flanks were shaved and cleaned with Betadine. A small incision (1 cm) was made through the skin and the muscles. The ovary was pulled through the incision. The oviduct was cleaved, and the ovary was removed. The skin and the muscles were sutured with a silk thread. This procedure was repeated on the contralateral side. In the sham/OVX group (without removal of ovary), all the above procedures were performed except the removal of the ovary (to assess the possible stress caused by surgery).

After termination of the ovariectomy, rats were immediately positioned in the stereotaxic instrument and cannulation of the LPGi nucleus was performed. A guide cannula (23 gauge) equipped with a 30 gauge stylet was embedded in the right LPGi [coordinates from bregma: AP: -12 mm, L: ±1.6 mm, DV: 10.4 mm (Paxinos and Watson, 2007)]. The guiding cannula was stocked to the skull with a stainless steel screw and acrylic cement (Dentimax, the Netherlands). All animals were left to recover for seven days before behavioral testing. The efficiency of ovariectomy was proven by examining

vaginal smears collected for four sequential days. Fundamentally, only rats were used for OVX experiments that showed the diestrus phase on all days.

Drugs and injections

Water-soluble cyclodextrin encapsulated 17β-estradiol [0.8 μmol; (Aloisi and Ceccarelli, 1999; Khakpay et al., 2014)], and NMDA receptor antagonist, AP5, [0.5 μmol; (Khakpay et al., 2010)], AMPA receptor antagonist, CNQX, (30 nmol; (Khakpay et al., 2010) were obtained from Sigma (Sigma Chemicals, St. Louis, MO, USA) and dissolved in normal saline. These doses were the final concentrations of the drugs used in experiments. Also, formalin was bought from Dr. Mojallaly's company [50 μl of 5%; (Ceccarelli et al., 2004; Khakpay et al., 2014)].

Intra-LPGi injections were done as described by Aloisi and Ceccarelli (1999). All treatments were unilaterally injected into the right LPGi nucleus through the guide cannula and an injection needle (30 gauge) attached to polyethylene tubing to a 0.5 μl Hamilton microsyringe (Hamilton, Switzerland). The tip of the injection needle was 0.2 mm outside the guide cannula and all drugs were applied in a volume of 500 nl. The needle was detached, and the stylet was replaced sixty seconds after infusing the substance.

Formalin test

The formalin test allows the evaluation of acute and persistent nociception. 50 μl of 5% formalin solution was injected into the intraplantar surface of the left hind paw by a 30 gauge needle (Khakpay et al., 2016). Next, the animals were instantaneously returned to the formalin test box, and the duration of flexing and licking behaviors were recorded for an hour (Wheeler-Aceto and Cowan, 1991, Aloisi et al., 1998; Khakpay et al., 2014). The data was blindly recorded by the researcher. Pain behaviors were monitored for 60 min; the duration of flexing and licking of the injected paw was measured at 5-min intervals starting at time 0. Two phases of flexing and licking behaviors were observed: phase 1 (early phase or acute phase) began immediately after formalin injection to 7 min (0–7 min) and phase 2 (late phase or persistent phase) began at time16 min to 60 min (16–60 min) (Mahmoudi and Zarrindast, 2002; Khakpay et al., 2010; Roca-Vinardell et al., 2018). By the end of the experiment, the rats were anesthetized by ketamine and xylazine and the brains were removed. Then, the brains were examined for the precise cannula placement in the LPGi nucleus. Only data acquired from animals with accurate cannula placement were considered in the analysis.

Experimental design

In all experiments, intra-LPGi microinjection of drugs took place 15 min before the formalin test. Then, formalin-induced flexing and licking behaviors were measured for 60 min. Each experimental group consisted of six female rats and each female rat was used only once. Doses of drugs were selected based on the pilot experiments and our previous works which are published in the scientific literature (Khakpay et al., 2016; Khakpay and Azaddar, 2017).

Experiment 1: This experiment was designed to assess the precise effect of surgical agents, cannulation of the LPGi nucleus, surgery with removal of ovary (OVX) or without removal of ovary (sham/OVX), and drug injection into the LPGi nucleus of the OVX rats. In this experiment, experimental groups were included as follows: control group (formalin test in intact animals), sham/CAN group (only cannulation of the LPGi nucleus), sham/OVX group (surgery without removal of ovary and cannulation of the LPGi nucleus), saline group (injection of saline into the LPGi nucleus of the OVX rats), and 17-beta-estradiol group (injection of 0.8 μmol 17-beta-estradiol (E2) into the LPGi nucleus of the OVX rats). In the intact groups, female rats were included in the diestrus phase of the estrus cycle.

Experiment 2: To find appropriate doses of glutamate receptor antagonists (NMDA and AMPA receptor antagonists) for pain behavior, the OVX rats were examined in the formalin test. Experimental groups were included as follows: control group (formalin test in the intact female rats), saline group (intra-LPGi microinjections of saline in the OVX rats), AP5 group (intra-LPGi infusion of 0.5 μmol AP5 in the OVX rats), and CNQX group (intra-LPGi injection of 30 nmol CNQX in the OVX rats). In this experiment, two control groups were used to analyze the data of the AP5 and CNQX groups.

Experiment 3: To detect possible interaction between 17β-estradiol and glutamate NMDA and AMPA receptors of the LPGi nucleus on pain coping behavior, the OVX rats were submitted to the formalin test. This experiment consisted of saline, E2 (0.8 μmol), E2/AP5 as well as E2/CNQX groups. In the E2/AP5 and E2/CNQX groups, 0.5 μmol AP5 and 30 nmol CNQX were microinjected in the LPGi nucleus, 15 min before the intra-LPGi microinjection of 17β-estradiol in the OVX rats (Khakpay et al., 2020). In these groups, AP5 or CNQX was injected 30 min before the formalin test. Two control groups were used for analyzing the data of the AP5 and CNQX groups.

At the end of the behavioral tests, rats were deeply anesthetized, and methylene blue solution (1%, 0.3 µl/rat) was injected into the guide cannula to confirm the correct cannula placement. The brain of each rat was removed and placed in formaldehyde (10%). After one week, the brain sections were histologically evaluated according to the atlas of Paxinos and Watson (2007). Only the data from the OVX rats with precise cannula implants into the right LPGi were included in the statistical analysis (Fig. 1).

Statistical analysis

The SPSS software was used for data analysis and data are presented as mean ± S.E.M. Sample size for all groups was performed which means that six female rats were selected randomly for each experimental group. The normality of data was checked by the Shapiro–Wilk normality tests. For the data with normal distribution, one-way and/or two-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test was done to compare treatments in terms of statistical significance. $P<0.05$ was considered statistically significant.

RESULTS

Effects of 17β-estradiol on flexing and licking responses induced by formalin

The effect of cannulation into the LPGi nucleus, ovariectomy, and intra-LPGi injection of saline and 17β-estradiol on the formalin-induced pain responses in the OVX rats was shown in the Fig. 2. One-way ANOVA and *post hoc* analysis revealed that animals belonging to the sham/CAN and sham/OVX groups had no significant effect on both the early ($F_{(3,20)}=2.761$, $P>0.05$) and late ($F_{(3,20)}=0.565$, $P>0.05$) phases of flexing response (Fig. 2A, B) as well as both the early ($F_{(3,20)}=1.304$, $P>0.05$) and late ($F_{(3,20)}=0.427$, $P>0.05$) phases of licking behavior (Fig. 2C, D) when compared with control and saline groups in the formalin test. So, these groups were not included in the results section.

Moreover in the OVX rats, the same analyses indicated that intra-LPGi injection of 17β-estradiol at a dose of 0.8 µmol decreased the flexing duration in both early ($F_{(2,15)}=4.005$, $P<0.05$) and late ($F_{(2,15)}=4.920$, $P<0.05$) phases of formalin test (Fig. 2E, F), while reduced the licking behavior only in the late phase ($F_{(2,15)}=5.899$, $P<0.05$) but not in the early phase ($F_{(2,15)}=0.307$, $P>0.05$) of formalin test (Fig. 2G, H) when compared with control and saline groups.

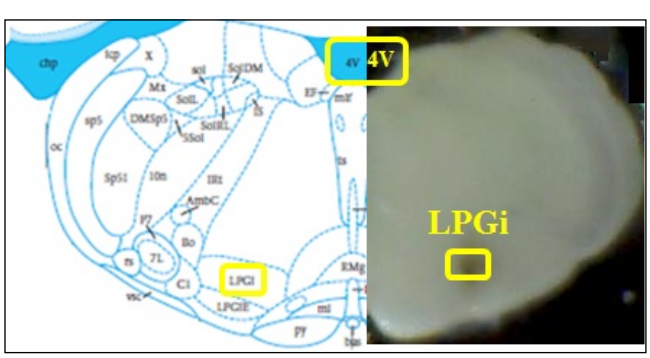

Fig. 1. The approximate site of the microinjection cannula tip in the LPGi nucleus for all rats included in the data analyses was taken from the atlas of Paxinos and Watson (Paxinos and Watson, 2007).

Effects of AP5 and CNQX on flexing and licking responses induced by formalin

In the previous study, the effect of several doses of AP5 (NMDA receptor antagonist) and CNQX (AMPA receptor antagonist) on the formalin-induced flexing and licking behaviors of male rats was examined (Khakpay et al., 2020). To avoid the pain modulatory effect of both AP5 and CNQX and its interaction with the analgesic effect of 17β-estradiol and upon previous study results, only the non-nociceptive dose of CNQX and AP5 was used in the current research (Khakpay et al., 2020). Two-way ANOVA analysis indicated that intra-LPGi infusion of AP5 (0.5 µmol) had no significant effect on the time-course of the flexing behavior [within-group comparison: AP5-effect $F_{(2,21)}=0.347$, $P>0.05$; time-course effect: $F_{(2,21)}=0.335$, $P>0.05$; AP5-time-course interaction: $F_{(2,21)}=0.912$, $P>0.05$; Fig. 3A] and the licking behavior [within-group comparison: AP5-effect: $F_{(2,21)}=0.974$, $P>0.05$; time-course effect: $F_{(2,21)}=0.738$, $P>0.05$; AP5-time-course interaction: $F_{(2,21)}=0.809$, $P>0.05$; Fig. 3C] in the formalin test. Moreover, one-way ANOVA showed that intra-LPGi microinjection of AP5 (0.5 µmol) had no significant effect on the flexing duration in both early ($F_{(2,15)}=0.264$, $P>0.05$)and late ($F_{(2,15)}=0.694$, $P>0.05$) phases of formalin test (Fig. 3B). Similarly, both early ($F_{(2,15)}=0.762$, $P>0.05$) and late ($F_{(2,15)}=0.727$, $P>0.05$) phases of the licking behavior were not affected by intra-LPGi administration of AP5 (Fig. 3D).

Correspondingly, two-way ANOVA showed that intra-LPGi injection of CNQX (30 nmol) had no significant effect on the time-course of the flexing behavior [within-group comparison: CNQX effect: $F_{(2,21)}=0.781$, $P>0.05$; time-course effect: $F_{(2,21)}=0.410$, $P>0.05$; CNQX-time-course interaction: $F_{(2,21)}=0.859$, $P>0.05$; Fig. 3E] and the licking behavior [within-group com-

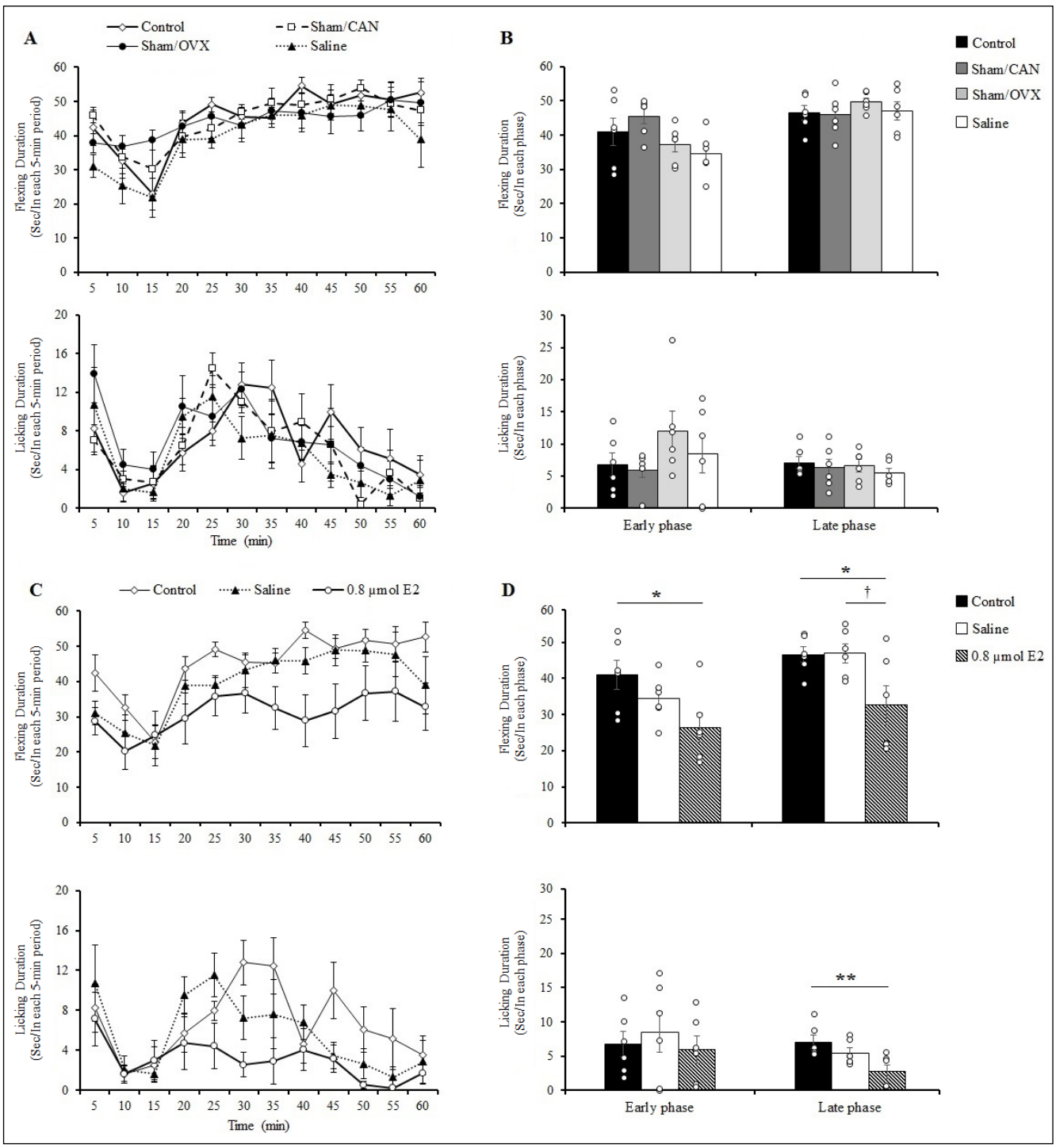

Fig. 2. Effect of cannulation into the LPGi nucleus, ovariectomy, and intra- LPGi injection of 17β-estradiol on the formalin-induced flexing and licking responses in the OVX rats (A, C, E, G (time-course) and B, D, F, H (drug-effect)). The sham/CAN (only cannulation of the LPGi nucleus) and sham/OVX groups (without removal of ovary) had no significant alterations with the control and saline groups during both the early and late phases of flexing duration (A (time-course) and B (drug-effect)). The groups of sham/CAN (only cannulation of the LPGi nucleus) and sham/OVX (without removal of ovary) did not display any significant differences with the control and saline groups during both phases of licking behavior (C (time-course) and D (drug-effect)). Intra-LPGi injection of 17β-estradiol (0.8 µmol) reduced the flexing duration in both the early and late phases of the formalin test (E (time-course) and F (drug-effect)). Injection of 17β-estradiol (Intra-LPGi, 0.8 µmol) decreased the licking behavior only in the late phase but not in the early phase of the formalin test (G (time-course) and H (drug-effect)). The nociceptive responses are presented by mean ± SEM of flexing and licking duration of 6 rats per group (n=6). *p<0.05 and **p<0.01 indicated a significant difference from the control group. †p<0.05 showed a significant difference from the saline group. 17β-estradiol=0.8µmol E2; CAN=cannulation of the LPGi nucleus and OVX=ovariectomy.

parison: CNQX effect: $F_{(2,21)}$=0.519, P>0.05; time-course effect: $F_{(2,21)}$=0.265, P>0.05; CNQX-time-course interaction: $F_{(2,21)}$=0.507, P>0.05; Fig. 3G] in the formalin test. Also, one-way ANOVA displayed that intra-LPGi microinjection of CNQX (30 nmol) did not alter the flexing duration in both early ($F_{(2,15)}$=2.040, P>0.05) and late ($F_{(2,15)}$=0.195, P>0.05) phases of formalin test (Fig. 3F). Also, both early ($F_{(2,15)}$=0.191, P>0.05) and late ($F_{(2,15)}$=0.976, P>0.05) phases of the licking behavior were not significantly changed by intra-LPGi microinjection of CNQX (Fig. 3H).

Effects of NMDA and AMPA receptors antagonists on the anti-nociceptive effect of 17β-estradiol

Fig. 4 displayed the effect of intra-LPGi co-treatment of AP5 and CNQX along with 17β-estradiol on the flexing and licking responses induced by formalin in the OVX rats. Two-way ANOVA indicated that intra-LPGi co-administration of AP5 along with 17β-estradiol increased the flexing duration at 5 min compared to the control group [within-group comparison: AP5/E2 effect: $F_{(2,21)}$=18.80, P<0.01; time-course effect: $F_{(2,21)}$=8.333, P<0.05; AP5/E2-time-course interaction: $F_{(2,21)}$=21.130, P<0.001; Fig. 4A] in the formalin test. The same analysis revealed that these injections had no significant effect on the time-course of the licking behavior [within-group comparison: AP5/E2 effect: $F_{(2,21)}$=1.133, P>0.05; time-course effect: $F_{(2,21)}$=0.886, P>0.05; AP5/E2-time-course interaction: $F_{(2,21)}$=0.289, P>0.05; Fig. 4C] in the formalin test. Furthermore, one-way ANOVA and *post hoc* analysis revealed that intra-LPGi injection of AP5 (0.5 µmol) before 17β-estradiol (0.8 µmol) injection significantly blocked the analgesic effect of 17β-estradiol on the flexing duration in the early ($F_{(2,15)}$=8.187, P<0.01] and the late ($F_{(2,15)}$=6.152, P<0.05) phases of formalin test (Fig. 4B). Although, these injections did not change licking behavior in the early phase ($F_{(2,15)}$=1.777, P>0.05; one-way ANOVA], but significantly reversed the reduction of licking behavior induced by17β-estradiol in the late phase ($F_{(2,15)}$=5.482, P<0.05; one-way ANOVA] of formalin test (Fig. 4D).

In addition, two-way ANOVA exhibited that intra-LPGi co-injection of CNQX and 17β-estradiol had no significant effect on the time-course of the flexing behavior [within-group comparison: CNQX/E2 effect: $F_{(2,21)}$=0.782, P>0.05; time-course effect: $F_{(2,21)}$=0.418, P>0.05; CNQX/E2-time-course interaction: $F_{(2,21)}$=0.164, P>0.05; Fig. 4E] and the licking behavior [within-group comparison: CNQX/E2 effect: $F_{(2,21)}$=0.217, P>0.05; time-course effect: $F_{(2,21)}$=0.983, P>0.05; CNQX/E2-time-course interaction: $F_{(2,21)}$=0.586, P>0.05; Fig. 4G] in the formalin test. Furthermore, one-way ANOVA indicated that intra-LPGi microinjection of CNQX (30 nmol) before 17β-estradiol (0.8 µmol) microinjection reversed the analgesic effect of 17β-estradiol on the flexing duration only in the early phase ($F_{(2,15)}$=4.660, P<0.05) but not in the late phase ($F_{(2,15)}$=3.330, P>0.05) of formalin test (Fig. 4F). Also, these injections reversed the reduction of the licking behavior induced by17β-estradiol in the late phase ($F_{(2,15)}$=5.631, P<0.05; one-way ANOVA) but not in the early phase ($F_{(2,15)}$=0.557, P>0.05; one-way ANOVA) of formalin test (Fig. 4H).

DISCUSSION

In this study, intra-LPGi injection of 17β-estradiol was used to evaluate the effect of this neuroactive steroid on the centrally mediated nociceptive responses in the OVX rats. The results indicated that the microinjection of 0.8 µmol 17β-estradiol into the LPGi nucleus attenuated the flexing duration in both phases but decreased licking behavior only at the late phase of the formalin test in the OVX rats, indicating an analgesic effect.

It is well accepted that female rodents have a lower pain threshold in the experimental models of hot thermal (Wala et al., 2001; Chesler et al., 2002; Terner et al., 2003a; 2003b; Sternberg et al., 2004), chemical (Aloisi et al., 1994; Gaumond et al., 2002; Barrett et al., 2003), inflammatory (Bradshaw et al., 2000; Dina et al., 2001; Cook and Nickerson, 2005), and mechanical nociception (Barrett et al., 2002; Bourquin et al., 2006) tests. Females have a longer licking and flexing duration in the formalin-produced inflammatory pain (Aloisi et al., 1998). Aloisi et al. (2010) demonstrated that treatment with estradiol or testosterone differently affected the behavioral and hormonal parameters in the two sexes. Female gonadal hormones affect and modulate behavioral and neuronal responses to a repeated nociceptive stimulus (Palmeira et al., 2011).

Furthermore, the pain response of female rats changes as a function of the estrus cycle (Frye and Walf, 2004). In female rats, there are natural variations in the estradiol levels along with the estrus cycle: low during metestrus and diestrus; during proestrus, estrogens significantly surge, and finally, there is a brief temporary peak of estradiol on proestrus (Hernandez-Leon et al., 2018). Several researchers have analyzed pain sensitivity across the estrus cycle. However, there are inconsistencies in their findings. Two studies of female rats have indicated reduced analgesic responses to morphine during the late proestrus

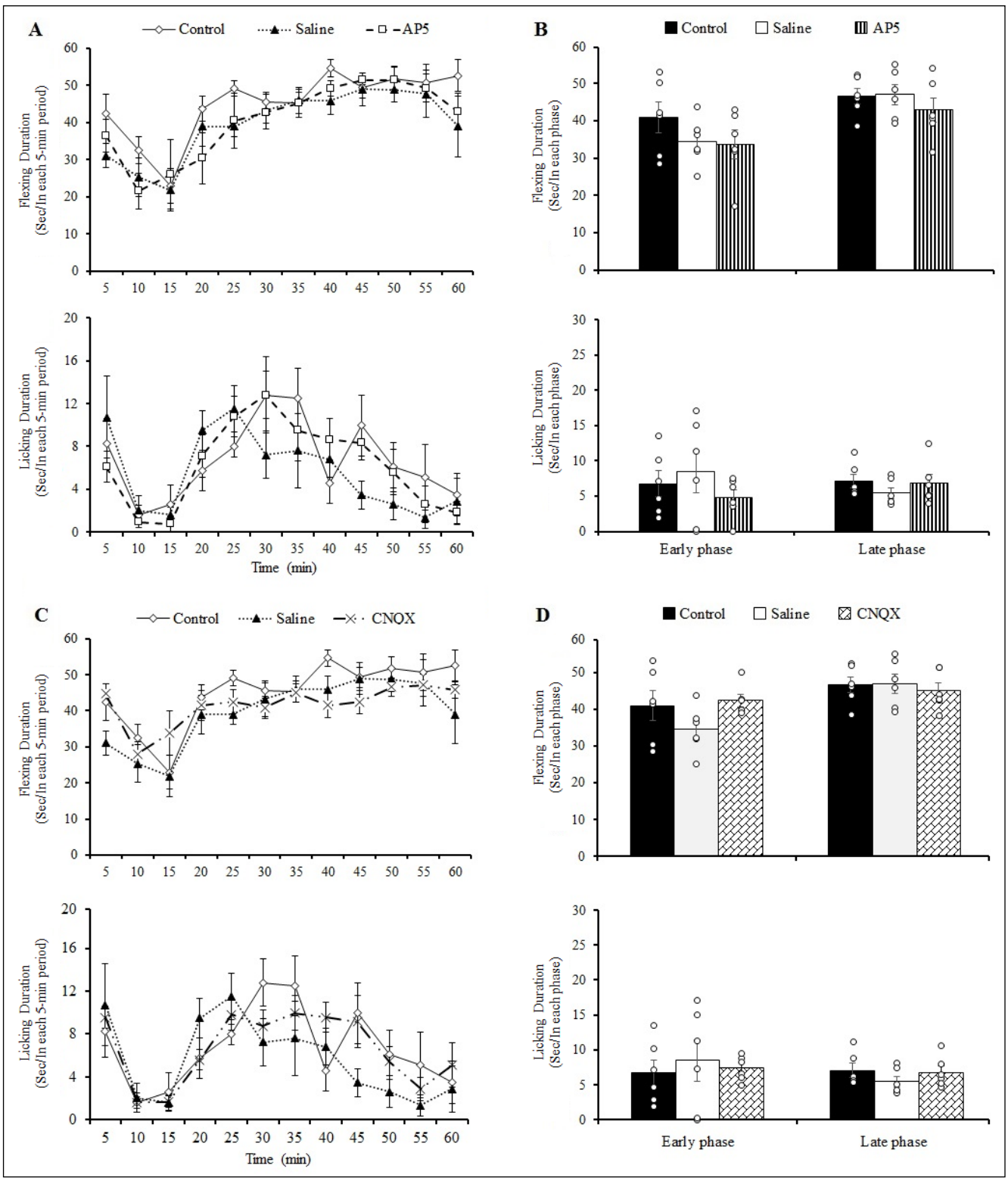

Fig. 3. Effect of intra-LPGi infusion of AP5 and CNQX on flexing and licking responses induced by formalin in the OVX rats (A, C, E, G (time-course) and B, D, F, H (drug-effect)). Intra-LPGi injection of AP5 (0.5 μmol) had no significant effect on the flexing duration in both the early and late phases of the formalin test (A (time-course) and B (drug-effect)). Treatment of the LPGi nucleus with AP5 (0.5 μmol) did not alter both phases of licking behavior in the formalin test (C (time-course) and D (drug-effect)). Application of CNQX (Intra-LPGi, 30 nmol) did not change the flexing duration in both the early and late phases of the formalin test (E (time-course) and F (drug-effect)). Intra-LPGi microinjection of CNQX (30 nmol) did not alter both the early and late phases of licking behavior in the formalin test (G (time-course) and H (drug-effect)) (n=6).

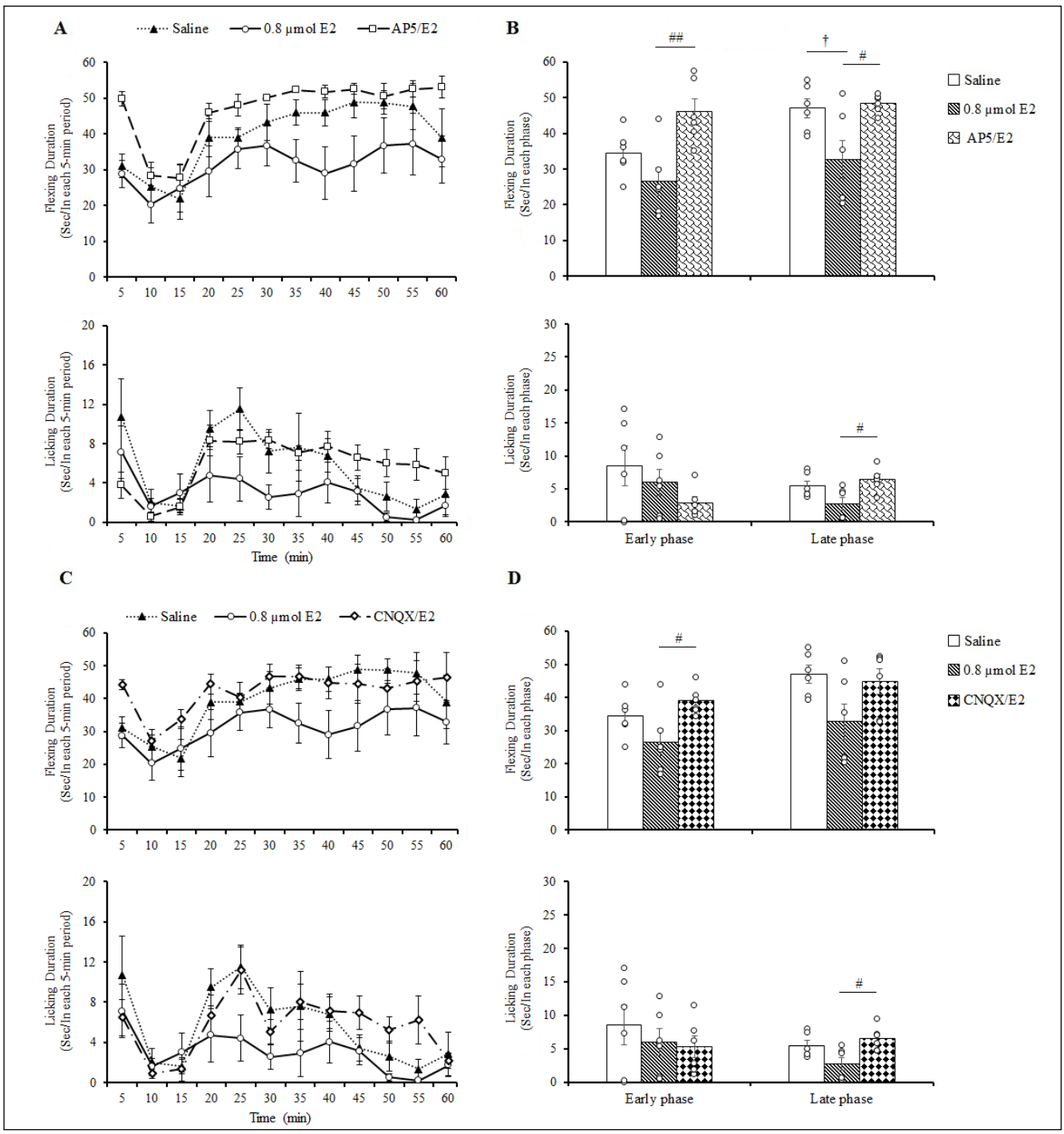

Fig. 4. Effect of pre-treatment of LPGi nucleus with AP5 and CNQX along with 17β-estradiol on flexing and licking responses induced by formalin in OVX rats (A, C, E, G (time-course) and B, D, F, H (drug-effect)). Intra-LPGi injection of AP5 (0.5 µmol) before 17β-estradiol (Intra-LPGi, 0.8 µmol) injection blocked the analgesic effect of 17β-estradiol on the flexing duration in both the early and late phases of the formalin test (A (time-course) and B (drug-effect)). Pre-treatment of LPGi nucleus with AP5 (0.5 µmol) along with 17β-estradiol (0.8 µmol) did not change licking behavior in the early phase but reversed the reduction of 17β-estradiol-induced licking behavior in the late phase of the formalin test (C (time-course) and D (drug-effect)). Intra-LPGi injection of CNQX (30 nmol) before 17β-estradiol (0.8 µmol) microinjection reversed the analgesic effect of 17β-estradiol on the flexing duration only in the early but not in the late phases of the formalin test (E (time-course) and F (drug-effect)). Pre-treatment of the LPGi nucleus with CNQX (30 nmol) along with 17β-estradiol (0.8 µmol) antagonized the decrement of licking behavior produced by 17β-estradiol in the late phase but not in the early phase of the formalin test (G (time-course) and H (drug-effect)). Data are presented as mean ± SEM for six rats (n=6). #p<0.05 and ##p<0.01 indicated a significant difference from the 17β-estradiol group. †p<0.05 indicated a significant difference from the saline group. 17β-estradiol=0.8 µmol E2, 17β-estradiol/AP5=E2/AP5 and 17β-estradiol/CNQX=E2/CNQX.

phase (Banerjee et al., 1983) in comparison to other cycle phases (Berglund and Simpkins, 1988). Another study indicated slightly greater analgesic magnitude during proestrus following intracerebroventricular infusion of morphine (Kepler et al., 1989).

Balthazart and Ball (2006) reported that the serum level of estradiol can vary dramatically, but the change occurs slowly over a matter of days, not minutes. Recently, Kato and coworkers (2013) indicated that the concentration of 17β-estradiol was 4 nM in the hippocampus of the female rats during proestrus, whereas the circulating concentration was only 0.1 nM. A growing body of researches confirming neuronal de novo estradiol synthesis and the subcellular localization of aromatase enzyme in the synaptic terminals has led to the suggestion that estradiol may act as a neurosteroid as well as a gonadal steroid, and some researches displayed its neuromodulatory effects (Luine, 2014; McCarthy, 2008). As mentioned, estradiol availability changes during the estrus cycle and so, its analgesic effect may significantly depend on the estrus cycle phase. In this study, OVX female rats were used to eliminate the centrally effect of estradiol content in different phases of the estrus cycle. The single estradiol dose used in this study exerted an analgesic effect in the formalin test. The OVX female rats were used as an animal model to eliminate the hormonal variations of the estrus cycle on the nociceptive response (Ji et al., 2003). Although some studies have reported no differences in the pain sensitivity after ovariectomy, many reports have indicated thermal, mechanical, and inflammatory hyperalgesia in the OVX rats (Li et al., 2014). Moreover, the formalin pain scores were enhanced in the OVX rats, which were significantly counteracted by estrogen replacement (Li et al., 2014; Mannino et al., 2007). Consistently, previous research has demonstrated that estradiol significantly decreased formalin-induced nociceptive responses in the OVX rats (Kuba et al., 2006; Mannino et al., 2007; Hunter et al., 2011; Palmeira et al., 2011; Li et al., 2014).

The role of estradiol in acute pain responses has been extensively studied. However, these studies have reported no change, an increase, or a decrease in nociception, suggesting that estradiol could be either analgesic or pro-nociceptive (Kuba et al., 2006). For example, the administration of 17β-estradiol increased (Frye et al., 1992), decreased (Ratka and Simpkins, 1991), or did not affect (Dawson-Basoa and Gintzler, 1993) tail-flick test latency. The results of the previous research in our laboratory indicated that the administration of 17β-estradiol into the LPGi nucleus decreased the formalin-induced responses, including paw jerking, flexing, and licking behaviors in the male rats (Khakpay and Azaddar, 2017; Khakpay et al., 2016).

As mentioned previously, steroids play pivotal physiological roles in the CNS, such as the modulation of pain sensitivity (Mensah-Nyagan et al., 2008; 2009; De Nicola et al., 2013). Steroids can exert their effects by coupling to intracellular/nuclear receptors, consequently influencing the transcription and signaling pathways (Baulieu, 2001; Schumacher et al., 2008). Also, steroids modulate membrane excitability and synaptic transmission through their allosteric interaction with ionotropic receptors, e.g., $GABA_A$ and glutamate receptors (Coronel et al., 2016). Indeed the GABAergic brainstem neurons modulate the release of the endogenous opioid enkephalin in the spinal cord to regulate inputs from the sensory pain fibers (François et al., 2017). Silencing or stimulation of the dual GABA/enkephalinergic RVM neurons extensively enhanced or reduced behavioral sensitivity, respectively, to the heat and mechanical stimuli. These revealed that both GABA and enkephalin can induce presynaptic inhibition of the sensory afferents (Zhang et al., 2015). Moreover, pain-modulatory pathways receive input from the ascending pain transmission pathways. Zhang et al. (2015), using optogenetics with whole-cell patch-clamp recording and *in vivo* single-cell recording, indicated that there are direct functional connections from the parabrachial complex to physiologically determined pain-modulating neurons of the RVM. Also, they reported an identified nociceptive synapse in the RVM that could be investigated in relevant physiologic contexts, and set a point for dissection about the relations between nociceptive transmission and nociceptive regulation in the transition from acute to chronic pain (Chen et al., 2017).

Recent studies revealed that the glutamatergic synapses not only play central role in the sensory transmission, including pain and itch transmission but also participate in the nociceptive sensitization at different levels of the brain. Between the pain-related brain areas, all three primary forms of ionotropic receptors (NMDA, AMPA, and KA receptors) have been recognized (Zhuo, 2017). The critical role of the NMDA receptors in tonic/chronic pain and hyperalgesia is now revealed (Coderre, 1993). The NMDA receptors are involved in the development of plasticity, which participates in the persistent pain responses. Thus, the development of the late/chronic phase of formalin-produced inflammatory pain is the product of NMDA receptor stimulation during the early/acute phase (Danysz and Parsons, 1998). Also, intra-periaqueductal grey matter administration of AP5 relieved behavioral responses induced by formalin in rats (Vaccarino et al., 1997).

Intrathecal injection of NMDA and AMPA antagonists reduced pain behavior (Coderre and Melzack, 1992; Näsström et al., 1992; Wang and Goffer, 2010).

Gordon and Soliman (1996) reported that intrathecal injection of CNQX inhibited the full development of the thermal hyperalgesia for up to 10 days when administered immediately before peripheral nerve injury. Also, intrathecal CNQX blocked the full development of the thermal hyperalgesia up to 3 days after peripheral nerve injury (Gordon and Soliman, 1996). Moreover, intra-LPGi administration of CNQX attenuated the late phase of nociceptive behaviors in the rat formalin test (Khakpay and Azaddar, 2017). Electrophysiological recordings of wide-dynamic-range dorsal horn neurons in the formalin-injected rats exhibited that the tonic response of these neurons was significantly suppressed by the NMDA receptor antagonists, including AP5, ketamine, and MK-801. However, the phasic response of these neurons was decreased, meaninglessly (Eisenberg et al., 1993). Therefore, we tried to find a dose of AP5 and CNQX without any significant effect on nociception.

In the present study, intra-LPGi administration of 0.5 µmol AP5 and 30 nmol CNQX failed to induce any significant nociceptive response (Fig. 3). Therefore, these doses of the drugs were selected to assess whether the NMDA and the AMPA receptors are involved in the pain-coping behavior of 17β-estradiol in the LPGi nucleus of the OVX female rats. Interestingly, AP5 significantly antagonized the analgesic effect of 17β-estradiol on flexing behavior in both phases of the formalin test. In contrast, CNQX antagonized the decline of 17β-estradiol-evoked flexing duration only in the early phase of the formalin test in the OVX rats. Although AP5 and CNQX did not block the anti-nociceptive effect of 17β-estradiol in the early phase of licking response, they significantly antagonized the 17β-estradiol-induced analgesic effect in the late phase of licking response of formalin test in the OVX rats. It may be concluded that the analgesic effect of 17β-estradiol in formalin-induced inflammatory pain is mediated through interaction with membrane-bound receptors, probably the glutamate (NMDA and AMPA) receptors in the OVX rats.

The levels of neurosteroids like 17β-estradiol can be changed in the OVX rats. This change can influence receptor expression or neurotransmitter release (Partridge and Valenzuela, 2001). Studies indicated that the density of NMDA receptors in the estrus phase of female rats is higher than in the diestrus phase, but this variation is not significant. Also, female rats in the diestrus have markedly higher AMPA receptor densities than female rats in the estrus (Palomero-Gallagher et al., 2003). So as mentioned above, the OVX female rats were used to exclude the hormonal alterations of the estrus cycle on the nociceptive response.

The effects of ovarian steroids withdrawal and subsequent treatment on the density of NMDA and AMPA receptors in the rat hippocampus are well demonstrated (Palomero-Gallagher et al., 2003). Oberlander and Woolley (2017) indicated that different estrogen receptors modulate glutamate signaling, both in the presynaptic and postsynaptic level in the hippocampus. It has been reported that intra-CA1 injection of 17β-estradiol increased synaptic excitability by increasing the amplitude of AMPA receptor-mediated responses (Wong and Moss, 1992). Also, estradiol stimulates the expression of NMDA and AMPA receptors of the hypothalamic neurons (Cyr et al., 2001; Diano et al., 1997). In contrast to intact female animals, the density of NMDA receptors is sensitive to variation hormonal levels in the OVX rats (Woolley et al., 1997). The modulatory effect of estradiol on the AMPA receptor expression has been reported to be site-specific in rats (Cyr et al., 2001). Furthermore, the expression and phosphorylation of the NR1 subunit of the NMDA receptor can be increased by estradiol which can increase the spinal processing of visceral nociception in the OVX rats (Tang et al., 2008). It can be postulated that the anti-nociceptive effect of intra-LPGi 17β-estradiol on inflammatory pain may be mediated *via* the NMDA and AMPA receptors in the OVX female rats. However, it needs more investigation by molecular and electrophysiological approaches to clarify the mechanism of this analgesic effect.

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REFERENCES

Aloisi A, Ceccarelli I (1999) Role of gonadal hormones in formalin-induced pain responses of male rats: modulation by estradiol and naloxone administration. Neuroscience 95: 559–566.

Aloisi AM, Affaitati G, Ceccarelli I, Fiorenzani P, Lerza R, Rossi C, Giamberardino MA (2010) Estradiol and testosterone differently affect visceral pain-related behavioural responses in male and female rats. Eur J Pain 14: 602–607.

Aloisi AM, Albonetti ME, Carli G (1994) Sex differences in the behavioural response to persistent pain in rats. Neurosci Lett 179: 79–82.

Aloisi AM, Ceccarelli I, Lupo C (1998) Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. Brain Res Bull 47: 57–62.

Andrezik JA, Chan-Palay V, Palay SL (1981) The nucleus paragigantocellularis lateralis in the rat. Anat Embryol 161: 373–390.

Aston-Jones G, Chiang C, Alexinsky T (1991) Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. Prog Brain Res 88: 501–520.

Azcoitia I, Arevalo MA, Garcia-Segura LM (2017) Neural-derived estradiol regulates brain plasticity. J Chem Neuroanat 89: 53–59.

Azcoitia, I, Sierra A, Veiga S, Honda S, Harada N, Arcia-Segura LM (2001) Brain aromatase is neuroprotective. J Neurobiol 47: 318–329.

Azhdari-Zarmehri H, Reisi Z, Vaziri A, Haghparast A, Shaigani P, Haghparast A (2013) Involvement of orexin-2 receptors in the ventral tegmental area and nucleus accumbens in the antinociception induced by the lateral hypothalamus stimulation in rats. Peptides 47: 94–98.

Azhdari-Zarmehri H, Semnanian S, Fathollahi Y (2015) Orexin-a modulates firing of rat rostral ventromedial medulla neurons: an in vitro study. Cell J 17: 163–170.

Azhdari-Zarmehri H, Semnanian S, Fathollahi Y, Pakdel FG (2014) Tail flick modification of orexin-a induced changes of electrophysiological parameters in the rostral ventromedial medulla. Cell J 16: 131.

Bailey DJ, Ma C, Soma KK, Saldanha CJ (2013) Inhibition of hippocampal aromatization impairs spatial memory performance in a male songbird. Endocrinology 154: 4707–4714.

Balthazart J, Ball GF (2006) Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci 29: 241–249.

Banerjee P, Chatterjee TK, Ghosh JJ (1983) Ovarian steroids and modulation of morphine-induced analgesia and catalepsy in female rats. Eur J Pharmacol 96: 291–294.

Barrett AC, Smith ES, Picker MJ (2002) Sex-related differences in mechanical nociception and antinociception produced by μ-and κ-opioid receptor agonists in rats. Eur J Pharmacol 452: 163–173.

Barrett AC, Smith ES, Picker MJ (2003) Capsaicin-induced hyperalgesia and μ-opioid-induced antihyperalgesia in male and female Fischer 344 rats. J Pharmacol Exp Ther 307: 237–245.

Baulieu E (2001) Neurosteroids, their role in brain physiology: neurotrophicity, memory, aging. Bulletin de l'Academie nationale de medecine 185: 349.

Berglund A, Simpkins JW (1988) Alterations in brain opiate receptor mechanisms on proestrous afternoon. Neuroendocrinology 48: 394–400.

Bleakman D, Alt A, Nisenbaum ES (2006) Glutamate receptors and pain. Semin Cell Dev Biol 17: 592–604.

Bourquin AF, Süveges M, Pertin M, Gilliard N, Sardy S, Davison AC, Decosterd I (2006) Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. Pain 122: 14.

Bradshaw H, Miller J, Ling Q, Malsnee K, Ruda M (2000) Sex differences and phases of the estrous cycle alter the response of spinal cord dynorphin neurons to peripheral inflammation and hyperalgesia. Pain 85: 93–99.

Ceccarelli I, Fiorenzani P, Grasso G, Lariviere WR, Massafra C, Massai L, Aloisi AM (2004) Estrogen and μ-opioid receptor antagonists counteract the 17β-estradiol-induced licking increase and interferon-γ reduction occurring during the formalin test in male rats. Pain 111: 181–190.

Chakraborty TR, Gore AC (2004) Aging-related changes in ovarian hormones, their receptors, and neuroendocrine function. Exp Biol Med 229: 977–987.

Chen Q, Roeder Z, Li MH, Zhang Y, Ingram SL, Heinricher MM (2017) Optogenetic evidence for a direct circuit linking nociceptive transmission through the parabrachial complex with pain-modulating neurons of the rostral ventromedial medulla (RVM) eNeuro 4: ENEURO.0202–0217.2017.

Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS (2002) Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. Neurosci Biobehav Rev 26: 907–923.

Coderre TJ (1993) The role of excitatory amino acid receptors and intracellular messengers in persistent nociception after tissue injury in rats. Mol Neurobiol 7: 229–246.

Coderre TJ, Melzack R (1992) The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 12: 3665–3670.

Cook CD, Nickerson MD (2005) Nociceptive sensitivity and opioid antinociception and antihyperalgesia in Freund's adjuvant-induced arthritic male and female rats. J Pharmacol Exp Ther 313: 449–459.

Coronel MF, Labombarda F, González SL (2016) Neuroactive steroids, nociception and neuropathic pain: A flashback to go forward. Steroids 110: 77–87.

Cyr M, Ghribi O, Thibault C, Morissette M, Landry M, Di Paolo T (2001) Ovarian steroids and selective estrogen receptor modulators activity on rat brain NMDA and AMPA receptors. Brain Res Rev 37: 153–161.

Da Silva LFS, Walder RY, Davidson BL, Wilson SP, Sluka KA (2010) Changes in expression of NMDA-NR1 receptor subunits in the rostral ventromedial medulla modulate pain behaviors. Pain 151: 155–161.

Danysz W, Parsons CG (1998) Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol Rev 50: 597–664.

Dawson-Basoa MB, Gintzler AR (1993) 17-β-estradiol and progesterone modulate an intrinsic opioid analgesic system. Brain Res 601: 241–245.

Diano S, Naftolin F, Horvath TL (1997) Gonadal steroids target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: a morphological and biochemical study 1. Endocrinology 138: 778–789.

Dina OA, Aley K, Isenberg W, Messing RO, Levine JD (2001) Sex hormones regulate the contribution of PKCε and PKA signalling in inflammatory pain in the rat. Eur J Neurosci 13: 2227–2233.

Eisenberg E, Vos BP, Strassman AM (1993) The NMDA antagonist memantine blocks pain behavior in a rat model of formalin-induced facial pain. Pain 54: 301–307.

Erami E, Azhdari-Zarmehri H, Ghasemi-Dashkhasan E, Esmaeili MH, Semnanian S (2012) Intra-paragigantocellularis lateralis injection of orexin-A has an antinociceptive effect on hot plate and formalin tests in rat. Brain Res 1478: 16–23.

De Nicola AF, Coronel F, I Garay, L, Gargiulo-Monachelli G, Claudia Gonzalez Deniselle, M, L Gonzalez S, Schumacher M (2013) Therapeutic effects of progesterone in animal models of neurological disorders. CNS Neurol Disord Drug Targets 12: 1205–1218.

Fields HL, Heinricher MM, Mason P (1991) Neurotransmitters in nociceptive modulatory circuits. Ann Rev Neurosci 14: 219–245.

François A, Low SA, Sypek EI, Christensen AJ, Sotoudeh C, Beier KT, Scherrer G (2017) A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. Neuron 93: 822–839.

Frye CA, Bock BC, Kanarek RB (1992) Hormonal milieu affects tailflick latency in female rats and may be attenuated by access to sucrose. Physiol Behav 52: 699–706.

Frye CA, Walf AA (2004) Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. Behav Neurosci 118: 306.

Gaumond I, Arsenault P, Marchand S (2002) The role of sex hormones on formalin-induced nociceptive responses. Brain research 958: 139–145.

Gaunitz C, Schüttler A, Gillen C, Allgaier C (2002) Formalin-induced changes of NMDA receptor subunit expression in the spinal cord of the rat. Amino Acids 23: 177–182.

Gordon FT, Soliman MR (1996) The effects of estradiol and progesterone on pain sensitivity and brain opioid receptors in ovariectomized rats. Hormones Behav 30: 244–250.

Guan Y, Guo W, Robbins MT, Dubner R, Ren K (2004) Changes in AMPA receptor phosphorylation in the rostral ventromedial medulla after inflammatory hyperalgesia in rats. Neurosci Lett 366: 201–205.

Hernandez-Leon A, De la Luz-Cuellar YE, Granados-Soto V, González--Trujano ME, Fernández-Guasti A (2018) Sex differences and estradiol involvement in hyperalgesia and allodynia in an experimental model of fibromyalgia. Hormones Behav 97 39–46.

Hunter DA, Barr GA, Amador N, Shivers KY, Kemen L, Kreiter CM, Quinones-Jenab V (2011) Estradiol-induced antinociceptive responses on formalin-induced nociception are independent of COX and HPA activation. Synapse 65: 643–651.

Ji Y, Murphy AZ, Traub RJ (2003) Estrogen modulates the visceromotor reflex and responses of spinal dorsal horn neurons to colorectal stimulation in the rat. J Neurosci 23: 3908–3915.

Kato A, Hojo Y, Higo S, Komatsuzaki Y, Murakami G, Yoshino H, Kawato S (2013) Female hippocampal estrogens have a significant correlation with cyclic fluctuation of hippocampal spines. Front Neural Circuits 7: 149.

Kepler KL, Kest B, Kiefel JM, Cooper ML, Bodnar RJ (1989) Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracerebroventricular morphine in rats. Pharmacol Biochem Behav 34: 119–127.

Khakpay R, Azaddar M (2017) Role of the AMPA receptors of paragigantocellularis lateralis nucleus in the inflammatory pain modulation in male rat. Physiol Pharmacol 21: 147–154.

Khakpay R, Azaddar M, Khakpai F (2016) The antinociceptive effect of 17β-estradiol in the nucleus paragigantocellularis lateralis of male rats may be mediated by the NMDA receptors. Physiol Pharmacol 20: 122–129.

Khakpay R, Azaddar M, Khakpai F (2020) Involvement of glutamate receptors of the paragigantocellularis lateralis nucleus in the pain modulatory effect of 17β-estradiol in male rats. Acta Neurol Belg 120: 653–660.

Khakpay R, Barani S, Hatami Nemati H (2014) The antinociceptive effect of 17β-estradiol in the paragigantocellularis lateralis of male rats is mediated by estrogenic receptors. Physiol Pharmacol 18: 215–223.

Khakpay R, Semnanian S, Javan M, Janahmadi M (2010) The effect of intra-locus coeruleus injection of 17beta-estradiol on inflammatory pain modulation in male rat. Behav Brain Res 214: 409–416.

Kuba T, Kemen LM, Quinones-Jenab V (2005) Estradiol administration mediates the inflammatory response to formalin in female rats. Brain Res 1047: 119–122.

Kuba T, Wu HBK, Nazarian A, Festa ED, Barr GA, Jenab S, Quinones-Jenab V (2006) Estradiol and progesterone differentially regulate formalin-induced nociception in ovariectomized female rats. Hormones Behav 49: 441–449.

Li LH, Wang ZC, Yu J, Zhang YQ (2014) Ovariectomy results in variable changes in nociception, mood and depression in adult female rats. PloS One 9: e94312.

Loyd DR, Murphy AZ (2008) Androgen and estrogen (alpha) receptor localization on periaqueductal gray neurons projecting to the rostral ventromedial medulla in the male and female rat. J Chem Neuroanat 36: 216–226.

Luine VN (2014) Estradiol and cognitive function: past, present and future. Horm Behav 66: 602–618.

Mahmoudi M, Zarrindast MR (2002) Effect of intracerebroventricular injection of GABA receptor agents on morphine-induced antinociception in the formalin test. J Psychopharmacol 16: 85–91.

Mannino CA, South SM, Quinones-Jenab V, Inturrisi CE (2007) Estradiol replacement in ovariectomized rats is antihyperalgesic in the formalin test. J Pain 8: 334–342.

Mason P (1999) Central mechanisms of pain modulation. Curr Opin Neurobiol 9: 436–441.

McCarthy MM (2008) Estradiol and the developing brain. Physiol Rev 88: 91–134.

Mensah-Nyagan A, Kibaly C, Schaeffer V, Venard C, Meyer L, Patte-Mensah C (2008) Endogenous steroid production in the spinal cord and potential involvement in neuropathic pain modulation. J Steroid Biochem Mol Biol 109: 286–293.

Mensah-Nyagan AG, Meyer L, Schaeffer V, Kibaly C, Patte-Mensah C (2009) Evidence for a key role of steroids in the modulation of pain. Psychoneuroendocrinology 34: S169-S177.

Näsström J, Karlsson U, Post C (1992) Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. EurJ Pharmacol 212: 21–29.

Normandin JJ, Murphy AZ (2008) Nucleus paragigantocellularis afferents in male and female rats: organization, gonadal steroid receptor expression, and activation during sexual behavior. J Comp Neurol 508: 771–794.

Oberlander JG, Woolley CS (2017) 17β-estradiol acutely potentiates glutamatergic synaptic transmission in the hippocampus through distinct mechanisms in males and females. J Neurosci 37: 12314–12327.

Palmeira CC, Ashmawi HA, Posso Ide P (2011) Sex and pain perception and analgesia. Rev Bras Anestesiol 61: 814–828.

Palomero-Gallagher N, Bidmon HJ, Zilles K (2003) AMPA, kainate, and NMDA receptor densities in the hippocampus of untreated male rats and females in estrus and diestrus. J Comp Neurol 459: 468–474.

Partridge LD, Valenzuela CF (2001) Neurosteroid-induced enhancement of glutamate transmission in rat hippocampal slices. Neurosci Lett301: 103–106.

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates (6th ed.) Academic Press, London, UK.

Potes CS, Neto FL, Castro-Lopes JM (2006) Administration of baclofen, a gamma-aminobutyric acid type B agonist in the thalamic ventrobasal complex, attenuates allodynia in monoarthritic rats subjected to the ankle-bend test. J Neurosci Res 83: 515–523.

Ratka A, Simpkins JW (1991) Effects of estradiol and progesterone on the sensitivity to pain and on morphine-induced antinociception in female rats. Hormones Behav 25: 217–228.

Roca-Vinardell A, Berrocoso E, Llorca-Torralba M, García-Partida JA, Gibert-Rahola J, Mico JA (2018) Involvement of 5-HT1A/1B receptors in the antinociceptive effect of paracetamol in the rat formalin test. Neurobiol Pain 3: 15–21.

Saldanha CJ, Rohmann KN, Coomaralingam L, Wynne RD (2005) Estrogen provision by reactive glia decreases apoptosis in the zebra finch (Taeniopygia guttata). Develop Neurobiol 64: 192–201.

Schumacher M, Sitruk-Ware R, De Nicola AF (2008) Progesterone and progestins: neuroprotection and myelin repair. Curr Opin Pharmacol 8: 740–746.

Shamsizadeh A, Soliemani N, Mohammad-Zadeh M, Azhdari-Zarmehri H (2014) Permanent lesion in rostral ventromedial medulla potentiates swim stress-induced analgesia in formalin test. Iran J Basic Med Sci 17: 209.

Soleimani N, Erami E, Abbasnejad M, Ali S, Hassan AZ (2013) Effect of transient inactivation of rostral ventromedial medulla on swim stress induced analgesia in formalin test in rats. Physiol Pharmacol 17: 116–124.

Sternberg WF, Smith L, Scorr L (2004) Nociception and antinociception during the first week of life in mice: sex differences and test dependence. J Pain 5: 420–426.

Tang B, Ji Y, Traub RJ (2008) Estrogen alters spinal NMDA receptor activity via a PKA signaling pathway in a visceral pain model in the rat. Pain 137: 540–549.

Terner JM, Barrett A, Cook C, Picker M (2003a) Sex differences in (−)-pentazocine antinociception: comparison to morphine and spiradoline in four rat strains using a thermal nociceptive assay. Behav Pharmacol 14: 77–85.

Terner JM, Lomas LM, Smith ES, Barrett AC, Picker MJ (2003b) Pharmacogenetic analysis of sex differences in opioid antinociception in rats. Pain 106: 381–391.

Vaccarino AL, Clemmons HR, Mader GJ, Magnusson JE (1997) A role of periaqueductal grey NMDA receptors in mediating formalin-induced pain in the rat. Neurosci Lett 236: 117–119.

Wala EP, Sloan JW, Jing X, Holtman Jr JR (2001) The effects of diazepam dependence and withdrawal on morphine-induced antinociception and changes in locomotion in male and female rats. Pharmacol Biochem Behav 69 475–484.

Wang J, Goffer Y (2010) AMPA receptors and pain—A future therapeutic intervention? Tech Reg Anesth Pain Manag 14: 59–64.

Wheeler-Aceto H, Cowan A (1991) Neurogenic and tissue-mediated components of formalin-induced edema: evidence for supraspinal regulation. Agents Actions 34: 264–269.

Willis W, Coggeshall R (2004) Sensory mechanisms of the spinal cord. Vol 1. Primary afferent neurons and the spinal dorsal horn. In: New York: Kluwer Academic/Plenum Publishers.

Wong M, Moss RL (1992) Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. J Neurosci 12: 3217–3225.

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA (1997) Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J Neurosci 17: 1848–1859.

Yang K, Ma WL, Feng YP, Dong YX, Li YQ (2002) Origins of GABAB receptor-like immunoreactive terminals in the rat spinal dorsal horn. Brain Res Bull 58: 499–507.

Zhang QG, Wang R, Tang H, Dong Y, Chan A, Sareddy GR, Brann DW (2014) Brain-derived estrogen exerts anti-inflammatory and neuroprotective actions in the rat hippocampus. Mol Cell Endocrinol 389: 84–91.

Zhang, Y, Zhao, S, Rodriguez, E, Takatoh, J, Han, BX, Zhou, X, Wang, F (2015) Identifying local and descending inputs for primary sensory neurons. J Clin Invest 125: 3782–3794.

Zhuo M (2017) Ionotropic glutamate receptors contribute to pain transmission and chronic pain. Neuropharmacology 112: 228–234.