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Trigeminal neuroplasticity after pulpitis and oral administration of paracetamol in rats

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ABSTRACT

Experimental stimulation of dental pulp nociceptors induces plastic changes in the trigeminal subnucleus caudalis (Vc) through increased intracellular calcium levels and discharge of nociceptive afferent fibers. Acetaminophen (paracetamol) is a potent and selective inhibitor of COX-2 within the central nervous system (CNS) and may act in association with the cannabinoid system and the descending serotonergic pathway. ***Aim:*** *In this context, the main objective of this study is to analyze the plastic changes induced by paracetamol in the nociceptive circuit of the Vc following unilateral dental pulp inflammation (pulpitis) in rats.* ***Material and Method:*** *Twenty-one female Wistar rats, 50 days old, were divided into three groups. In the experimental group 1 (EG1), a unilateral pulpitis was induced in the left mandibular first molar, and animals received no further treatment. In group 2 (EG2), the procedure was the same as in EG1, but 48 h later, animals received paracetamol in 3 daily doses (300 mg/kg each) every 4 h for 2 days. The control group (CG) received no treatment. After euthanasia, liver, hemimandibles and brainstem samples were analyzed. The results were analyzed using Student's t-test or ANOVA ($p<0.05$).* ***Results:*** *5-hydroxytryptamine transporter (5HT-T) analysis in the ipsilateral Vc demonstrated that paracetamol administration increased plexus length and varicosity density compared with EG1 and CG ($p<0.05$). Paracetamol also increased cannabinoid CB1 receptor (CB1r) expression ($p<0.05$), and decreased the number of calbindin D28K positive projection neurons ($p<0.05$).* ***Conclusion:*** *Following experimental pulpitis, oral administration of paracetamol induces plastic changes in the serotonergic and endocannabinoid systems within the Vc. This plasticity is reflected by a reduced number of fusiform calbindin D28K projection neurons in lamina I, associated with modulation of orofacial nociceptive processing.*

Keywords: *acetaminophen - receptor, cannabinoid - CB1- serotonin plasma membrane transport proteins - trigeminal caudal nucleus - nociception - pulpitis*

Neuroplasticidad trigeminal luego de una pulpitis y administración oral de paracetamol en ratas

RESUMEN

La estimulación experimental de los nociceptores de la pulpa dental induce cambios plásticos en el subnúcleo caudal del trigémino (Vc) generando un aumento de los niveles de calcio intracelular y la descarga de las fibras aferentes nociceptivas. El paracetamol es un potente inhibidor selectivo de la COX-2 en el sistema nervioso central (SNC) y puede actuar en asociación con el sistema cannabinoide y la vía serotoninérgica descendente. ***Objetivo:*** *el objetivo principal de este estudio es analizar los cambios plásticos inducidos por el paracetamol en el circuito nociceptivo del Vc tras una inflamación unilateral de la pulpa dental (pulpitis) en ratas.* ***Materiales y métodos:*** *21 ratas Wistar hembras, de 50 días de edad, se dividieron en tres grupos. En el grupo experimental 1 (GE1), se indujo una pulpitis unilateral en el primer molar mandibular izquierdo, y los animales no recibieron ningún tratamiento adicional. En el grupo 2 (GE2), el procedimiento fue el mismo que en GE1, pero 48 h después, los animales recibieron paracetamol en 3 dosis diarias (300 mg/kg cada una) cada 4 h durante 2 días. El grupo control (GC) no recibió ningún procedimiento. Tras la eutanasia, se analizaron muestras de hígado, hemimandíbulas y tronco encefálico. Los resultados se analizaron mediante la prueba t de Student o ANOVA ($p < 0,05$).* ***Resultados:*** *En el Vc ipsilateral del GE2 se demostró que la administración de paracetamol aumentó la longitud del plexo y la densidad de varicosidades del transportador de 5-hidroxitriptamina (5HT-T) en comparación con el EG1 y el CG ($p < 0,05$). También se demostró mayor expresión del receptor cannabinoide CB1 (CB1r) y menor número de neuronas de proyección positivas para calbindina D28K ($p < 0,05$).* ***Conclusión:*** *Tras una pulpitis experimental, la administración oral de paracetamol induce cambios plásticos en los sistemas serotoninérgico y endocannabinoide dentro del Vc. Esta neuroplasticidad se refleja en una reducción del número de neuronas de proyección fusiformes positivas para calbindina D28K en la lámina I, asociada a la modulación del procesamiento nociceptivo orofacial.*

Palabras clave: *paracetamol - receptor cannabinoide - CB1 - proteínas de transporte de serotonina en la membrana plasmática - núcleo caudal del trigémino - nocicepción - pulpitis*

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INTRODUCTION

Prostaglandins play an essential role in processing inflammatory pain, not only by sensitizing the peripheral terminals of primary afferent fibers, but also by increasing input of nociceptive information in the central nervous system (CNS)[1]. The sensory information of orofacial structures is relayed by the trigeminal system, which comprises principal, mesencephalic and spinal nuclei. Nociceptive information is processed in the spinal subnucleus oralis (Vo), interpolaris (Vi) and caudalis (Vc), depending on the orofacial region involved. Experimental stimulation of orofacial nociceptors has been shown to induce plastic changes (neuroplasticity) at various levels of nociceptive pathways[2,3]. Afferent projections from mandibular tooth pulp terminate in the dorsomedial region of the Vc, which has been identified as the first center involved in orofacial nociceptive modulation[4]. Glutamatergic trigeminal ganglion neurons carry the nociceptive information at the superficial laminae I and II and activate glutamatergic receptors. From laminae I (LI), those nociceptive messages are transmitted by fusiform projection neurons to other regions of the brainstem and to the thalamus[4,5]. The majority of fusiform neurons in LI project to supraspinal targets and more than half co-express glutamate and calbindin immunoreactivity. Calbindin may therefore serve as a marker of projection neurons and contribute to buffering intracellular calcium increases induced by discharge of nociceptive afferent fibers[5]. These transient changes in local Vc circuits are critical for maintaining balance between excitatory and inhibitory neurons in nociceptive pathways[6,7]. Neurons in substantia gelatinosa or lamina II (LII) are considered interneurons that process sensory information and do not project out of Vc. In addition to local modulation by the Vc, the descending system presents components functionally related with those of the opioid, endocannabinoid, noradrenergic and serotonergic systems, and play an important role in the orofacial nociceptive pathway[8,9].

Dental clinical evidence shows that patients with pulpitis frequently undergo analgesic therapy to mitigate treatment-associated pain. Acetaminophen (paracetamol) is an effective analgesic medication which is metabolized in the liver and can thus cause liver injury at high doses[10]. In terms of functional effects, paracetamol exerts weak inhibition of peripheral COX-1 and COX-2 linked to the inflammatory response following injury[11,12], but acts predominantly at the CNS. It also produces antinociceptive effects through interactions with serotonergic and endocannabinoid pathways[13-15]. Descending facilitatory and inhibitory serotoninergic fibers originated in the midbrain periaqueductal gray (PAG) and ventromedial medulla (VMM) descend and make contacts with primary afferents and interneurons at laminae of Vc[8,9]. The 5-hydroxytryptamine transporter (5HT-T) modulates neurotransmission by facilitating the removal of serotonin from synapses through neurotransmitter reuptake by presynaptic terminals[16]. In turn, the effects of endocannabinoids are mediated by receptors CB1 or CB2 and are responsible of physiological responses as analgesia and synaptic plasticity, among others[17]. Recent research has demonstrated that cannabinoid CB1 receptor (CB1r) is the most abundant in CNS and is expressed in glutamatergic, GABAergic, serotonergic, cholinergic, noradrenergic and dopaminergic neurons[18]. The activation of these receptors present in both the plasmatic membrane and on the outer mitochondrial membrane, decreases the release of glutamate and GABA[14,18,19]. Given the limited evidence available of the action of serotonin and CB1r on Vc cells, this study analyzed paracetamol-induced plastic changes in the trigeminal Vc nociceptive circuit after unilateral pulpitis (inflammation of the dental pulp) in rats.

We hypothesize that oral administration of paracetamol after pulpitis induces neuroplastic changes in the Vc through serotonergic and endocannabinoid mechanisms. These changes are reflected by alterations in 5HT-T expression, CB1 receptor distribution, and in the number of calbindin D28K-positive neurons. These mechanisms are likely interconnected rather than independent, as evidence suggests that paracetamol metabolites (e.g., AM404) simultaneously engage cannabinoid receptors and serotonergic pathways within central pain circuits[14, 15].

Current evidence indicates that paracetamol analgesia involves a multi-target central mechanism, including indirect activation of CB1 receptors and facilitation of descending serotonergic pathways, rather than isolated actions on COX enzymes.

MATERIALS AND METHOD

Animals

Animals were housed in galvanized steel cages, with three animals per cage, in a temperature-controlled room (21-24°C) under 12-hour light/dark cycle. Food (Standard diet rat-mouse chow – Cooperación) and water were provided ad libitum. Our protocol followed the internationally accepted standards of the Guidelines for the Care and Use of Laboratory Animals (NIH Publication 1985, and the National Academy of Science, US) and was approved by the Ethics Committee of the School of Dentistry at Universidad de Buenos Aires (Cicual-Odon/FOUBA 006/2023). Twenty-one female Wistar rats, 50 days old, weighing 120-140 g were randomly assigned to one of three groups of seven animals each (n=7), using a simple randomization method. In experimental group 1 (EG1), a unilateral dental pulp injury was induced in the left mandibular first molar, and animals received no further treatment. In experimental group 2 (EG2), the procedure was the same as in EG1, but 48 h later animals received paracetamol antinociceptive treatment, similar to the analgesia used when the nociceptive pathway is activated by inflammatory tissue injury in humans. The control group (CG) received no treatment.

Surgical procedure of molar access cavity

Animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg, Holliday Laboratory) and xylazine (10 mg/kg König Laboratory). In the pulpitis groups, i.e., EG1 and EG2, enamel and dentin were removed from the left mandibular first molar using a 1/4 round bur at low speed to expose the mesial horn of the pulp chamber (ipsilateral). The cavity was then sealed with phosphate cement (Harvard; Richter and Hoffmann, Berlin, Germany) to prevent bacterial contamination and stimulate pulp inflammation through direct contact with cement. After the procedure, animals were monitored for discomfort or pain, and body weight was followed up until euthanasia. Only in the first 24 h after surgery, EG1 and EG2 animals showed decreased activity and ate less than the previous day. In EG2, 900 mg/kg/day paracetamol (Termofren™ drops, Roemmers, Buenos Aires, Argentina) for 2d [10,20,21]. Paracetamol was administrated orally starting 48 h after pulp injury to simulate delayed clinical analgesic intervention. Animals received three doses (300 mg/kg each) per day (every 4 h starting at 8 am), resulting in a 12-hour drug-free interval. On day 4, EG2 and CG animals were anesthetized and 500 µl blood was collected from the rat tail vessels for analysis of serum transaminase levels (GPT: alanine transaminase and GOT: aspartate transaminase) and to evaluate the potential hepatotoxic effect of paracetamol. Serum transaminase levels in control animals were used as reference values.

Fixation and dissection

On day 4 after molar pulp injury, all animal groups were euthanized with anesthesia overdose and transcardially perfused through the left ventricle with cold saline solution (Ringer's sodium chloride - potassium chloride - calcium chloride) plus 50 IU heparin and 60 ml cold paraformaldehyde (Merck, Rahway, NJ, US) 4% (w/v) in 0.1 M phosphate buffer, pH 7.3-7.5. After euthanasia, animals were dissected and liver, hemimandibles and brainstem samples collected. Livers were removed and processed using routine histologic techniques. Paraplast (Sigma-Aldrich, Burlington, MA, US) sections of approximately 8-µm thickness were prepared and stained with hematoxylin and eosin (H&E). To detect glycogen deposition in hepatocytes some sections were processed through periodic acid-Schiff (PAS) histochemistry. The hemimandibles were decalcified in 10% EDTA/phosphate-buffered saline (PBS) 1X for 5 wk. and embedded in Paraplast. Mesiodistal longitudinal sections of approximately 8-µm thickness were obtained from the mandibular first molars, and sections were stained with H&E. After brainstem dissection, the right ventral side was marked with a blade to identify the contralateral side. Brainstems were cryoprotected in sucrose/PBS 30% (w/v), and frozen coronal sections (30 µm) from the C4 to -5.6 interaural level[22] were collected as floating sections into PBS/azide.

Immunofluorescence

Sections were incubated in blocking solution (3% normal sheep serum, Tris Buffer saline (TBS), 0.3% Triton X-100) for 2-4 hours at room temperature. At that time, sections were incubated for 48 hours at 4°C with combined primary antibodies: mouse monoclonal anti-5-hydroxytryptamine transporter (RRID Millipore Cat# MAB1564, RRID:AB_94220 1:1000), rabbit anti-calbindin D28K (1:5000; RRID Lot Nº 03, Swant Cat# CB38, RRID:AB_2721225) and rabbit anti-CB1r (1:3000; RRID Cayman Chemical Cat#

10006590, RRID:AB_10098690) in 1% normal sheep serum, 0.1% Triton X-100, TBS. Negative controls were performed by replacing primary antibodies with goat serum. After 40 min washing with TBS, rhodamine red (TRITC 1:2000 Jackson Immuno Research) or fluorescein isothiocyanate (FITC 1:2000 Jackson Immuno Research) conjugated secondary antibodies were used. Sections were incubated for 3 hours, and then washed with TBS for 30 min. Cell nuclei were labeled with the fluorescent DNA marker Hoechst 33342. Finally, sections were mounted on glass slides previously dipped in 1% gelatin, air dried and slides were coverslipped using TBS:glycerol (1:3) for UV microscopic observation.

Image analysis

Photomicrographs were acquired with a Zeiss Axiophot light microscope equipped with epifluorescence, alternating between FITC and rhodamine filter sets. Images were captured at 24-bit resolution (8 bpp × 3 channels) using an Olympus Q-color 5 camera. Histomorphometric analysis was performed on digital photomicrograph from histological sections using Image Pro Plus 6 software (Media Cybernetics, Silver Springs, MD, US). Image acquisition and analyses were performed by an observer blinded to group allocation. The following parameters were studied:

On livers: histological features on H&E stained and PAS histochemistry sections.

On left mandibular first molars: percentage of pulp inflammation volume per total cameral pulp volume (2D) (IV/PV_{2D}%) and histological features.

On dorsomedial area and two levels of ipsilateral Vc per animal:

I. 5HT-T plexus: determined as the total length of fibers (in μm) and quantification of 5HT-T varicosities (in number of varicosities) / area of 0.01 mm^2 in laminae I, II and III).
II. Calb+ 5HT-T: percentage of calbindin D28k positive neurons with 5HT-T varicosities around the soma / total calbindin D28k positive neurons per area of 0.01 mm^2 in laminae I, II and III, in %.
III. CB1r or Calbindin D28k positive cells: quantification of fusiform somas compatible with the morphology of projection neurons in LI and quantification of smaller somas compatible with modulatory interneurons in LII / area of 0.05 mm^2

Statistical analysis

Data are shown as means ± SEM. Data were statistically compared by Student t-test or two-way analyses of variance (ANOVA), followed by the post hoc Bonferroni test. Statistical differences were considered significant for p-values below 0.05. All statistical analyses were performed with Graph Pad Prism (version 5.03; GraphPad Software, Williston, VT, UD).

RESULTS

Analysis of hepatotoxicity

To evaluate hepatotoxicity associated with paracetamol, the livers of CG and EG2 were processed using routine histological techniques with H&E (Fig. 1A - C) and examined for glycogen deposits using PAS (Fig. 1D - F) staining. After paracetamol administration, the hepatic tissue and cell morphology showed normal histological appearance. Hepatocytes showed positive PAS staining for intracellular glycogen deposits. In the portal space, the morphology was preserved and exhibited no evidence of inflammatory infiltrate. Biochemical serum final transaminase levels (UI/L) exhibited no significant differences between EG2 and CG (final GPT EG2: 68.5 ± 12.0; CG: 78.5 ± 27.6; final GOT EG2: 292 ± 11.3; CG: 250 ± 19.8; $p>0.05$).

Analysis of left mandibular first molar in situ

In CG (Fig. 2 A), the molars showed normal histological features, with no signs of inflammatory process in the pulp or periodontal ligament. Sections of left mandibular first molar in EG1 and EG2 revealed an acute inflammatory infiltrate in the coronal pulp in direct contact with the sealing material 4 d post injury. Inflammatory reactions observed in both groups were similar in terms of the percentage of volume pulp inflammation in relation to the total volume cameral pulp (IV/PV_{2D}%) (EG1: 29% ± 2.92; EG2: 28% ± 2.41; $p>0.05$) and histological features (Fig. 2 B and C). This histopathological reaction may be compatible with a pulpitis diagnosis and the consequent nociceptive stimulation.

Analysis of 5HT-T expression in the Vc

Descending serotonergic fibers showed varicosities along their path and 5HT-T was expressed in these varicosities. 5HT-T analysis revealed that the descending fibers were distributed as a plexus

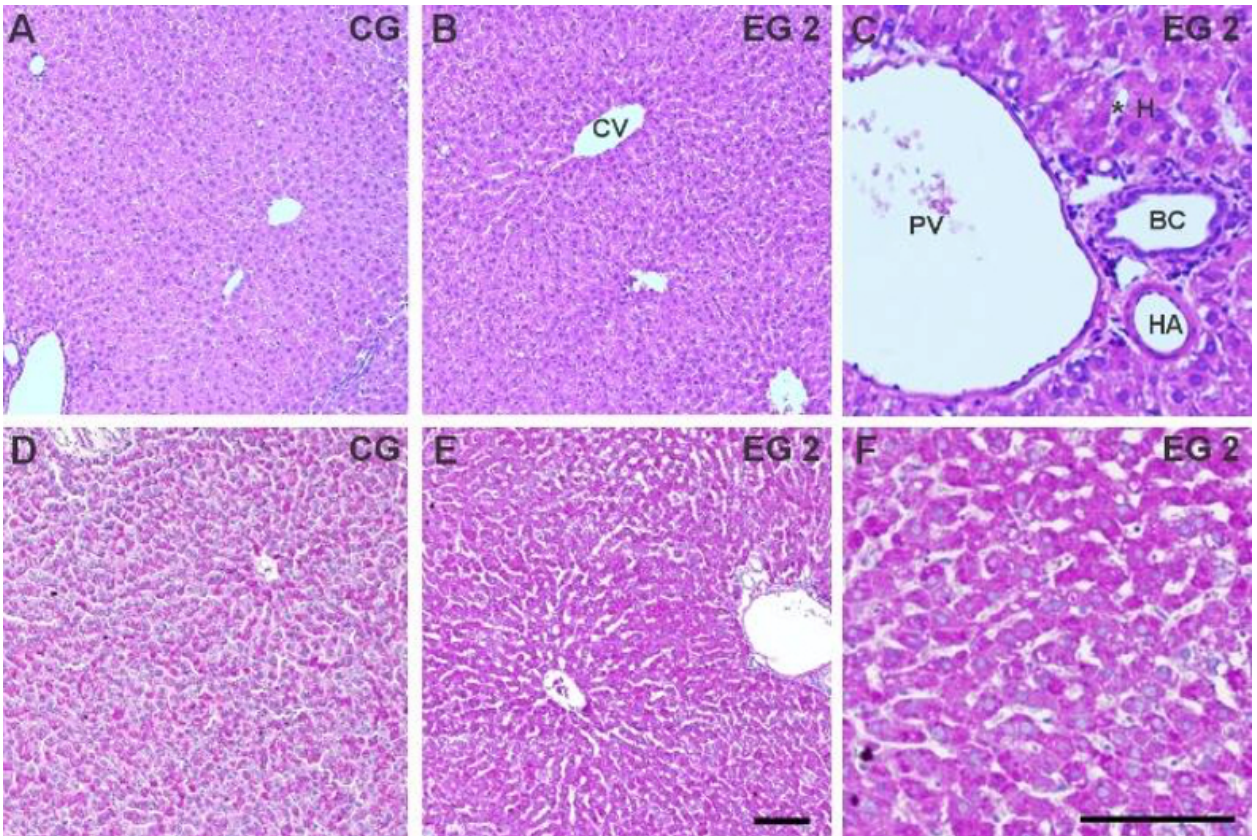

*Fig. 1: Microphotographs of the liver from representative animal specimens from CG (A and D) and EG2 (B, C, E and F) processed with H&E (A - C) and PAS (D - F). In both groups, the hepatic acinus and portal space display normal histological appearance (C) and hepatocytes retained intracellular glycogen deposits (F). HA: hepatic artery, PV: portal vein, BD: bile duct, CV: central vein, H: hepatocytes and *: sinusoidal capillaries and Kupffer cell. The scale bar represents 100 µm*

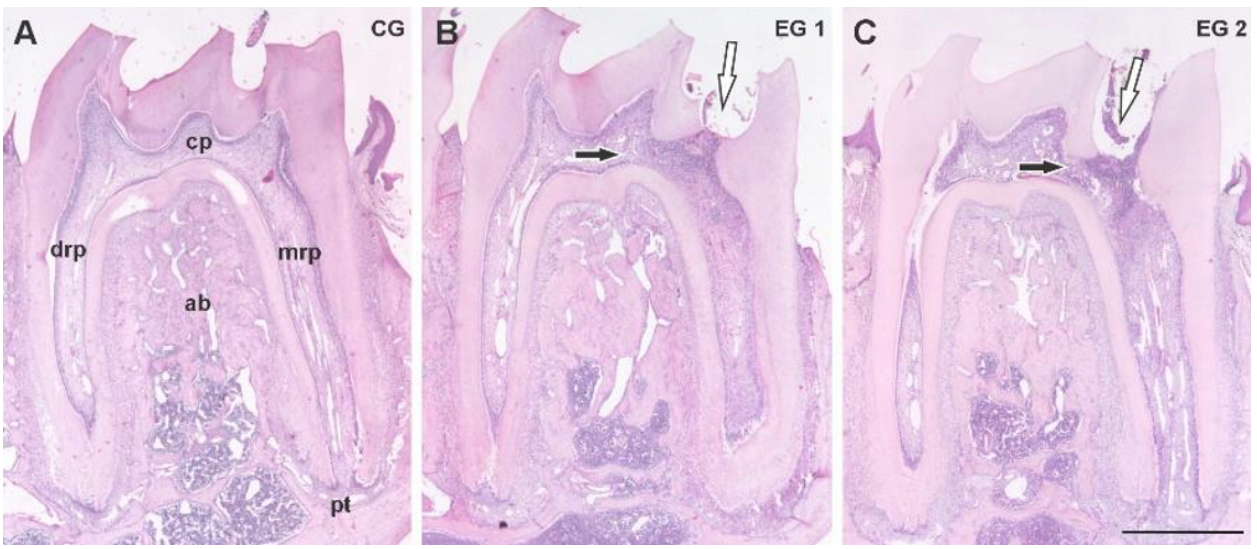

Fig. 2: Sections showing control (A) and experimental left mandibular first molar (B and C) in the alveolar bone (ab) stained with H&E. B and C show the mesial cavity (black arrow), and the extent of the inflammatory process (white arrow) in the cameral pulp (cp). The inflammatory reactions in EG1 (B) and EG2 (C) show numerous inflammatory cells, mainly lymphocytes and macrophages. Note that the mesial root pulp (mrp), distal root pulp (drp) and the periapical tissues (pt) appeared free of morphological signs of inflammation (A – C). The scale bar represents 1000 µm.

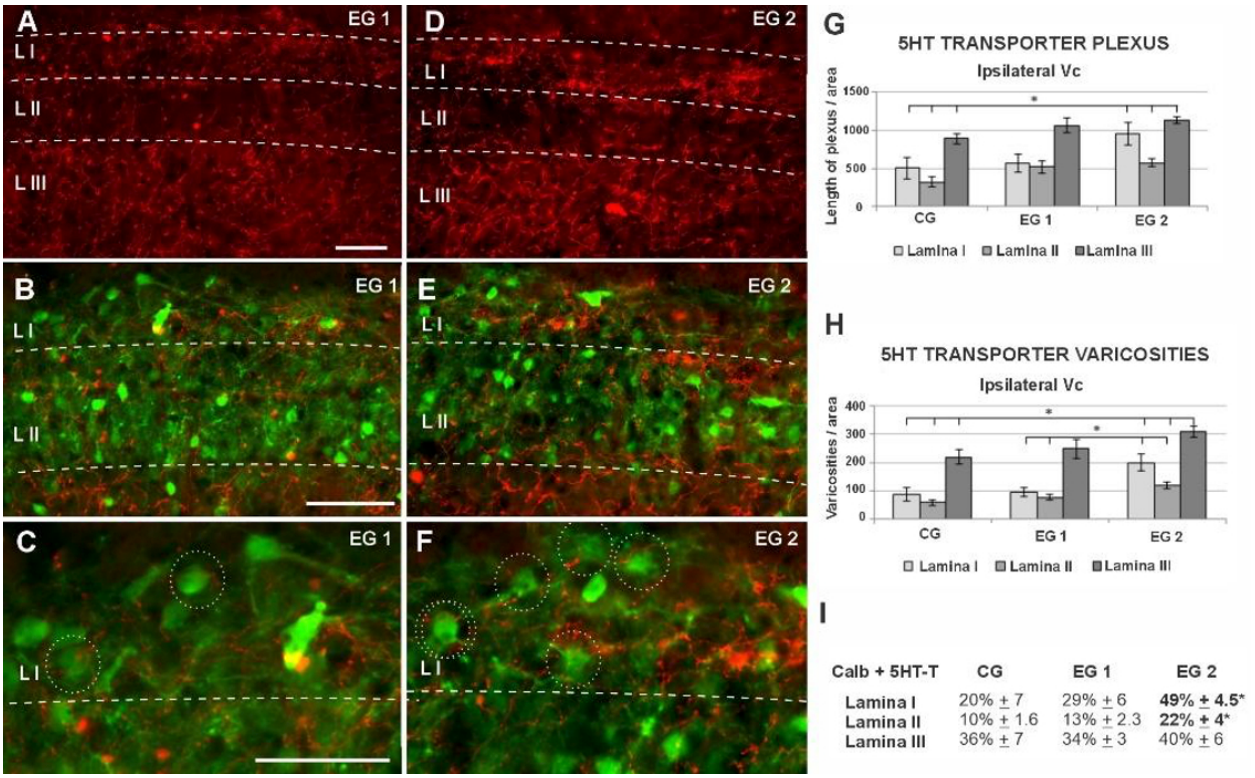

*Fig. 3: Analysis of the 5HT-T in the laminae of the ipsilateral Vc to molar pulpitis (A and D). The length of the plexus (G) and the quantification of its varicosities (H) in superficial laminae (LI and LII) and deeper lamina (LIII) are represented in the bar graphs for CG, EG1 and EG2. B-C and E-F show the co-expression of calbindin D28K (FITC) and 5HT-T varicosities (rhodamine). The table (I) presents the mean percentage + SEM of calbindin D28K neurons with 5HT-T varicosities around the soma for each lamina of the Vc in all groups. The scale bar represents 50 µm. * $p<0.05$*

in the Vc, primarily in LI and III, with a minimal presence in LII (Fig. 3 A and D). Comparisons of plexus length in each lamina of the ipsilateral Vc rendered significantly greater length in LI, II and III of EG2 compared to CG (Fig. 3 G). Paracetamol administration significantly increased the number of 5HT-T positive varicosities in LI (CG: 88.13 ± 24.40; EG1: 95.36 ± 14.47; EG2: 200.36 ± 28.99; $p<0.05$) and II (CG: 57.92 ± 10.78; EG1: 76.79 ± 9.43; EG2: 118.39 ± 12.48; $p<0.05$) (Fig. 3 A, D and H).

Furthermore, 5HT-T varicosities associated with calbindin D28K positive neurons were quantified. In LI, projection neurons showed a fusiform soma and two long primary extensions emerging from each end of the soma. LII showed smaller neurons compatible with the morphology of modulatory interneurons (Fig. 3 B-C, E-F). EG2 demonstrated a significant increase of varicosities associated with calbindin D28K positive somas in LI (CG: 20% ± 7; EG1: 29% ± 6; EG2: 49% ± 4.5; $p<0.05$) and LII (CG: 10% ± 1.6; EG1: 13% ± 2.3; EG2: 22% ± 4; $p<0.05$) (Fig. 3 I).

Analysis of CB1r expression in the Vc

CB1r expression was observed in the perinuclear cytoplasm and at the beginning of the neuronal processes in both projection neurons (P) and interneurons (M). Furthermore, the distribution of CB1r in nociceptive LI-II and V was studied (Fig. 4 A - C). Expression analysis of CB1r in the superficial laminae demonstrated that the number of projection neurons located in LI on the ipsilateral side was significantly greater in EG2 than in EG1 or CG (CG: 3.67 ± 0.33; EG1: 2.6 ± 0.24; EG2: 6.0 ± 0.58; $p< 0.05$). In LII, no significant changes were observed in CB1r expression (CG: 26 ± 3.06; EG1: 18 ± 2.10; EG2: 20 ± 2.16; $p > 0.05$) (Fig 4 D, E and H, I).

Analysis of calbindin D28K expression in the Vc

The number of calbindin D28K positive fusiform neurons per area of LI in the ipsilateral Vc was significantly larger in EG1 than in EG2 or CG at 4 d post-injury (CG: 3.3 ± 0.63; EG1: 4.29 ± 0.47; EG2:3.0 ± 0.19; $p<0.05$). In contrast, calbindin D28K expression in smaller modulatory interneurons of

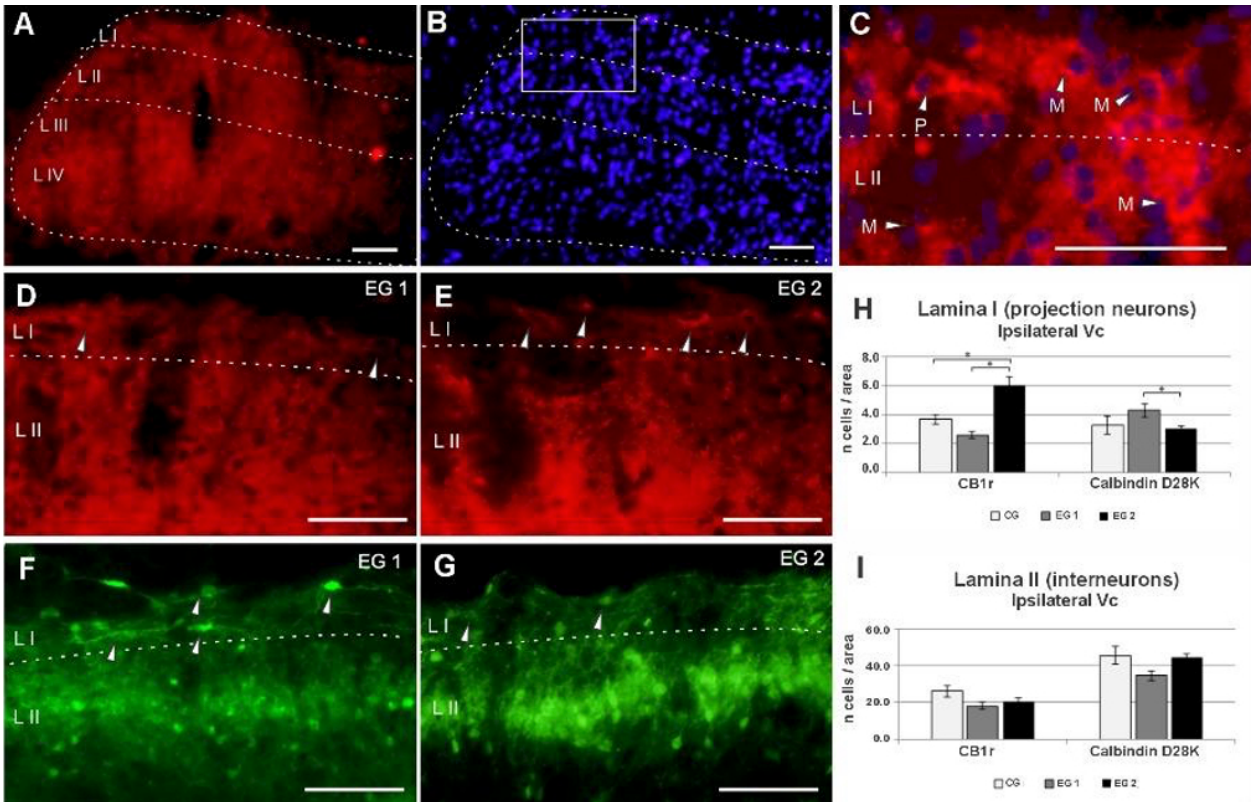

*Fig. 4: Coronal sections of the Vc using the immunofluorescence technique for CB1r (A) and fluorescent DNA marker Hoechst for cell nuclei labeling (B). Arrowhead in C indicate co-expression in projection (P) and modulatory (M) neurons LI and LII. Arrowheads indicate CB1r positive projection neurons (D and E) and calbindin D28K neurons (F and G) in the ipsilateral Vc of experimental groups. The bar graphs represent the number of positive projection neurons (G) and positive modulatory neurons (H) per area in LI or LII of each group. The scale bar represents 50 µm. * $p<0.05$*

LII showed non-significant differences (CG: 45.5 ± 6.19; EG1: 34.4 ± 10.34; EG2: 44.38 ± 7.85; $p > 0.05$) (Fig. 4 F - I).

DISCUSSION

Paracetamol is occasionally used to manage pain before and after dental procedures. In EG2, paracetamol was administrated orally starting 48 h after pulp injury to simulate delayed clinical analgesic intervention, once pulp inflammation (pulpitis) had developed. To maximize antinociceptive efficacy, the EG2 animals received three doses (300 mg/kg each) per day (every 4 h), resulting in a 12-hour drug-free interval. The dosing used in this study is higher than typical clinical doses in humans, however, such doses are commonly employed in experimental models[20,21] to ensure central pharmacological effects. Because 90–95% of paracetamol undergoes hepatic biotransformation and its toxicity increases with dose[12], we examined liver histology and serum transaminases in both control and paracetamol treated rats to check for possible liver toxicity. Importantly, no hepatotoxicity was detected, consistent with previous studies showing that toxicity depends on metabolic saturation rather than dose alone under controlled conditions[10,12,21]. Histological assessment showed preserved lobular architecture and portal tracts in paracetamol-treated animals. Hepatocytes contained cytoplasmic glycogen, and there were no signs of necrosis or inflammation. Serum transaminase levels in EG2 remained within CG ranges. These findings confirm that, under our dosing regimen, rats do not develop paracetamol-induced liver injury[10]. Nevertheless, caution is warranted when extrapolating these findings to clinical settings.

Different tooth injuries induce varying degrees of neuroplastic change at the trigeminal nucleus, depending on severity. In this study we used a type II intermediate molar tooth injury, which caused partial pulp loss, controlled infection with an inflammatory reaction, and occasionally necrosis of

the pulp, but did not spread to adjacent territories such as periapical tissues[23]. Pulp inflammation was induced in the left mandibular first molar by exposing the mesial pulp chamber, and sealing with restorative material was then performed to mimic what may happen in the dental clinic procedures. Histopathological examination of the exposed pulp confirmed the development of pulpitis. This inflammatory response generates nociceptive signals that are conveyed predominantly via Aδ and C primary afferent fibers originated from the trigeminal ganglion and carry nociceptive information to the Vc[4].

In recent years, we have studied neurons in LI, II, and V of the rat Vc and their modulatory role in the orofacial nociceptive pathway after pulpitis[7]. Our analysis focused on female rats, testing the hypothesis of sex-based differences in nociceptive thresholds. The literature indicates that these responses are highly genotype-dependent; for instance, while Sprague-Dawley and Long-Evans strains exhibit dimorphic sensitivity, Wistar-Kyoto rats often show no significant difference between sexes[24]. Consequently, the present study evaluates the potential antinociceptive effect of paracetamol in female Wistar rats. Within this framework, both EG1 and EG2 groups displayed a similar degree of pulp inflammation, indicating that paracetamol exerts a weak inhibitory effect on peripheral pulp inflammation—an observation consistent with its limited efficacy in other inflamed tissues[11,12]. In contrast, recent evidence indicates that the central analgesic effects of paracetamol are mediated by its metabolite AM404, which activates CB1 receptors and interacts with serotonergic pathways within central nociceptive circuits[14,15,25.] Nevertheless, how oral paracetamol modulates synaptic and cellular plasticity within the Vc in an odontogenic pulpitis model remains to be determined.

Paracetamol administration is associated with remodeling of the serotonergic pathways in the superficial dorsal laminae of the Vc. EG2 exhibited a significant increase in both the length of 5HT-T positive plexus and the density of 5HT-T–immunoreactive varicosities, indicative of enhanced serotonin reuptake capacity[16]. Co-localization analysis further revealed that these 5HT-T varicosities formed close appositions nearby calbindin-D28k–positive neurons. These findings suggest modulation of nociceptive processing rather than direct evidence of functional inhibition.

Moreover, the cannabinoid system modulates synaptic transmission within SNC[17]. Activation of CB1r in neurons in the trigeminal complex inhibits neurotransmitter release, leading to a decrease in nociceptive responses, or pain signaling[26]. Therefore, CB1r activation contributes to antinociception by blocking the progression of the pain pathway.

In line with Farquhar-Smith et al.[27], our immunohistochemical analysis revealed CB1r localization in the perinuclear cytoplasm of neurons within the superficial laminae of the Vc. EG2 showed a notable increase in CB1r immunoreactivity, specifically in ipsilateral LI projection neurons, that receive central projections from trigeminal primary afferent neurons innervating the mandibular division. Animal studies have demonstrated that calbindin D28K, a calcium-binding protein, is expressed by distinct neuronal populations in the Vc, suggesting different functional properties among these neurons. Small multipolar calbindin-positive neurons in LI and II modulate nociceptive input, whereas fusiform calbindin-positive neurons in LI constitute ascending projection cells targeting the medial thalamus and other pain-related subnuclei[5,28]. Multiple Ca^{2+} channels and Ca^{2+} influx are involved in the glutamatergic synaptic transmission[29]. Consistent with our preceding study[7], enhanced calbindin D28K expression in LI fusiform neurons, supports the activation of the ascending orofacial nociceptive pathway after pulpitis. In contrast, the present study demonstrates that oral paracetamol administration produces a significant reduction in the number of calbindin D28K–positive projection neurons in LI of the Vc. This decrease likely reflects enhanced presynaptic inhibition mediated by the concomitant increase of 5HT-T and CB1r expression in EG2, resulting in reduced calcium-dependent neurotransmitter release. In agreement with these findings, previous studies of excitatory synaptic transmission have indicated that presynaptic action of CB1r and 5HTr reduce directly or indirectly Ca^{2+} influx and glutamate release[29,30].

CONCLUSIONS

In conclusion, our findings indicate that after experimental activation of dental pulp nociceptors (pulpitis), oral administration of paracetamol produces plastic changes that can be observed at the level of the serotonergic and endocannabinoid

systems of the Vc. This plasticity is expressed as a lower number of fusiform calbindin D28K projection neurons in lamina I. The reduction in the number of calbindin D28K-positive neurons in lamina I may reflect adaptive neuroplasticity associated with the modulation of orofacial nociceptive processing.

From a clinical perspective, these findings support the concept that paracetamol may exert part of its analgesic effect through central modulation of trigeminal nociceptive circuits, which is particularly relevant in odontogenic pain conditions such as pulpitis.

A major limitation of this study is the absence of behavioral nociceptive assessment. Further research including behavioral and electrophysiological approaches, as well as co-expression analyses of fusiform neurons with glutamate, CB1r or 5HT receptor subtypes, are needed to validate the functional relevance of these findings. Elucidating these mechanisms may contribute to optimizing the central antinociceptive effects of paracetamol and analgesic strategies in dental practice.

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CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship and/or publication of this article.

REFERENCES

1. Ito S, Okuda-Ashitaka E, Minami T. 2001 Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res. 2001 Dec;41(4):299-332. https://doi.org/10.1016/S0168-0102(01)00289-9. PMID: 11755218.
2. Sato T, Kitagawa J, Ren K, Tanaka H, Tanabe A, Watanabe T, Mitsuhashi Y, Iwata K. Activation of trigeminal intranuclear pathway in rats with temporomandibular joint inflammation. J Oral Sci. 2005 Jun;47(2):65-9. https://doi.org/10.2334/josnusd.47.65. PMID: 16050485.
3. Khan A, Hargreaves KM. Animal models of orofacial pain. Methods Mol Biol. 2010;617:93-104. https://doi.org/10.1007/978-1-60327-323-7_8. PMID: 20336416
4. Sessle BJ. Peripheral and central mechanisms of orofacial inflammatory pain. Int Rev Neurobiol. 2011; 97:179-206. https://doi.org/10.1016/B978-0-12-385198-7.00007-2. PMID: 21708311
5. Gamboa-Esteves FO, Kaye JC, McWilliam PN, Lima D, Batten TF. Immunohistochemical profiles of spinal lamina I neurones retrogradely labelled from the nucleus tractus solitarii in rat suggest excitatory projections. Neuroscience. 2001;104(2):523-38. https://doi.org/10.1016/S0306-4522(01)00071-9. PMID: 11377852.
6. Mody I. Aspects of the homeostaic plasticity of GABAA receptor-mediated inhibition. J Physiol. 2005 Jan 1;562(Pt 1):37-46. https://doi.org/10.1113/jphysiol.2004.077362. Epub 2004 Nov 4. PMID: 15528237; PMCID: PMC1665492.
7. Canzobre MC, Paganelli AR, Ríos H. Effect of periapical inflammation on calcium binding proteins and ERK in the trigeminal nucleus. Acta Odontol Latinoam. 2019 Aug 1;32(2):103-110. English. PMID: 31664301
8. Millan MJ. Descending control of pain. Prog Neurobiol. 2002 Apr;66(6):355-474. https://doi.org/10.1016/S0301-0082(02)00009-6. PMID: 12034378.
9. Sommer C. Serotonin in pain and analgesia: actions in the periphery. Mol Neurobiol. 2004 Oct;30(2):117-25. https://doi.org/10.1385/MN:30:2:117. PMID: 15475622.
10. McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicol Appl Pharmacol. 2012 Nov 1;264(3):387-94. https://doi.org/10.1016/j.taap.2012.08.015. Epub 2012 Aug 23. PMID: 22980195; PMCID: PMC3478469.
11. Alloui A, Chassaing C, Schmidt J, Ardid D, Dubray C, Cloarec A, Eschalier A. Paracetamol exerts a spinal, tropisetron-reversible, antinociceptive effect in an inflammatory pain model in rats. Eur J Pharmacol. 2002 May 17;443(1-3):71-7. https://doi.org/10.1016/S0014-2999(02)01578-9. PMID: 12044794.
12. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology. 2013 Jun;21(3):201-32. https://doi.org/10.1007/s10787-013-0172-x. Epub 2013 May 30. PMID: 23719833
13. Klinger-Gratz PP, Ralvenius WT, Neumann E, Kato A, Nyilas R, Lele Z, Katona I, Zeilhofer HU. Acetaminophen Relieves Inflammatory Pain through CB1 Cannabinoid Receptors in the Rostral Ventromedial Medulla. J Neurosci. 2018 Jan 10;38(2):322-334. https://doi.org/10.1523/JNEUROSCI.1945-17.2017. Epub 2017 Nov 22. PMID: 29167401; PMCID: PMC6596108.
14. Ohashi N, Kohno T. Analgesic Effect of Acetaminophen: A Review of Known and Novel Mechanisms of Action. Front Pharmacol. 2020 Nov 30;11:580289. https://doi.org/10.3389/fphar.2020.580289. PMID: 33328986; PMCID: PMC7734311.

15. Mallet C, Desmeules J, Pegahi R, Eschalier A. An Updated Review on the Metabolite (AM404)-Mediated Central Mechanism of Action of Paracetamol (Acetaminophen): Experimental Evidence and Potential Clinical Impact. J Pain Res. 2023 Mar 29;16:1081-1094. https://doi.org/10.2147/JPR.S393809. PMID: 37016715; PMCID: PMC10066900.
16. Hains BC, Everhart AW, Fullwood SD, Hulsebosch CE. Changes in serotonin, serotonin transporter expression and serotonin denervation supersensitivity: involvement in chronic central pain after spinal hemisection in the rat. Exp Neurol. 2002 Jun;175(2):347-62. https://doi.org/10.1006/exnr.2002.7892. PMID: 12061865.
17. Xu JY, Chen C. Endocannabinoids in synaptic plasticity and neuroprotection. Neuroscientist. 2015 Apr;21(2):152-68. https://doi.org/10.1177/1073858414524632. Epub 2014 Feb 25. PMID: 24571856; PMCID: PMC4143501.
18. Lutz B. 2020. Neurobiology of cannabinoid receptor signaling. Dialogues Clin Neurosci. 2020 Sep;22(3):207-222. https://doi.org/10.31887/DCNS.2020.22.3/blutz. PMID: 33162764; PMCID: PMC7605026
19. Topuz RD, Gündüz Ö, Karadağ ÇH, Ulugöl A. Non-opioid Analgesics and the Endocannabinoid System. Balkan Med J. 2020 Oct 23;37(6):309-315. Epub 2020 Jun 19. PMID: 32551466; PMCID: PMC7590544. https://doi.org/10.4274/balkanmedj.galenos.2020.2020.6.66
20. Bonnefont J, Alloui A, Chapuy E, Clottes E, Eschalier A. Orally administered paracetamol does not act locally in the rat formalin test: evidence for a supraspinal, serotonin-dependent antinociceptive mechanism. Anesthesiology. 2003 Oct;99(4):976-81. https://doi.org/10.1097/00000542-200310000-00034. PMID: 14508334.
21. Hoshijima H, Hunt M, Nagasaka H, Yaksh T. Systematic Review of Systemic and Neuraxial Effects of Acetaminophen in Preclinical Models of Nociceptive Processing. J Pain Res. 2021 Nov 12;14:3521-3552. https://doi.org/10.2147/JPR.S308028. PMID: 34795520; PMCID: PMC8594782
22. Paxinos,G. and Watson,C. The Rat Brain in Stereotaxic Coordinates, Compact third edition. San Diego, CA, USA: Academic Press; 1997.
23. Byers MR, Närhi MV. Dental injury models: experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. Crit Rev Oral Biol Med. 1999;10(1):4-39. https://doi.org/10.1177/10454411990100010101. PMID: 10759425.
24. Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF. Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. Neurosci Biobehav Rev. 2000 May;24(3):375-89. https://doi.org/10.1016/S0149-7634(00)00015-4. PMID: 10781697.
25. Courade JP, Caussade F, Martin K, Besse D, Delchambre C, Hanoun N, Hamon M, Eschalier A, Cloarec A. Effects of acetaminophen on monoaminergic systems in the rat central nervous system. Naunyn Schmiedebergs Arch Pharmacol. 2001 Dec;364(6):534-7. https://doi.org/10.1007/s002100100484. PMID: 11770008.
26. Akerman S, Holland PR, Goadsby PJ. Cannabinoid (CB1) receptor activation inhibits trigeminovascular neurons. J Pharmacol Exp Ther. 2007 Jan;320(1):64-71. https://doi.org/10.1124/jpet.106.106971. Epub 2006 Oct 3. PMID: 17018694.
27. Farquhar-Smith WP, Egertová M, Bradbury EJ, McMahon SB, Rice AS, Elphick MR. Cannabinoid CB(1) receptor expression in rat spinal cord. Mol Cell Neurosci. 2000 Jun;15(6):510-21. https://doi.org/10.1006/mcne.2000.0844. PMID: 10860578
28. Egea J, Malmierca E, Rosa AO, del Barrio L, Negredo P, Nuñez A, López MG. Participation of calbindin-D28K in nociception: results from calbindin-D28K knockout mice. Pflugers Arch. 2012 Mar;463(3):449-58. https://doi.org/10.1007/s00424-011-1063-x. Epub 2011 Dec 3. PMID: 22134771.
29. Godino Mdel C, Torres M, Sánchez-Prieto J. CB1 receptors diminish both Ca(2+) influx and glutamate release through two different mechanisms active in distinct populations of cerebrocortical nerve terminals. J Neurochem. 2007 Jun;101(6):1471-82. https://doi.org/10.1111/j.1471-4159.2006.04422.x. Epub 2007 Feb 5. PMID: 17286592.
30. Nishijo T, Suzuki E, Momiyama T. Serotonin 5-HT1A and 5-HT1B receptor-mediated inhibition of glutamatergic transmission onto rat basal forebrain cholinergic neurones. J Physiol. 2022 Jul;600(13):3149-3167. https://doi.org/10.1113/JP282509. Epub 2022 Jun 8. PMID: 35598305.