Biochemical and Biophysical Research Communications 744 (2025) 151178

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hormonal mechanisms in the paraventricular nuclei associated with hyperalgesia in Parkinson's disease model rats

Shengsen Yang [a,*], Nayuka Usami [a], Shiou−Ling Lu [b], Wakana Oda [a], Hiroharu Maegawa [a], Hitoshi Niwa [a], Chiho Kudo [a]

[a] Department of Dental Anesthesiology, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka, 565-0871, Japan
[b] Department of Oral Cellular Biology, Center for Frontier Oral Science, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka, 565-0871, Japan

A B S T R A C T

Pain is a major non-motor symptom of Parkinson's disease (PD). The relationship between hyperalgesia and neuropeptides originating from paraventricular nucleus (PVN) in 6-hydroxydopamine (6-OHDA) rats has already been investigated for oxytocin (OXT), but not yet for arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH). The present study aimed to investigate the alterations in these neuropeptides following nociceptive stimulation in PD model rats and to examine the mechanisms of hyperalgesia. Dopaminergic nigrostriatal lesions were induced by injecting 6-OHDA into the medial forebrain bundle. Subcutaneous formalin injection into the vibrissa pad was performed in rats as a nociceptive stimulus in the orofacial region. Dopamine depletion's effect on nociception was assessed by counting the p-ERK-immunoreactive (-IR) cells in the trigeminal spinal subnucleus caudalis (Vc). The PD model rats induced by 6-OHDA injection (6-OHDA rats) showed a significantly higher number of p-ERK-IR cells in the Vc than the sham rats, confirming hyperalgesia in 6-OHDA rats. Then, we investigated the immunohistochemical responses to OXT, AVP, and CRH cells in the PVN and examined the changes in blood levels of these neuropeptides. As a result, formalin injection increased neuronal activity and blood levels of OXT and CRH in sham rats, but these were suppressed in the 6-OHDA rats. Contrarily, neuronal activity and blood level of AVP were unaffected by nociceptive stimuli and were significantly lower in 6-OHDA rats than in sham rats. Our findings suggest that OXT and CRH suppression is linked to hyperalgesia in PD, whereas AVP does not directly influence the observed hyperalgesia.

1. Introduction

Hyperalgesia is a common non-motor symptom in patients with Parkinson's disease (PD) [1–6]. Recent studies have shown that hyperalgesia in PD patients is mainly associated with pathological changes in the central nervous system, including the loss of dopaminergic neurons and alterations in the pain modulation pathways [4,7]. More than half of PD patients report chronic pain, including spontaneous pain and hyperalgesia [1,2,4,6], which severely impact their quality of life and are often clinically overlooked, leading to inadequate management and treatment [2,8].

PD model rats induced by 6-hydroxydopamine (6-OHDA) injections into the medial forebrain bundle (MFB) reportedly cause hyperalgesia [3–6]. The altered neuronal activity in the paraventricular nucleus (PVN) is reported to be associated with hyperalgesia in PD model rats induced by 6-OHDA injection (6-OHDA rats) [4,9]. We have previously demonstrated that the subcutaneous (SC) injection of formalin into the vibrissa pad of 6-OHDA rats increased their face rubbing behavior and the number of *c*-Fos-immunoreactive (-IR) cells in the trigeminal spinal subnucleus caudalis (Vc), indicating hyperalgesia [4–6]. Additionally, the SC injection of formalin into the vibrissa pad increased the neuronal activity of oxytocin (OXT) in the PVN and blood OXT levels in sham rats, but these were suppressed in 6-OHDA rats [4]. We also reported that the intracisternal administration of OXT alleviated hyperalgesia in 6-OHDA rats, suggesting that the observed hyperalgesia could be caused by the suppression of the analgesic effect of OXT originating from the PVN [4,5].

The PVN contains neurosecretory cells that produce OXT, arginine vasopressin (AVP), and corticotropin-releasing hormone (CRH), which are neuropeptides known to have analgesic properties [10,11]. The relationship between hyperalgesia and OXT originating from the PVN in 6-OHDA rats has already been investigated [4], but those for AVP and CRH have not been examined yet [12,13]. Thus, the present study aimed to investigate the immunohistochemical responses to OXT, AVP, and CRH cells in the PVN and the changes in the serum levels of these neuropeptides following nociceptive stimulation in PD model rats and to

* Corresponding author.
E-mail addresses: shengsenyang@gmail.com (S. Yang), usami.nayuka.dent@osaka-u.ac.jp (N. Usami), lu.shiouling.dent@osaka-u.ac.jp (S. Lu), oda.wakana.dent@osaka-u.ac.jp (W. Oda), maegawa.hiroharu.dent@osaka-u.ac.jp (H. Maegawa), niwa.hitoshi.dent@osaka-u.ac.jp (H. Niwa), kudo.chiho.dent@osaka-u.ac.jp (C. Kudo).

https://doi.org/10.1016/j.bbrc.2024.151178
Received 3 November 2024; Received in revised form 14 November 2024; Accepted 11 December 2024
Available online 12 December 2024

examine the mechanisms underlying hyperalgesia in PD.

2. Materials and methods

2.1. Animals

Altogether, 24 male Wistar rats (Japan SLC, Inc., Shizuoka, Japan; weight: 150–200 g) were used in the present study. The rats were housed under a controlled environment with a 12-h dark/light cycle, with unrestricted access to food and water. The study was approved by the Osaka University Graduate School of Dentistry Animal Care and Use Committee, and all experimental procedures adhered to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Induction of 6-OHDA lesions

The procedures for inducing 6-OHDA lesions in rats have been described previously [4–6]. Briefly, the rats were anesthetized by the intraperitoneal (IP) administration of a saline solution containing midazolam (2.0 mg/kg, Sandoz, Tokyo, Japan), medetomidine (0.375 mg/kg, Zenoaq, Fukushima, Japan), and butorphanol (2.5 mg/kg, Meiji Seika Pharma, Tokyo, Japan). Unilateral nigrostriatal lesions were introduced by administering 6-OHDA (Sigma, St. Louis, MO, USA; 15 μg in 5 μl of sterile saline containing 0.01 % ascorbic acid) into their left MFB (henceforth referred to as the ipsilateral side). The sham rats received saline injections into the left MFB.

2.3. Rotational behavior test

At 2 weeks after 6-OHDA injection into the MFB (MFB injection), the rats were placed in a cylindrical container (300 mm in diameter), and IP administration of methamphetamine (3 mg/kg, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) was performed to trigger a rotational behavior. The animals exhibiting $\geq$5 turns/minute were referred to as 6-OHDA rats [14].

2.4. Formalin SC injection

The rats were divided randomly into two groups as follows: formalin- and saline-injected groups. At 3 weeks after the MFB injection, the rats were anesthetized using IP sodium pentobarbital (50 mg/kg) and received a 50-μl SC injection of 4 % formalin or saline into the vibrissa pad on the ipsilateral side [4–6]. At 5 min after the SC injection, under deep anesthesia with the IP injection of sodium pentobarbital, 3.0 ml of blood was collected from the hearts of the rats, then they were perfused.

2.5. Immunohistochemistry

Intracardial perfusion was performed using 150-ml of 0.02 M phosphate-buffered saline (PBS, pH 7.4) followed by 500-ml 4 % (w/v) paraformaldehyde in PBS. The brains were then post-fixed at 4 °C for 24 h and afterwards transferred to 30 % sucrose in 0.01-M PBS at 4 °C for 48 h. Serial coronal sections were subsequently prepared at a 40-μm thickness using a freezing microtome.

Immunoreactivity analyses for tyrosine hydroxylase (TH) in the striatum and substantia nigra and for p-ERK in the Vc were performed [4–6]. The sections were incubated for 20 min with 0.3 % hydrogen peroxide in methanol, followed by rising in PBS. The sections were blocked for 30 min at room temperature (RT) with 1 % normal horse serum (NHS, Vector Labs, Burlingame, CA, USA) and 0.1 % Triton X-100 in PBS. The sections were then incubated overnight at 4 °C with specific primary antibodies, 1 % NHS and 0.1 % Triton X-100 in PBS; mouse anti-TH antibody (1:8000 dilution, T2928, Sigma-Aldrich, St. Louise, MO, USA) or mouse anti-p-ERK antibody (1:500 dilution, sc-136,521, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA). After the primary antibodies incubations, the sections were rinsed in PBS and incubated for 1 h at RT with a biotinylated horse anti-mouse antibody (1:200 dilution, Vector Labs, Burlingame, CA, USA). The sections were then rinsed in PBS and incubated for 1 h with an avidin–biotin-peroxidase complex (1:200, ABC Elite kit, Vector Labs). The sections were finally treated with the DAB Substrate kit (Sigma-Aldrich), mounted on glass slides, air-dried, dehydrated, and cover-slipped.

2.6. Double immunofluorescence

Double immunofluorescent labeling for p-ERK/OXT, p-ERK/AVP, and p-ERK/CRH in the sections containing the PVN was achieved by incubating the sections with specific primary antibodies [4]. The sections were blocked for 1 h at RT with 10 % NGS and 0.3 % Triton X-100 in PBS. The sections were then incubated overnight at 4 °C with three pattern of primary antibodies, 2 % NGS and 0.3 % Triton X-100 in PBS; a mixture of rabbit anti-OXT antibody (1:1000, MAB5296, Merck Millipore, Burlington, MA, USA) and mouse anti-p-ERK antibody (1:200 dilution, sc-136,521, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) for the first pattern; a mixture of rabbit anti-AVP antibody (1:1000, AB1565, Merck Millipore) and mouse anti-p-ERK antibody (1:200 dilution, sc-136,521) for the second pattern; a mixture of rabbit anti-CRH antibody (1:500, C5348, Sigma-Aldrich) and mouse anti-p-ERK antibody (1:200 dilution, sc-136,521) for the third pattern. The sections were then rinsed in PBS and incubated for 2 h at RT with secondary antibodies, which are a mixture of goat anti-rabbit Alexa Fluor 488 (1:200, Invitrogen, Carlsbad, CA, USA) and goat anti-mouse Alexa Fluor 568 (1:200, Invitrogen) and 2 % NGS in PBS. The sections were finally rinsed in PBS, mounted on glass slides and coverslipped. Negative control sections confirmed the specificity of staining, as omission of primary antibodies resulted in no detectable labeling.

2.7. Cell counting

Anatomical locations within the Vc and PVN were identified based on the Paxinos and Watson atlas [15]. Images were acquired using a light microscope (BX51, Olympus, Tokyo, Japan) or a confocal laser microscope (TCS SP8, Leica Microsystems) at 10× magnification. In each rat, cell counts were performed on five sections in the Vc and three sections in the PVN, following established methods [4–6]. The cell number was counted by one examiner who was blinded to the experimental procedures.

To quantify the proportion of OXT, AVP, and CRH neurons activated by the SC injection of formalin into the vibrissa pad, the percentage of OXT-IR, AVP-IR, and CRH-IR neurons expressing p-ERK protein in the PVN was calculated using the following formula [4]:

$$\left(\frac{\text{number of OXT-, AVP-, or CRH-IR neurons co-localized with p-ERK protein}}{\text{number of OXT-, AVP-, or CRH-IR neurons}}\right) \times 100\ (\%)$$

2.8. Serum concentration of OXT, AVP, and CRH

Experiments were conducted between 9 a.m. and 3 p.m. to control for circadian rhythm effects. The serum samples were separated through centrifugation (KITMAN-18, TOMY DIGITAL BIOLOGY Co. Ltd., Tokyo, Japan) at 4 °C and 1600×*g* for 15 min and stored in aliquots at −70 °C until analysis. The serum OXT, AVP, and CRH levels were quantified using specific enzyme immunoassay kit (OXT: ADI-900-153A-0001, Enzo Life Science Inc., NY, USA, AVP: ADI-900-017 A, Enzo Life Science Inc., CRH: CEA835RA, CLOUD-CLONE CORP., TX, USA) [4]. Luminescence counts were measured using the iMark™ Microplate Absorbance Spectrophotometer (Bio-Rad Laboratories Inc., CA, USA).

2.9. Statistical analysis

Data are expressed as mean ± standard error. An unpaired *t*-test was used to compare the difference in the immunohistochemical data after formalin injection between the 6-OHDA and sham rats, while other results were analyzed using two-way analysis of variance followed by Bonferroni's test for multiple comparisons where relevant. All statistical analyses were conducted with the statistical software, SPSS (IBM, Statistics ver. 24, IL, USA), and statistical significance was defined as $p\text{-value} < 0.05$.

3. Results

3.1. TH immunoreactivity

TH immunohistochemical staining was performed on all rats to confirm dopamine depletion by unilateral 6-OHDA administration into the MFB. The sham rats exhibited high TH immunoreactivity in the striatum and substantia nigra on both sides [Fig. 1A (a and b)]. Contrarily, the 6-OHDA rats showed a significant decrease in TH immunoreactivity in the striatum and substantia nigra ipsilateral to 6-OHDA administration [Fig. 1A (c and d)].

3.2. p-ERK expression in the Vc

Immunohistochemical staining for p-ERK in the Vc (Fig. 1B) was performed, and the number of p-ERK-IR cells in the superficial layers of the Vc was counted. In the saline-injected group, the 6-OHDA and sham rats showed no significant differences in the number of p-ERK-IR cells in the Vc. However, in the formalin-injected group, the 6-OHDA rats exhibited significant increase in the number of p-ERK-IR cells in the Vc as compared to the sham rats (30.3 ± 1.8 vs. 21.3 ± 2.7, $p\text{-value} < 0.05$, Fig. 1C).

3.3. Co-expression of p-ERK and OXT in the PVN

Fluorescent double staining of OXT-IR and p-ERK-IR cells in the PVN was performed (Fig. 2A, p-ERK-IR cells: a-d, OXT-IR cells: e-h, merged images: i-l). In the sham rats, the percentage of OXT-IR neurons co-localized with p-ERK protein in the PVN was significantly higher in the formalin-injected group than in the saline-injected group [F (1,20) = 19.717, p-value <0.01; Fig. 2B], whereas, in the 6-OHDA rats, no significant difference was observed between the two groups (40.85 ± 3.75 vs. 42.37 ± 1.99; Fig. 2B). Moreover, in the formalin-injected group, the percentage of OXT-IR neurons co-localized with p-ERK protein in the PVN was significantly lower in the 6-OHDA rats than in the sham rats [F (1,20) = 17.197, $p\text{-value} < 0.01$; Fig. 2B].

3.4. Co-expression of p-ERK and CRH in the PVN

Fluorescent double staining of CRH-IR and p-ERK-IR cells in the PVN was performed (Fig. 2C, p-ERK-IR cells: a-d, CRH-IR cells: e-h, merged images: i-l). In the sham rats, the percentage of CRH-IR neurons co-localized with p-ERK protein in the PVN was significantly higher in the formalin-injected group than in the saline-injected group [F (1,20) = 14.263, $p\text{-value} < 0.01$; Fig. 2D], whereas, in the 6-OHDA rats, no significant difference was observed between saline- and formalin-injected groups (44.53 ± 3.21 vs. 41.97 ± 3.00; Fig. 2D). In the formalin-injected group, the percentage of CRH-IR neurons co-localized with p-ERK protein in the PVN was significantly lower in the 6-OHDA rats than in the sham rats [F (1,20) = 24.08, $p\text{-value} < 0.01$; Fig. 2D].

3.5. Co-expression of p-ERK and AVP in the PVN

Fluorescent double staining of AVP-IR and p-ERK-IR cells in the PVN was performed (Fig. 3A, p-ERK-IR cells: a-d, AVP-IR cells: e-h, merged images: i-l). In both the saline- and formalin-injected groups, the co-localization rate was significantly lower in the 6-OHDA rats than in the sham rats [saline-injected group: F (1,20) = 109.29, $p\text{-value} < 0.01$; formalin-injected group: F (1,20) = 79.99, $p\text{-value} < 0.01$; Fig. 3B].

3.6. Effect of SC formalin injection on serum OXT concentration

In the sham rats, the serum OXT concentration was significantly higher in the formalin-injected group than in the saline-injected group [F (1,20) = 6.57, $p\text{-value} < 0.05$, Fig. 4A]. Conversely, in the 6-OHDA rats, no significant difference in serum OXT concentration was observed between the saline- and formalin-injected groups (2040.26 ± 78.88 vs. 2631.76 ± 155.13, Fig. 4A). Moreover, in the formalin-injected group, the serum OXT concentration was lower in the 6-OHDA rats than in the sham rats [F (1,20) = 14.077, $p\text{-value} < 0.01$; Fig. 4A].

3.7. Effect of SC formalin injection on serum CRH concentration

In the sham rats, the serum CRH concentration was significantly higher in the formalin-injected group than in the saline-injected group [F (1,20) = 19.266, $p\text{-value} < 0.01$, Fig. 4B]. Conversely, in the 6-OHDA rats, no significant difference in serum CRH concentration was observed between the saline- and formalin-injected groups (95.66 ± 3.34 vs.88.89 ± 4.56, Fig. 4B). In the formalin-injected group, the serum CRH concentration was significantly lower in the 6-OHDA rats than in the sham rats [F (1,20) = 21.24, $p\text{-value} < 0.01$, Fig. 4B].

3.8. Effect of SC formalin injection on serum AVP concentration

In both the saline- and formalin-injected groups, the serum AVP concentration was significantly lower in the 6-OHDA rats than in the sham rats [saline-injected group: F (1,20) = 14.6, $p\text{-value} < 0.01$; formalin-injected group: F (1,20) = 14.61, $p\text{-value} < 0.01$, Fig. 4C]. In both the sham and 6-OHDA rats, no significant difference was observed between the saline- and formalin-injected groups [sham rats: (839.66 ± 35.76 vs. 798.07 ± 35.42; 6-OHDA rats: 672.43 ± 26.41 vs. 630.79 ± 24.49; Fig. 4C).

4. Discussion

OXT, AVP, and CRH are the neuropeptides known to be synthesized in the PVN [10,11,16]. Both OXT and CRH exhibit analgesic effects and are known to be enhanced by neuronal activity; their synthesis and secretion are also promoted by nociceptive stimuli [4,17]. In the present study, nociceptive stimuli enhanced the neuronal activity of producing cells and increased the serum OXT and CRH levels in sham rats. Contrarily, both the enhancement in neuronal activity and the increase in the serum concentrations of OXT and CRH were suppressed in 6-OHDA rats. This suggests that the synthesis and secretion of OXT and CRH promoted by nociceptive stimuli are suppressed in 6-OHDA rats, potentially leading to reduced analgesic effects of OXT and CRH and resulting in hyperalgesia. However, there were no changes in AVP serum

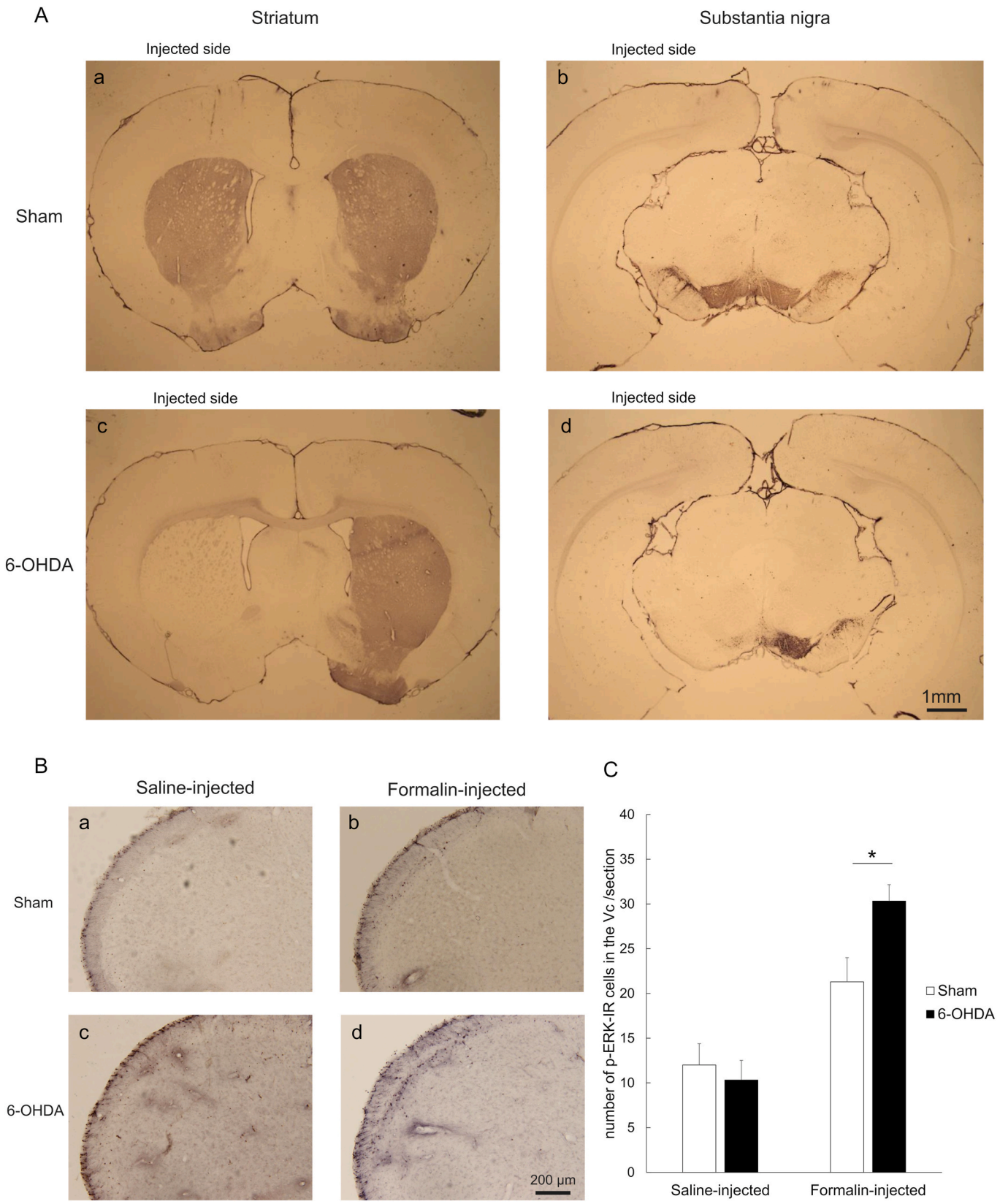

Fig. 1. Effect of 6-hydroxydopamine (6-OHDA) injection and response to formalin injection into the vibrissa pad. (A) No alterations in TH immunoreactivity were detected on the saline-injected side within the (a) striatum and (b) substantia nigra. However, a reduction in TH immunoreactivity was noted in the (c) striatum and (d) substantia nigra on the 6-OHDA-injected side. (B) Photomicrographs of the immunohistochemical staining of p-ERK in the trigeminal spinal subnucleus caudalis in the (a, c) saline-injected and (b, d) formalin-injected groups. (C) In the formalin-injected group, the number of p-ERK-immunoreactive (-IR) cells was significantly higher in the 6-OHDA rats than in the sham rats. *p-value < 0.05.

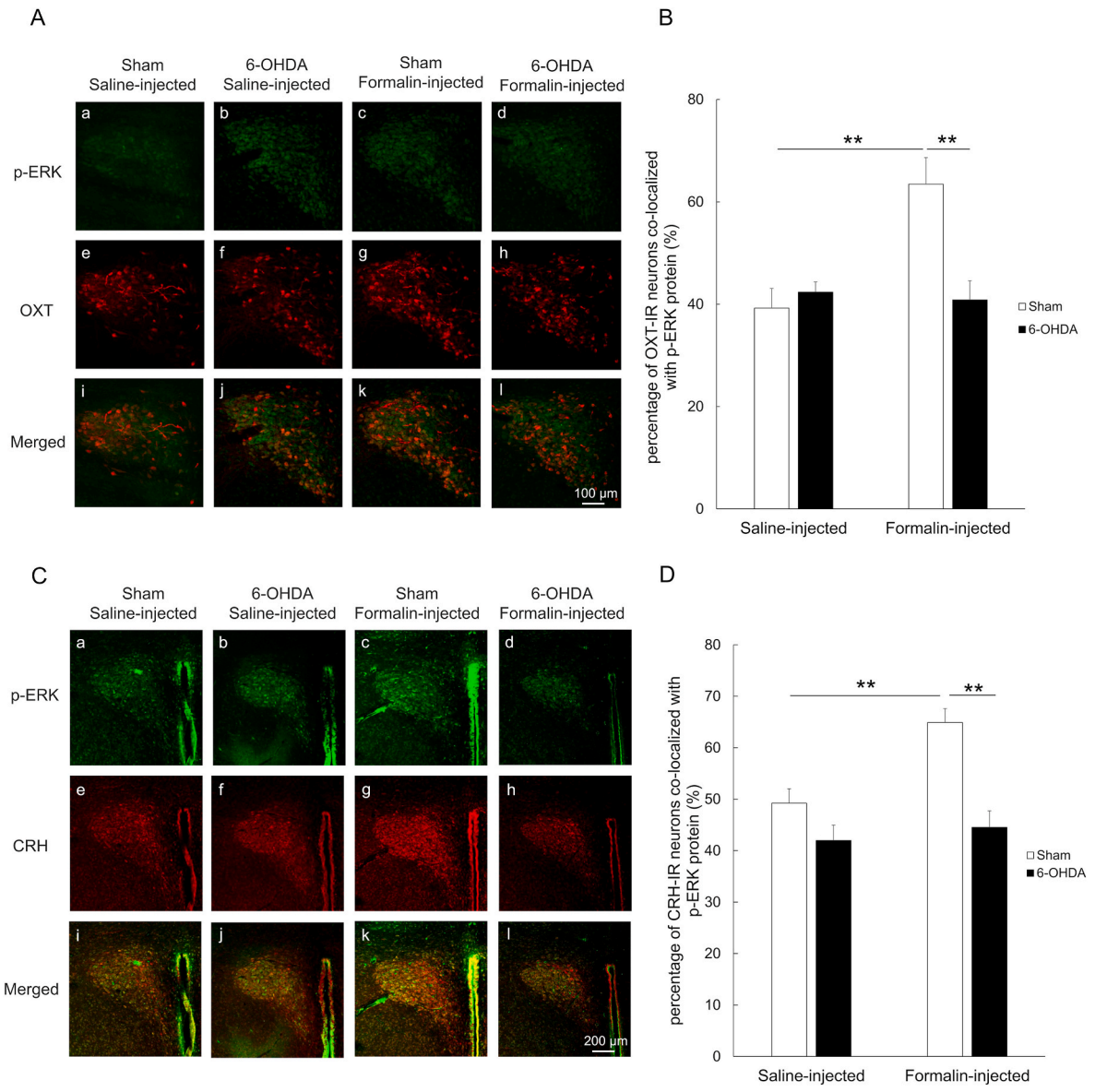

Fig. 2. Co-expression of p-ERK and oxytocin (OXT), and p-ERK and corticotropin-releasing hormone (CRH) in the PVN. (A) Photomicrographs of p-ERK-IR cells (a–d), OXT-IR cells (e–h) in the PVN, and merged images (i–l). (B) Changes in the percentage of OXT-IR neurons co-localized with p-ERK protein in the PVN. ***p-value* < 0.01. (C) Photomicrographs of p-ERK-IR cells (a–d), CRH-IR cells (e–h) in the PVN, and merged images (i–l). (D) Changes in the percentage of CRH-IR neurons co-localized with p-ERK protein in the PVN. ***p-value* < 0.01.

concentrations or neuronal activity in response to nociceptive stimuli in both sham and 6-OHDA rats. This suggests that AVP is unaffected by nociceptive stimuli and may not be involved in PD-associated hyperalgesia.

There are various theories regarding the analgesic mechanism of OXT. OXT is known to exhibit analgesic effects by stimulating the descending pain modulation pathways in the spinal cord and brainstem, particularly through its interactions with the periaqueductal gray and locus coeruleus regions [4,18,19]. This activation promotes the release of opioids within these areas, thereby inhibiting pain transmission [20–22]. Additionally, OXT enhances the analgesic effects by promoting the release of endogenous opioid peptides, such as β-endorphins [22]. OXT also reduces the inflammatory responses, alleviating inflammation-related pain, which is a process associated with the inhibition of pro-inflammatory factors, such as tumor necrosis factor-alpha and interleukin-6 [19,20]. Furthermore, OXT influences pain perception by modulating the brain regions associated with emotional processing, including the prefrontal cortex and amygdala, particularly affecting emotional pain [21].

CRH plays a key role in the body's stress response by activating the hypothalamic-pituitary-adrenal axis, which leads to the release of glucocorticoids [12,13]. These glucocorticoids alleviate pain by reducing inflammation and increasing pain tolerance, a mechanism often referred to as stress-induced analgesia [13,17]. Additionally, CRH is known to modulate the endogenous opioid system and activate the descending pain pathways, leading to enhanced pain inhibition [17,23]. Furthermore, although CRH is known to reduce pain during acute stress, the prolonged release of CRH during chronic stress may actually heighten pain perception [12,24]. This dual effect highlights the complex role of CRH in the nervous system, where it can either promote or inhibit pain depending on the duration and context of the stress [12,24]. To confirm the involvement of suppressed OXT and CRH synthesis or secretion in PD-related hyperalgesia, behavioral experiments involving the OXT and CRH administrations are necessary.

Regarding AVP in PD patients, PD patients are known to have low AVP levels, which tend to cause urinary incontinence [25,26]. This

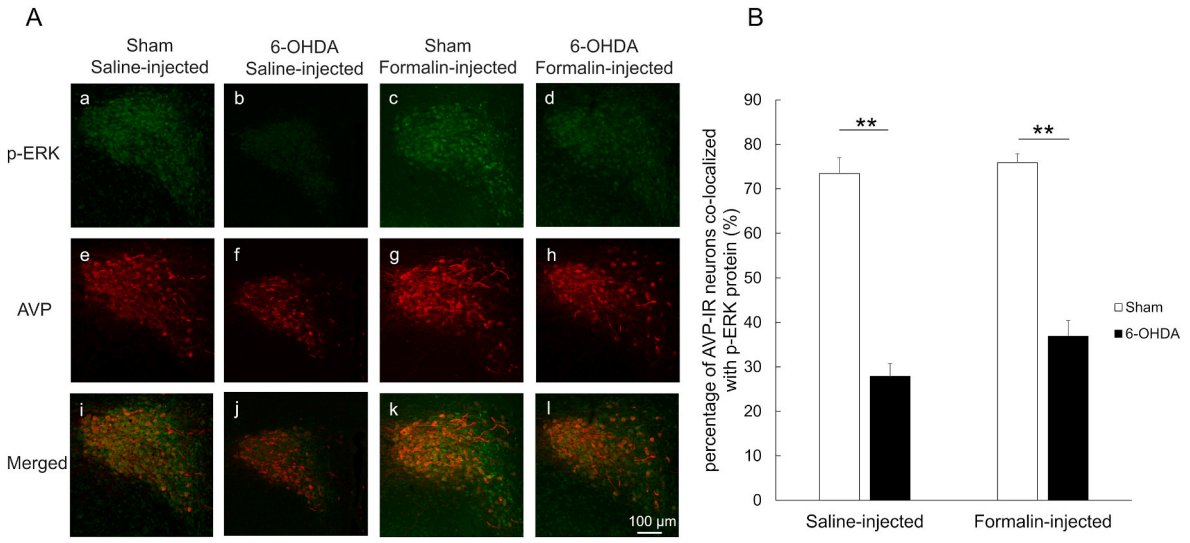

Fig. 3. Co-expression of p-ERK and arginine vasopressin (AVP) in the PVN. (A) Photomicrographs of p-ERK-IR cells (a–d), AVP-IR cells in the PVN (e–h), and merged images (i–l). (B) Changes in the percentage of AVP-IR neurons co-localized with p-ERK protein in the PVN. **p-value <0.01.

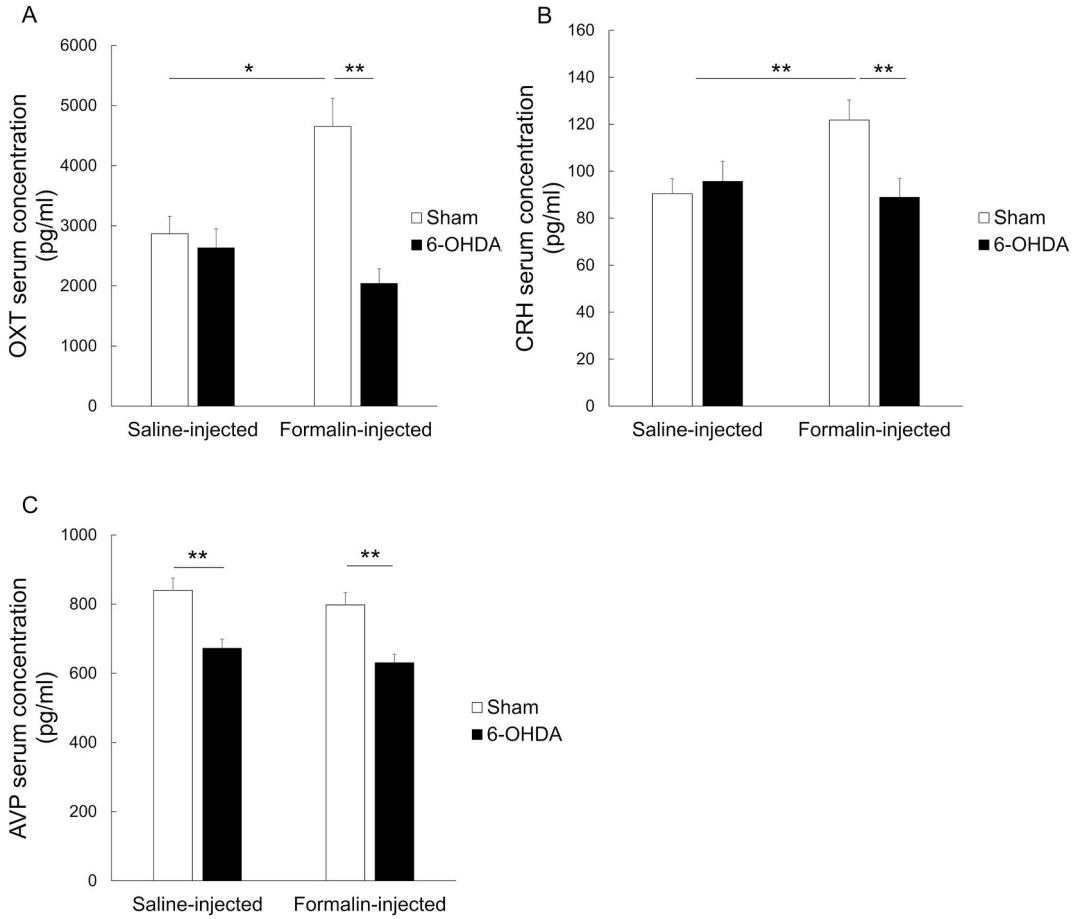

Fig. 4. Changes in the serum OXT, AVP, and CRH concentrations. (A) Our data show a significantly higher OXT concentration in the formalin-injected sham rats than in the saline-injected sham rats, whereas no significant difference was observed in the 6-OHDA rats. Additionally, the OXT concentration was significantly lower in the formalin-injected 6-OHDA rats than in the formalin-injected sham rats. (B) In the sham rats, the CRH concentration was significantly higher in the formalin-injected group than in the saline-injected group. However, no significant difference was found between the two groups in the 6-OHDA rats. In the formalin-injected groups, the CRH concentration was significantly lower in the 6-OHDA rats than in the sham rats. (C) In both the saline- and formalin-injected groups, the serum AVP concentration was significantly lower in the 6-OHDA rats than in the sham rats. No significant difference in the AVP concentration was observed between the saline- and formalin-injected groups in either the sham or 6-OHDA rats. * *p-value* < 0.05, ***p-value* < 0.01.

decrease is considered to be caused by the renin–angiotensin (AT) –aldosterone system, which regulates AVP synthesis and release [25]. There are three subtypes of AT receptors in the brain, with the AT1 receptor being predominantly distributed in the striatum and substantia nigra, which control the AVP release [8]. In PD patients, the damage to the striatum and substantia nigra affects the function of the AT1 receptor, thereby influencing AVP synthesis and secretion [8]. In this study, the neuronal activity and serum concentration of AVP did not change in response to the noxious stimuli in either the sham or 6-OHDA rats, and they consistently remained lower in PD rats. These results are consistent with the findings of previous studies reporting low AVP levels in PD patients [25], suggesting that AVP does not directly affect hyperalgesia in PD rats. Given that AVP receptors are known to exert analgesic effects in coordination with opioids and CRH [27], the reduced AVP levels in PD rats may lead to alterations in the AVP receptors, potentially affecting the analgesic pathways. However, further research is required to investigate the changes in AVP receptors in PD rats.

In conclusion, the enhanced neuronal activity and increased serum concentrations of OXT and CRH in response to nociceptive stimuli were suppressed in PD rats. These findings suggest that the reduced analgesic effects of OXT and CRH in response to nociceptive stimuli may contribute to hyperalgesia in PD rats. Our data could shed light on the mechanisms underlying hyperalgesia in PD and potentially form the basis for future methods to alleviate hyperalgesia.

CRediT authorship contribution statement

Shengsen Yang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nayuka Usami:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shiou—Ling Lu:** Writing – review & editing, Software. **Wakana Oda:** Writing – review & editing, Resources, Project administration. **Hiroharu Maegawa:** Writing – review & editing, Project administration, Methodology. **Hitoshi Niwa:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chiho Kudo:** Writing – review & editing, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by JST SPRING [Grant Number JPMJSP2138] and JSPS KAKENHI [Grant Number 20K10177].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2024.151178.

References

[1] K.R. Chaudhuri, A.H. Schapira, Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment, Lancet Neurol. 8 (2009) 464–474, https://doi.org/10.1016/S1474-4422(09)70068-7.
[2] M.G. Tansey, R.L. Wallings, M.C. Houser, M.K. Herrick, C.E. Keating, V. Joers, Inflammation and immune dysfunction in Parkinson disease, Nat. Rev. Immunol. 22 (2022) 657–673, https://doi.org/10.1038/s41577-022-00684-6.
[3] W. Dieb, O. Ouachikh, F. Durif, A. Hafidi, Lesion of the dopaminergic nigrostriatal pathway induces trigeminal dynamic mechanical allodynia, Brain Behav 4 (2014) 368–380, https://doi.org/10.1002/brb3.214.
[4] N. Usami, H. Maegawa, M. Hayashi, C. Kudo, H. Niwa, Changes in the analgesic mechanism of oxytocin can contribute to hyperalgesia in Parkinson's disease model rats, PLoS One 19 (2024) e0300081, https://doi.org/10.1371/journal.pone.0300081.
[5] H. Maegawa, N. Adachi, H. Hanamoto, C. Kudo, H. Niwa, Bilateral Parkinson's disease model rats exhibit hyperalgesia to subcutaneous formalin administration into the vibrissa pad, PLoS One 14 (2019) e0225928, https://doi.org/10.1371/journal.pone.0225928.
[6] H. Maegawa, Y. Morimoto, C. Kudo, H. Hanamoto, A. Boku, M. Sugimura, T. Kato, A. Yoshida, H. Niwa, Neural mechanism underlying hyperalgesic response to orofacial pain in Parkinson's disease model rats, Neurosci. Res. 96 (2015) 59–68, https://doi.org/10.1016/j.neures.2015.01.006.
[7] J. Lipski, R. Nistico, N. Berretta, E. Guatteo, G. Bernardi, N.B. Mercuri, l-DOPA: a scapegoat for accelerated neurodegeneration in Parkinson's disease? Prog. Neurobiol. 94 (2011) 389–407, https://doi.org/10.1016/j.pneurobio.2011.06.005.
[8] J.W. Wright, J.W. Harding, Importance of the brain angiotensin system in Parkinson's disease, Parkinson's Dis. 2012 (2012) 1–14, https://doi.org/10.1155/2012/860923.
[9] C. Tassorelli, M.-T. Armentero, R. Greco, R. Fancellu, G. Sandrini, G. Nappi, F. Blandini, Behavioral responses and Fos activation following painful stimuli in a rodent model of Parkinson's disease, Brain Res. 1176 (2007) 53–61, https://doi.org/10.1016/j.brainres.2007.08.012.
[10] B. Savić, D. Murphy, N. Japundžić-Žigon, The paraventricular nucleus of the hypothalamus in control of blood pressure and blood pressure variability, Front. Physiol. 13 (2022) 858941, https://doi.org/10.3389/fphys.2022.858941.
[11] J.I. Wamsteeker Cusulin, T. Füzesi, A.G. Watts, J.S. Bains, Characterization of corticotropin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus of Crh-IRES-Cre mutant mice, PLoS One 8 (2013) e64943, https://doi.org/10.1371/journal.pone.0064943.
[12] J.P. Herman, J.G. Tasker, Paraventricular hypothalamic mechanisms of chronic stress adaptation, Front. Endocrinol. 7 (2016), https://doi.org/10.3389/fendo.2016.00137.
[13] S.A. Mousa, P.C. Bopaiah, C. Stein, M. Schäfer, Involvement of corticotropin-releasing hormone receptor subtypes 1 and 2 in peripheral opioid-mediated inhibition of inflammatory pain, Pain 106 (2003) 297–307, https://doi.org/10.1016/S0304-3959(03)00302-6.
[14] A. Björklund, S.B. Dunnett, The amphetamine induced rotation test: a re-assessment of its use as a tool to monitor motor impairment and functional recovery in rodent models of Parkinson's disease, J. Parkinsons Dis. 9 (2019) 17–29, https://doi.org/10.3233/JPD-181525.
[15] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, sixth ed., Elsevier. Academic Press, Cambridge, 2007, p. 1.
[16] J.S. Purba, Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression, Arch. Gen. Psychiatr. 53 (1996) 137, https://doi.org/10.1001/archpsyc.1996.01830020055007.
[17] W.R. Lariviere, R. Melzack, The role of corticotropin-releasing factor in pain and analgesia, Pain 84 (2000) 1–12, https://doi.org/10.1016/S0304-3959(99)00193-1.
[18] R.A. Domenici, A.C.P. Campos, S.T. Maciel, M.B. Berzuino, M.S. Hernandes, E. T. Fonoff, R.L. Pagano, Parkinson's disease and pain: modulation of nociceptive circuitry in a rat model of nigrostriatal lesion, Exp. Neurol. 315 (2019) 72–81, https://doi.org/10.1016/j.expneurol.2019.02.007.
[19] C.T. Wotjak, J. Ganster, G. Kohl, F. Holsboer, R. Landgraf, M. Engelmann, Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: new insights into the secretory capacities of peptidergic neurons, Neuroscience 85 (1998) 1209–1222, https://doi.org/10.1016/S0306-4522(97)00683-0.
[20] J.A. Rash, A. Aguirre-Camacho, T.S. Campbell, Oxytocin and pain: a systematic review and synthesis of findings, Clin. J. Pain 30 (2014) 453–462, https://doi.org/10.1097/AJP.0b013e31829f57df.
[21] P.-E. Juif, P. Poisbeau, Neurohormonal effects of oxytocin and vasopressin receptor agonists on spinal pain processing in male rats, Pain 154 (2013) 1449–1456, https://doi.org/10.1016/j.pain.2013.05.003.
[22] A. González-Hernández, G. Rojas-Piloni, M. Condés-Lara, Oxytocin and analgesia: future trends, Trends Pharmacol. Sci. 35 (2014) 549–551, https://doi.org/10.1016/j.tips.2014.09.004.

[23] E.J. Van Bockstaele, B.A.S. Reyes, R.J. Valentino, The locus coeruleus: a key nucleus where stress and opioids intersect to mediate vulnerability to opiate abuse, Brain Res. 1314 (2010) 162–174, https://doi.org/10.1016/j.brainres.2009.09.036.
[24] A. Wyns, J. Hendrix, A. Lahousse, E. De Bruyne, J. Nijs, L. Godderis, A. Polli, The biology of stress intolerance in patients with chronic pain—state of the art and future directions, J. Clin. Med. 12 (2023) 2245, https://doi.org/10.3390/jcm12062245.
[25] A. Batla, V. Phé, L. De Min, J.N. Panicker, Nocturia in Parkinson's disease: why does it occur and how to manage? Movement Disord. Clin. Pract. 3 (2016) 443–451, https://doi.org/10.1002/mdc3.12374.
[26] L. Yeo, R. Singh, M. Gundeti, J.M. Barua, J. Masood, Urinary tract dysfunction in Parkinson's disease: a review, Int. Urol. Nephrol. 44 (2012) 415–424, https://doi.org/10.1007/s11255-011-9969-y.
[27] K. Csabafi, K.E. Ibos, É. Bodnár, K. Filkor, J. Szakács, Z. Bagosi, A brain region-dependent alteration in the expression of vasopressin, corticotropin-releasing factor, and their receptors might be in the background of kisspeptin-13-induced hypothalamic-pituitary-adrenal axis activation and anxiety in rats, Biomedicines 11 (2023) 2446, https://doi.org/10.3390/biomedicines11092446.