

Anti-stress Effect of Octopus Cephalotocin in Rats

Ye-Ji Kim^{1,2}, Seonmi Jo³, Seung-Hyun Jung³ and Dong Ho Woo^{1,2*}

¹Research Center for Convergence Toxicology, Korea Institute of Toxicology, Daejeon 34114,

²Department of Human and Environmental Toxicology, University of Science and Technology, Daejeon 34114,

³Department of Genetic Resources, National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea

Cephalotocin is a bioactivity-regulating peptide expressed in octopus (*Octopus vulgaris*). The peptide sequence of cephalotocin is very similar to the peptide sequence of mammalian vasopressin, and cephalotocin has been proposed to mainly activate arginine vasopressin 1b receptor (Avpr1b) in the brain. However, the effects of cephalotocin on mammalian behavior have not been studied. In the current study, cephalotocin significantly reduced both the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs) recorded from not only cultured neuronal cells from postnatal Sprague–Dawley (SD) rats but also hippocampal slices from 4-week-old male C57BL/6 mice. Intraperitoneal (IP) injection did not affect the open field behaviors of C57BL/6 mice. Cephalotocin was directly infused into the hippocampus because the normalized Avpr1b staining intensity divided by the DAPI staining intensity indicated that Avpr1b expression tended to be high in the hippocampus. A hippocampal infusion of 1 mg/kg cephalotocin via an implanted cannula exerted an anti-stress effect, significantly reducing the immobility time in the tail suspension test (TST). The present results provide evidence that the effects of cephalotocin on the activity of hippocampal neurons are related to ameliorating stress, suggesting that cephalotocin may be developed as an anti-stress biomodulator that functions by affecting the brain.

Key words: Cephalotocin, SD rat, Tail suspension test, sEPSCs, Intrahippocampal infusion

INTRODUCTION

Arginine vasopressin (Avp) is an important regulator of stress. Vasopressin-deficient Brattleboro rats have been shown to exhibit lower stress levels than wild-type rats in a 5 min forced swim test but not after a 15 min test, suggesting that Avp is strongly correlated with stress regulation [1]. The Avp 1a receptor (Avpr1a) is related to vascular modulation [2, 3], the Avp 2 receptor (Avpr2) is related to renal duct water reabsorption [4], and the Avp 1b receptor (Avpr1b or V3R) is related to stress regulation. Avpr1b plays key roles in social memory, aggressive behavior and stress responses [5]. An acute 38°C treatment increases the blood Avp levels and decreases Avpr1b protein expression, suggesting that

Avpr1b is strongly related to stress regulation [6]. *In situ* hybridization histochemistry (ISHH) and RT-PCR have provided evidence suggesting that Avpr1b transcripts are expressed in the hippocampal CA3, CA2 and CA1 regions [7].

The sequence of an octopus-originated peptide, cephalotocin (CPT), is similar to that of Avp and oxytocin. CPT has the potential to interact with the human oxytocin receptor and the vasopressin receptor. The 397 amino acid sequence of the receptor of CPT shares close homology with the receptor of vasopressin and is a potential Gq protein-coupled receptor that activates Ca²⁺-activated Cl⁻ currents in the *Xenopus* oocyte expression system [8]. Thus, CPT is a potential agonist of oxytocin and vasopressin receptors. CPT has the potential to interact with the vasopressin receptor for two reasons. First, CPT varies from Avp only in the 4th and 9th amino acids and exhibits 78% sequence homology with oxytocin and vasopressin [9]. Second, Avp treatment increases the number of action potentials from CA1 pyramidal neurons in hippocampal slices by blocking the G protein-gated inwardly rectifying potassium (GIRK) channel and increases glutamate release in

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*To whom correspondence should be addressed.
TEL: 82-42-610-8243, FAX: 82-42-610-8252
e-mail: dongho.woo@kitox.re.kr

the subiculum [10]. We hypothesize that CPT may interact with Avp receptors and thereby modulate neuronal activity. Since natural and artificial analogs of vasopressin have already been used as antidiuretics, in this study, we explored the possible usefulness of CPT in stress control by focusing on its role in the brain.

Vasopressin treatment is not only involved in stress control by regulating hippocampal Avp1b levels [5, 7, 11] but also related to the physiological process of diuretic reabsorption [4]. As a result, we propose a link between brain stress and diuretic reabsorption. This study is the first to assess the effect of the peptide CPT generated from octopus genome information on stress regulation.

The purpose of the present study is to show for the first time the results and the process for determining whether CPT is effective in relieving stress in mammals. CPT decreases the frequency of glutamate release recorded from cultured rat hippocampal neurons and mouse hippocampal CA1 pyramidal neurons. Intraperitoneal injection does not change locomotion in the open field test (OFT), and intrahippocampal infusion significantly reduces the immobility time in the tail suspension test (TST).

MATERIALS AND METHODS

Peptide

As described in Table 1, high purity of CPT were synthesized by AnyGen (Gwangju, Korea) using solid-phase peptide synthesis. The purity and molecular masses of the peptides were determined using high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) (Shimadzu, Kyoto, Japan).

Preparation of primary cultures of rat hippocampal neurons

Primary cultures of hippocampal neurons were prepared from postnatal day (P) 0-P1 rats using a previously described method to record EPSCs [12, 13]. The hippocampus was dissected and incubated with Mg^{2+} - and Ca^{2+} -free HBSS (Gibco, Massachusetts). The dissected brains were trypsinized with 0.025% trypsin for 10 min at 37°C. Trypsin activity was stopped by adding DMEM containing 10% FBS and incubating the samples for 1 min at room temperature. The medium was decanted and replaced with neurobasal medium (Gibco, Massachusetts) containing 2% B27 supplement, 2 mM L-glutamine, and 1% penicillin–streptomycin (100 units/ml

penicillin and 100 µg/ml streptomycin, Gibco). The cell suspension was passed through a fire-polished Pasteur pipette and incubated for 2 min. The trituration process was repeated 7–10 times. The cells were passed through a cell strainer with a pore size of 100 µm (93100, SPL, Seoul, Korea) and then filtered through another cell strainer with a pore size of 40 µm (93040, SPL, Korea) to remove nontriturated tissue debris. The cells were plated at a density of 3×10^5 cells per cover glass. The cultures were maintained in a humidified atmosphere containing 5% CO_2 and 95% O_2 at 37°C for 14–20 days.

Preparation of mouse hippocampal slices

Subsequently, we performed experiments to record the action potentials (APs) and excitatory postsynaptic currents (EPSCs) in mouse hippocampal pyramidal neurons. Four-week-old C57BL/6 male mice were anesthetized with 20 mg/ml avertin (2,2,2-tribromoethanol, Sigma–Aldrich, MA, USA; cat. no. #T4, 840-2). The brain was quickly removed from each mouse and horizontally sectioned in artificial cerebrospinal fluid (ACSF) consisting of (in mM) 130 NaCl, 1.25 NaH_2PO_4 , 3.5 KCl, 24 $NaHCO_3$, 1.5 $CaCl_2$, 1.5 $MgCl_2$ and 10 glucose. Rostral-to-caudal 300-µm-thick brain slices containing the hippocampal region were cut using a vibratome (Leica VT1000 S, Wetzlar, Germany) and bubbled with 95% O_2 /5% CO_2 at room temperature.

Electrophysiology

Whole-cell patch-clamp recordings of cultured hippocampal neurons and mouse hippocampal slices under voltage clamp (holding potential -70 mV) were performed with a MultiClamp 700B amplifier and digitized with a Digidata 1322A data acquisition system (Molecular Devices, CA, USA). Borosilicate glass capillaries (cat. no. 1B150F-4, outer diameter, 1.50 mm; inner diameter, 1 mm; World Precision Instruments, FL, USA) with a tip resistance of 3–8 MΩ were pulled (P-97 micropipette puller, Sutter Instrument, Novato, CA, USA). The capillaries were filled with an intracellular solution composed of (in mM) 150 CsMeSO₄, 10 NaCl, 0.5 $CaCl_2$, and 10 HEPES (pH adjusted to 7.3 with CsOH and osmolarity adjusted to 310 mOsm/L). The extracellular solution used for the cell patch-clamp recording was composed of (in mM) 150 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose and 10 HEPES (pH adjusted to 7.4 with NaOH and osmolarity adjusted to 315–320 mOsm/L with sucrose), and the ACSF solution used

Table 1. Cephalotocin used in this study [9]

Name	Sequence	Purity	Working conc.	Species
Cephalotocin (CPT)	CYFRNCPIG-NH ₂ (disulfide bond)	>98%	1 mg/ml/kg	<i>O. vulgaris</i>

for the slice patch-clamp recording was composed of (in mM) 130 NaCl, 1.25 NaH₂PO₄, 3.5 KCl, 24 NaHCO₃, 1.5 CaCl₂, 1.5 MgCl₂ and 10 glucose (osmolality adjusted to 330~340 mOsm/L). QX 314 (0.5 mM) was used to inhibit voltage-gated sodium channels. Mini Analysis software (Synaptosoft, USA) was used to analyze the frequency and amplitude.

AP recordings of mouse hippocampal neurons were performed in the current clamp mode with a MultiClamp 700B amplifier and digitized with a Digidata 1322A data acquisition system (Molecular Devices, CA, USA). The glass capillaries were filled with an intracellular solution consisting of (in mM) 140 K-gluconate, 10 HEPES, 7 NaCl, 4 Mg-ATP, and 0.3 Na-GTP (pH adjusted to 7.4 with KOH and osmolality adjusted to 280~290 mOsm/L). Each patched cell was inspected in the current clamp mode, and neurons were subjected to 10 depolarizing current pulses in 20 pA increments for 0.5 s each at 2 s intervals; then, the membrane potentials, which were initially adjusted to -60 mV, were returned to the resting potential.

Immunohistochemistry (IHC) of rat brain tissue sections

Rats were anesthetized with 20 mg/ml avertin and perfused with 0.9% saline, followed by ice-cold 4% paraformaldehyde (PFA). The brains were postfixed in PFA overnight and 30% sucrose for 48 hrs at 4°C. Coronal sections were cut with a cryostat at 20 µm and attached to glass slides. The sections were washed with PBS and incubated for 1 hr with a blocking solution (0.5% Triton X-100, 0.5% BSA, and 5% normal goat serum in PBS). The sections were incubated with primary antibodies in blocking solution overnight on a shaker at 4°C. After washing with 0.5% Triton X-100 in PBS (PBST) twice and 0.5% Triton X-100 and 0.5% BSA in PBS (PBSTB) once for 10 min at room temperature, the sections were incubated with secondary antibodies in PBSTB for 1 hr at room temperature. After three washes with PBST and 1 wash with PBS for 10 min at room temperature, the sections were mounted with mounting medium containing DAPI (VECTASHIELD, CA, USA) and dried. A series of fluorescence images were obtained under a confocal microscope (Olympus, Tokyo, Japan) and analyzed using ImageJ

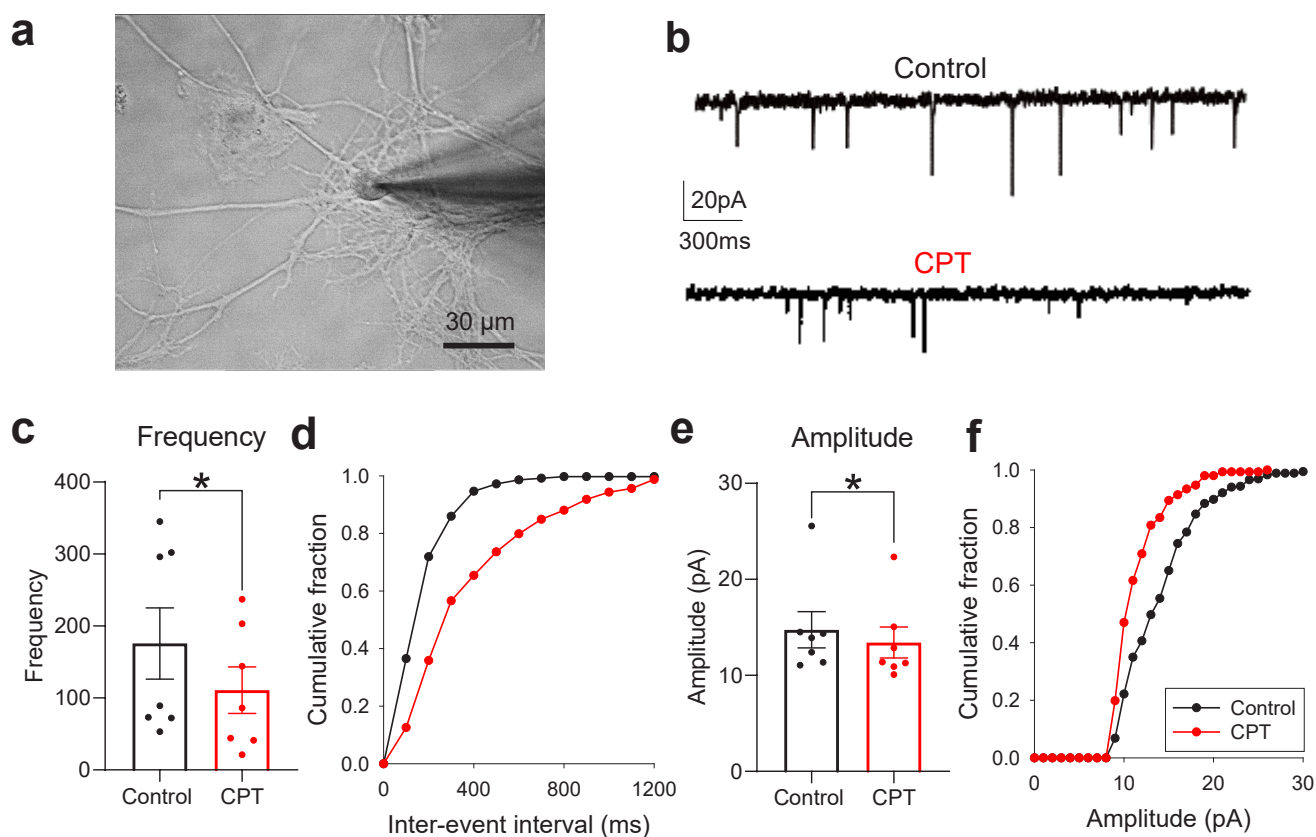


Fig. 1. CPT reduces both the frequency and amplitude of sEPSCs in rat hippocampal neurons. (a) Bright field image showing a glass pipette used for whole-cell patch-clamp recording of primary cultured rat hippocampal neurons. (b) Representative sEPSCs before (upper panel) and during the treatment with 10 µM CPT (lower panel). (c) Bar graph summarizing the frequencies (paired t test, *p=0.04, n=7). (d) Cumulative fraction of the interevent interval before (black line) and during the treatment with 10 µM CPT (red line). (e) Bar graph summarizing the amplitudes (paired t test, *p=0.04, n=7). (f) Cumulative fraction of the amplitude before (black line) and during the treatment with CPT (red line).

software. The primary antibody, anti-Avpr1b (AVR-011, Alomone, Jerusalem, Israel), was diluted 1:200, and the secondary antibody, Alexa Fluor 594-conjugated goat anti-rabbit (A11012, Invitrogen, MA, USA), was diluted 1:1,000.

Intraperitoneal injection (IP) and open field test (OFT)

Four-week-old C57BL/6 male mice were first acclimated for one week after acquisition before assessing their activity. The mice were grouped based on body weight. The mice were acclimated in polycarbonate cages with bedding. The mice were group-housed under a 12-h light/dark cycle at 25°C with free access to food and water. The mice were fasted for 12 hours to maximize the effect of the peptide on behavioral activity. First, 1 mg/ml or 5 mg/ml CPT or ethanol (EtOH, positive control) was intraperitoneally injected by a trained experimenter using a 26-gauge thick syringe after weighing the animal. The box used for the OFT was equipped with a recording camera. Each mouse was placed in the center of the box, habituated for 30 min and then tested for 1 hr after an intraperitoneal injection of 1 mg/ml/kg CPT with a 1 ml syringe. The tests were videotaped, and the distances traveled by the mice were analyzed using EthoVisionXT software (version 14, Noldus, Netherlands).

Stereotaxic surgery, CPT injection and the tail suspension test (TST) in rats

Four-week-old male Sprague–Dawley (SD) rats were obtained, and five-week-old rats were subjected to stereotaxic surgery and injected with CPT in the CA1 region of the hippocampus before the TST. The SD rats were single-housed under a 12-h light/dark cycle at 25°C with free access to food and water. The rats were anesthetized with 2% isoflurane and placed in a stereotaxic frame with sustained anesthesia during surgery. The scalp was incised from the frontal cranial bones to the back using a scalpel blade. Holes were drilled into the skull above the hippocampus (anterior/posterior, -2.0 mm; medial/lateral, -1.5 or +1.5 mm from the bregma) for the placement of the injection needle. An intracerebral guide cannula was inserted into the hippocampal CA1 region (dorsal/ventral, -1.7 mm), and a dummy cannula was inserted into the guide cannula to prevent blockage of the holes. After 5 days of recovery, 1 mg/2 μ l of CPT dissolved in saline solution was loaded and injected for 2 min into six-week-old SD rats to measure stress behaviors.

TST

We used rats that were stereotaxically injected in the hippocampal CA1 region to measure stress. After the injection, the rats were suspended from the tail above the floor with adhesive tape using

a traction force meter. The rats were placed in the middle of the horizontal plane, and the tests lasted for 10 min. The tests were videotaped and scored by a trained observer.

All animal protocols were performed in strict accordance with the institutional Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Korea Institute of Toxicology (1901-0028).

Data analysis

The experimental data are presented as the means \pm the experimental standard errors of the means (error bars), which were calculated using Excel 2016 software (Microsoft, WA, USA). The EC₅₀ (concentration producing a half-maximal response) and IC₅₀ (concentration causing half-maximal inhibition of the control agonist response) values were determined by 4-parameter logistic fitting of the dose–response curve using SigmaPlot 10.0 (Systat Software, CA, USA). The statistical analyses were performed using

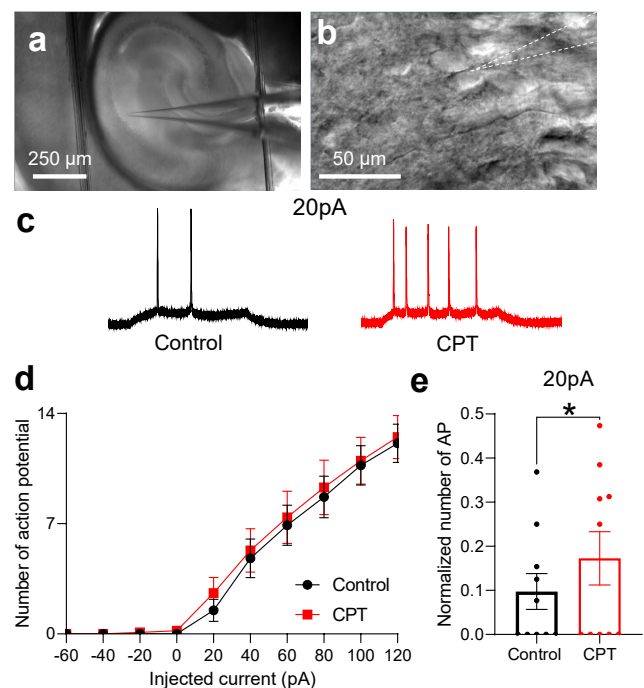


Fig. 2. CPT increases the number of APs generated by application of a +20-pA current to hippocampal CA1 pyramidal neurons from C57BL/6 mice. (a) Bright field image showing a glass pipette above the hippocampal CA1 region. (b) High-magnification image showing a glass pipette used for the whole-cell patch-clamp recording of CA1 pyramidal neurons. (c) Representative voltage trace in the current clamp mode before the treatment (left panel, black trace) and during the treatment with 10 μ M CPT (right panel, red trace). (d) Plot comparing the numbers of APs recorded during a 0.6 s current application using the current step protocol (black indicates before the CPT treatment; red indicates during the CPT treatment). (e) Summary bar graph comparing the numbers of APs recorded before and during the treatment with CPT (paired t test, * $p=0.04$, $n=10$).

GraphPad Prism version 8. The comparisons were performed using a paired t test, an unpaired t test or one-way ANOVA. Nonsignificant: ns, $p>0.05$; significant: * $p<0.05$, ** $p<0.01$, and *** $p<0.001$, as indicated in the individual figures.

RESULTS

CPT decreases both the frequency and amplitude of spontaneous EPSCs (sEPSCs) recorded from both cultured pyramidal neurons of SD rats and CA1 pyramidal neurons in C57BL/6 mouse brain slices

Avp modulates social memory via hippocampal Avpr1b-dependent changes [14], increases GABA release and reduces the excitability of hippocampal activity [15]. Avp increases the excitability of SD rat hippocampal neurons by increasing APs [10]. We investigated whether CPT, an Avp analog whose amino acid sequence

differs from that of Avp at only the 4th and 8th residues, changes excitability in rat hippocampal neurons (Fig. 1a). CPT (10 μ M) decreased the frequency of sEPSCs (Fig. 1b, c, right shifted red line in d, paired t test, * $p=0.04$). CPT also decreased the amplitude of sEPSCs (Fig. 1b, e, left-shifted red line in d and f, paired t test, * $p=0.04$). These results are inconsistent with a previous finding [10] suggesting that vasopressin substantially increases the number of APs. Then, CPT was applied to mouse hippocampal CA1 pyramidal neurons in the current clamp mode, and the number of APs was recorded (Fig. 2a, b). The number of APs increased in the presence of 10 μ M CPT and a 20-pA current (Fig. 2c, e, paired t test, * $p=0.03$). At least in terms of the quantity of APs, vasopressin and CPT produced different patterns in neuronal excitability. CPT was applied to mouse hippocampal neurons (Fig. 3a, b), and the sEPSC frequency in the CPT group was significantly lower than that in the non-CPT group (Fig. 3c, d, right-shifted red line in e,

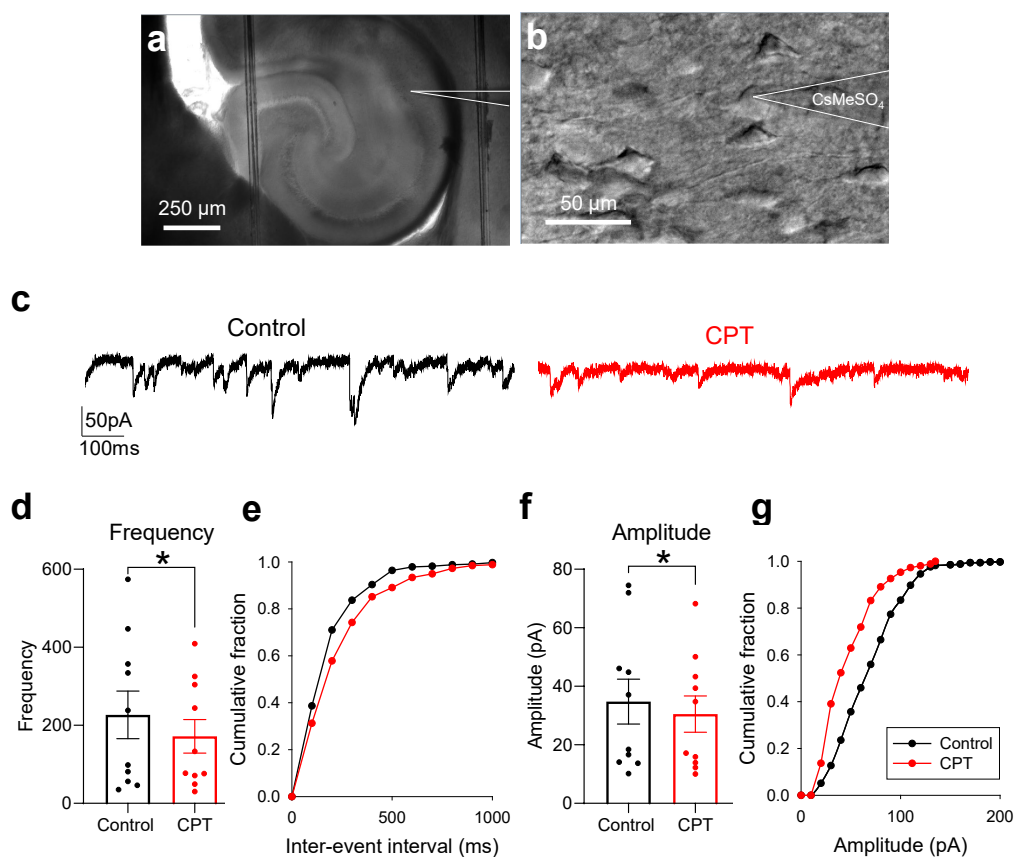


Fig. 3. CPT reduces the frequency of sEPSCs in hippocampal CA1 pyramidal neurons from C57BL/6 mice. (a) Bright field image showing a glass pipette above the hippocampal CA1 region. (b) High-magnification image showing a glass pipette used for whole-cell patch-clamp recording of CA1 pyramidal neurons. (c) Representative trace of sEPSCs before (left panel, black trace; internal solution: 150 mM CsMeSO₄ with 0.5 mM QX-314 for Na⁺ channel blockade; external solution: artificial cerebrospinal fluid [ACSF] containing 130 mM NaCl, V_h=-70 mV) and during the treatment with 10 μ M CPT (right panel, red trace; paired comparison with no treatment). (d) Bar graph summarizing the frequencies (paired t test, * $p=0.03$, n=10). (e) Cumulative fraction of the interevent interval before (black line) and during the treatment with 10 μ M CPT (red line). (f) Bar graph summarizing the amplitudes (paired t test, * $p=0.04$, n=10). (g) Cumulative fraction of the amplitude before (black line) and during the treatment with CPT (red line).

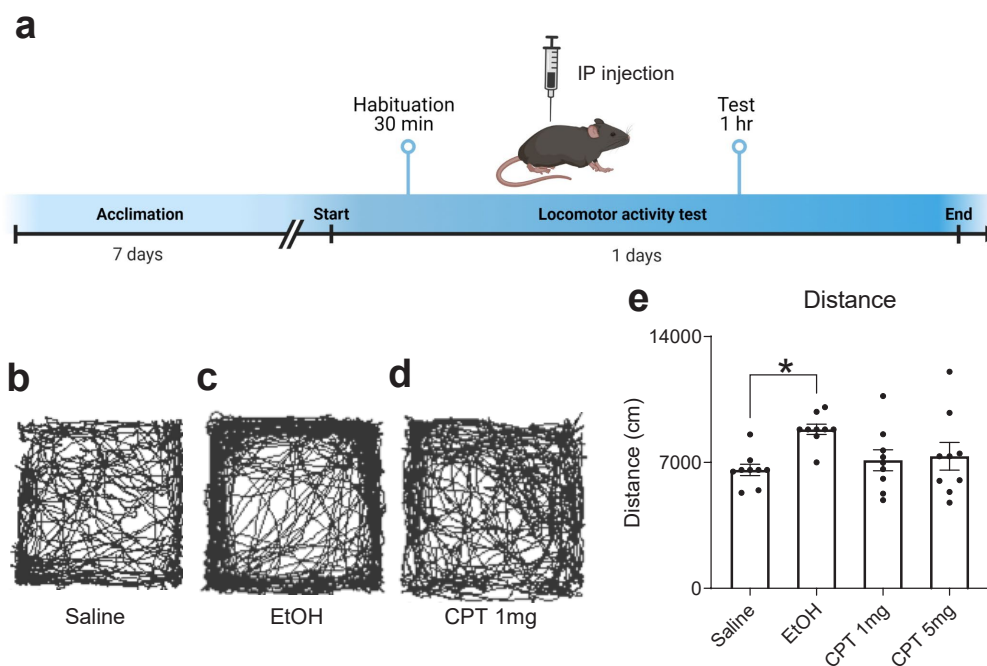


Fig. 4. IP injection of cephalotocin did not change locomotor activity. (a) Schematic diagram of the experimental schedule, which involved acclimation for 7 days and an IP injection of 1 mg/10 ml/kg CPT into 5-week-old SD male rats. (b–d) Representative summed traces of locomotor activity after injection with (b) saline (n=9), (c) ethanol (n=9), or (d) CPT (n=9). Bar graph summarizing the distances traveled (one-way ANOVA, * $p=0.02$, Dunnett's post-hoc test).

paired t test, * $p=0.03$). In addition, CPT decreased the amplitude of sEPSCs (Fig. 3c, f, left-shifted red line in g, paired t test, * $p=0.04$).

IP injection of 1 mg/kg CPT does not change locomotor activity

The effects of CPT on the behavior of mammals have not been reported. In the present study, the OFT was used to determine the effect of CPT on locomotor activity (Fig. 4a). An IP injection of 1 mg/kg CPT did not change locomotor activity according to the measurement of the distance traveled in the OFT (Fig. 4b, d, e) in contrast to an injection of ethanol, which significantly increased the distance traveled (Fig. 4b, c, e, one-way ANOVA, * $p=0.02$ saline and ethanol).

An intrahippocampal infusion of CPT significantly reduces the immobility time in the TST

We directly administered CPT into the brain because the intraperitoneal injection of CPT did not change locomotion, thus indicating that a systemic injection of CPT does not alter general locomotion. Thus, the reduced immobility following the intrahippocampal injection of CPT is not due to changes in motor function. Avpr1b (or V3R) is related to stress regulation. The expression of the Avpr1b transcript has been detected in many regions of the rodent nervous system, including the medulla, cerebellum, pons,

midbrain, caudate putamen, septum, hypothalamus, piriform cortex, amygdala, and parietal cortex. However, no expression has been observed in the vomeronasal organ, olfactory bulb, or thalamus [7]. In the current study, Avpr1b was mainly expressed in the hippocampal CA1 region (Fig. 5a~o). The number of Avpr1b-expressing cells was divided by the number of DAPI-expressing cells to determine the average number of Avpr1b-expressing cells (Fig. 5p~r). The intrahippocampal infusion was performed through an implanted cannula (Fig. 6a, b). We monitored the durations of mobility and immobility (Fig. 6c) during the indicated procedures (Fig. 6d). The mobility duration was increased in the presence of CPT (Fig. 6e, unpaired t test, ** $p=0.01$ saline & CPT). The immobility duration was significantly decreased in the presence of CPT (Fig. 6f, unpaired t test, ** $p=0.01$ saline & CPT).

DISCUSSION

Based on our results, an intrahippocampal infusion of CPT increases the mobile time in the TST through the molecular mechanism of reduced glutamate release in hippocampal pyramidal CA1 neurons.

The goal of this research was to determine whether CPT derived from octopus genomic data is bioavailable to mammals. The results of this study suggest that the peptide CPT synthesized ac-

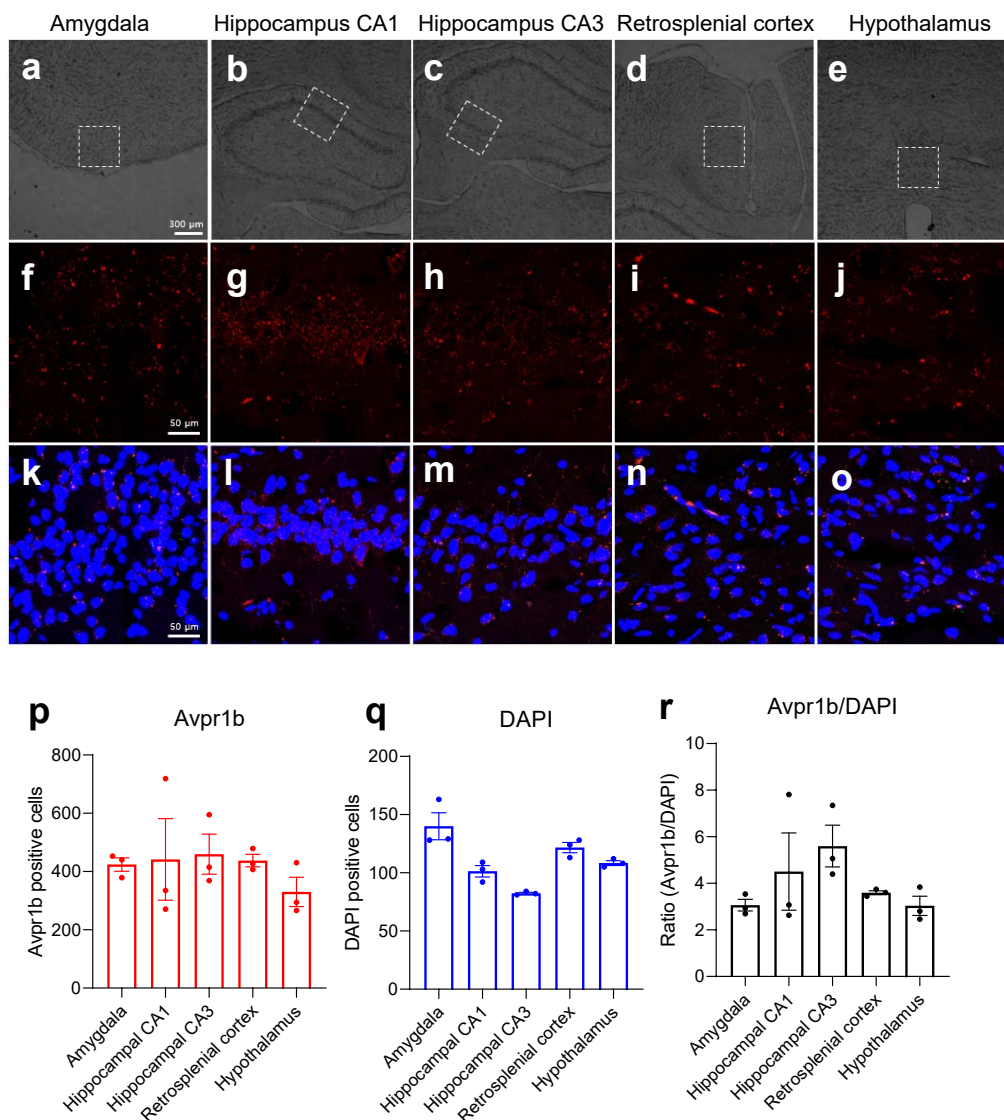


Fig. 5. Avpr1b was generally expressed at high levels in the hippocampus. (a) Bright field images of the amygdala, (b) hippocampal CA1 region, (c) hippocampal CA3 region, (d) retrosplenial cortex, (e) hypothalamus. (f) Avpr1b expression in the amygdala. (g) Avpr1b expression in the hippocampal CA1 region. (h) Avpr1b expression in the hippocampal CA3 region. (i) Avpr1b expression in the retrosplenial cortex. (j) Avpr1b expression in the hypothalamus. (k) DAPI and Avpr1b staining in the amygdala. (l) DAPI and Avpr1b staining in the hippocampal CA1 region. (m) DAPI and Avpr1b staining in the hippocampal CA3 region. (n) DAPI and Avpr1b staining in the retrosplenial cortex. (o) DAPI and Avpr1b staining in the hypothalamus. (p) Bar graph summarizing the number of Avpr1b puncta. (q) Bar graph summarizing the number of DAPI-stained. (r) Ratio (number of red puncta/number of blue-stained regions) showing that Avpr1b was expressed at high levels in the hippocampus.

According to previous octopus studies ameliorates stress by inhibiting glutamate release from hippocampal pyramidal neurons. Hippocampal stimulation with 1 s current trains of 1 s on / 1 s off (100 mA, 50~60 Hz, 1 ms pulse duration) for 10 min has been shown to increase the plasma corticosterone levels by 20% beginning at 5 min and continuing for 30 min [16]. In addition, monophasic stimulation with a 1.3 V current for 0.1 ms has been reported to reduce blood corticosterone levels [17]. These findings suggest that hippocampal electrical stimulation *in vivo* modulates the hip-

pocampal–pituitary–adrenal (HPA) axis. In this paper, an acute treatment with CPT reduced the stress levels by inhibiting presynaptic glutamate release and increasing CA1 APs.

The ventral region of the hippocampus is related to emotions, whereas the dorsal region is responsible for spatial memory [18]. We observed a discrepancy because CPT injection in the dorsal hippocampal region relieved stress, which is known to be mediated by the ventral hippocampal region. Nevertheless, extirpation of the dorsal hippocampus increased the blood levels of beta-

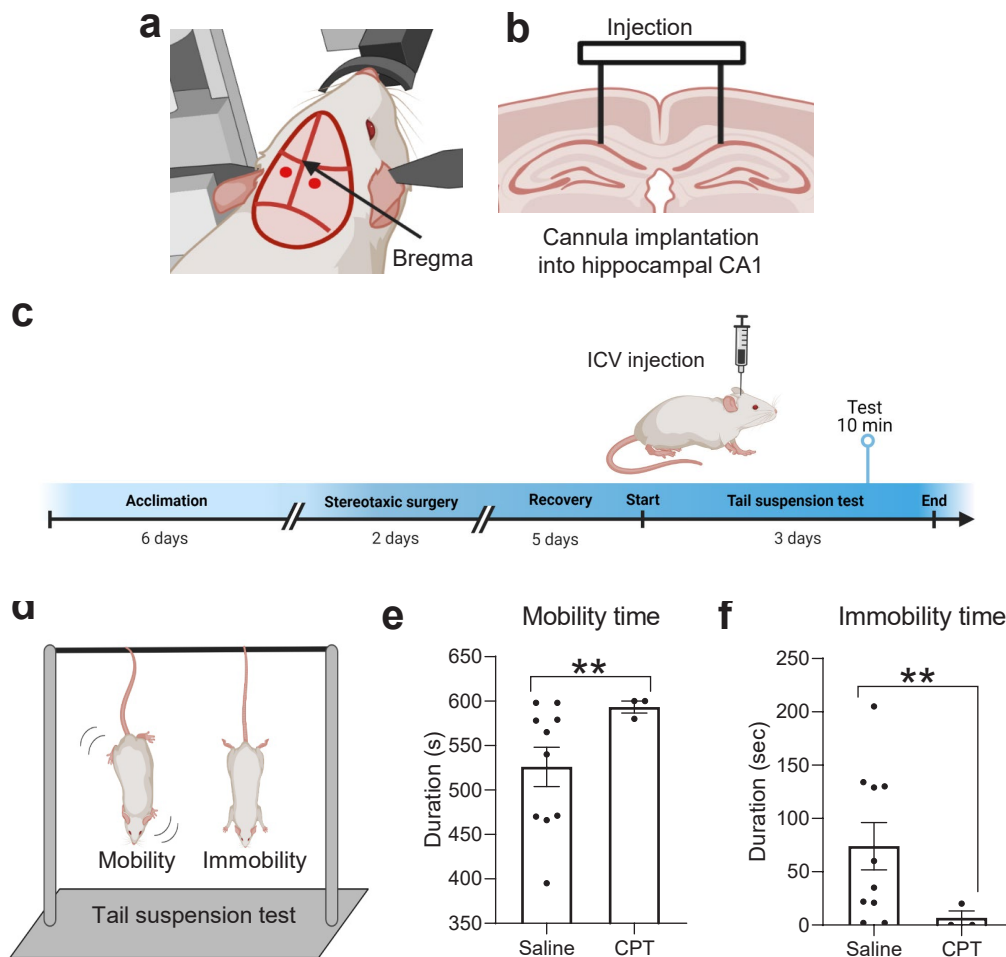


Fig. 6. An intrahippocampal infusion of CPT reduced immobility in the TST. (a) Stereotaxic surgery. (b) Cannula implantation for the infusion of CPT. (c) Timeline of the TST experiment. (d) Experimental explanation of TST. (e) Bar graph summarizing the mobility durations (unpaired t test, $**p=0.01$). (f) Bar graph summarizing the immobility durations (unpaired t test, $**p=0.01$).

endorphins, which is related to reduced stress [19]. The hippocampus performs a specific function in stress regulation in addition to memory and learning [20]. In addition, because the activation of Avpr1b regulates the release of stress hormones to relieve stress [21, 22] and the expression of Avpr1b in the hippocampus [7], the activity of Avpr1b in the hippocampus might regulate stress. We propose that dorsal hippocampal Avpr1b is involved in a specific mechanism that regulates stress.

The binding of plasma corticosterone secreted from the kidney adrenal cortex to the gonadotropin receptor (GR) in the hippocampus reduces stress levels through negative feedback [23]. Corticotropin-releasing hormone (CRH) neurons located in the paraventricular nucleus (PVN) release CRH and regulate ACTH release from the pituitary. ACTH activates the release of corticosterone from the adrenal cortex. Subsequently, the increased corticotropin levels bind GR, which, in turn, functions as a transcription

factor to repress the pathway and reduce stress levels [24]. Avp may act via negative feedback to reduce stress levels by facilitating the GR pathway. The reduced glutamate release in the hippocampal CA3 regulates the stress axis, namely, the hypothalamus-pituitary gland-adrenal cortex axis. The reduced glutamate release in the hippocampus may regulate hormone release from the PVN and pituitary gland. Glutamate release measured from hippocampal CA1 pyramidal neurons was also reduced. Consistently, our results are similar to previous findings.

Avp significantly increased the numbers of APs recorded from hippocampal CA1 pyramidal neurons from 20- to 30-day-old SD rats. An intracellular infusion of GDP- β -S, a nonhydrolyzable G protein, and U73122, a phospholipase C (PLC) inhibitor, does not increase Avp-induced excitability, suggesting that Avp-induced excitability requires PLC β [10]. In the current study, CPT decreased both the frequency and amplitude of sEPSCs in hippocampal

neurons from SD rats. Avp substantially increased the number of APs by inhibiting the inward rectifying potassium channel (GIRK, [10]), but CPT slightly increased the number of APs under only one condition (Fig. 2), suggesting that the affinity of CPT to Avp receptors differs from that of Avp. A possible explanation is the multireceptor activation of different subtypes of Avpr by CPT. For example, the interaction between CPT and Avpr1b is quite different in dissociating G proteins to regulate the mechanism of AP firing. High Avp1b expression is detected in the CA2 region of the dorsal hippocampus, while it is expressed at equal levels in the dorsal and ventral CA3, CA2 and CA1. Avpr1b expression has also been detected in dense fibers and numerous glial cells in the stratum oriens and stratum radiatum [25], suggesting that Avpr1b is expressed in the presynaptic membrane, postsynaptic membrane and glial membrane. Even if CPT possesses anti-stress properties, more research is needed to determine whether this peptide migrates into the brain. This study is important for the development of CPT as an anti-stress agent.

An intrahippocampal infusion of CPT, an Avp analog, exerted an anti-stress effect. The mechanism involved a reduction in glutamate release from the presynaptic terminals of hippocampal neurons. The CA1 CPT treatment mimicked Avp release from CRH neurons of the PVN in the presence of increasing stress levels during the TST. The primary significance of this work are that a peptide generated from octopus-derived genetic information exerts anti-stress effects in mammals and that the mechanism involves blockade of glutamate release in hippocampal neurons.

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COMPETING FINANCIAL INTERESTS

The South Korean government certified the patent (South Korea Patent 10-2239947) titled "Compositions for preventing, improving or treating cognition and stress-related disease comprising cephalotocin" on April 08, 2021, as a beneficiary. YJ Kim and DH Woo are listed as inventors in the patent application.

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AUTHOR CONTRIBUTIONS

YJK performed the in vitro and ex vivo electrophysiological experiments and rodent behavioral experiments. SJ and SHJ developed the initial concept and contributed to the experimental design based on a previous study investigating CPT (not yet published). DHW conceived the study design and wrote the manuscript.

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