รายงานวิจัยฉบับสมบูรณ์

โครงการ: ผลของคอร์ติคาลสเปรดดิงดีเพรสชันต่อการสื่อประสาทและการเปลี่ยนแปลงของการสื่อประสาทในฮิปโปแคมปัส

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รายงานวิจัยฉบับสมบูรณ์

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นักวิจัยที่ปรึกษา

สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และสำนักงานกองทุนสนับสนุนการวิจัย

สารบัญ

ABSTRACT

Cortical spreading depression (CSD) is a slowly propagated wave of depolarization on the cortex followed by a subsequent sustained suppression of spontaneous neuronal activity. CSD is originally linked to the aura phase of migraine. However, some clinical evidences suggest the links between CSD in migraine patients and associated symptoms such as amnesia.

This study aimed to investigate the alteration of hippocampal long-term plasticity and basal synaptic transmission induced by repetitive CSDs. There are some relations between migraine aura and amnesic attack. CSD may be responsible for this hippocampus-related symptom. However, the precise role of CSD on hippocampus activity has not been studied.

In the present study, Wistar rats were divided into 2 groups, namely CSD and control. Repetitive CSDs were induced *in vivo* by topical application of solid KCl on parietal cortex. Direct current potential was recorded in frontal cortex. Forty-five minutes later, ipsilateral hippocampus was removed and prepared for transverse hippocampal slice. A series of in vitro electrophysiological studies was performed to investigate hippocampal synaptic transmission and plasticity. In the other preparation, direct current potential was recorded in hippocampus during CSD induction.

The results showed that solid KCl application evoked repetitive CSDs. These SDs propagated to hippocampus as well. These SDs traveling across cortical and hippocampal tissues had an influence on hippocampal synaptic efficacy. Magnitude of hippocampal long-term potentiation (LTP) decreased in slice obtained from CSD-induced rat. Furthermore, slice obtained from CSD-induced rat showed a reduction in the excitability as evident by a decrease in post-synaptic AMPA receptor output.

In conclusions, repetitive CSDs altered hippocampal transmission by suppressing postsynaptic AMPA receptor function. This modulation could participate in CSD-induced LTP impairment. The finding from this study may explain hippocampus-related symptoms occurring during migraine attack.

Key words: Cortical spreading depression, hippocampus, long-term potentiation, synaptic transmission

บทคัดย่อ

ปรากฏการณ์คอร์ติคาลสเปรดดิงดีเปรสชัน (cortical spreading depression; CSD) เป็นปรากฏการณ์เปลี่ยนแปลงของประจุในผิวสมอง ทำให้เกิดการเพิ่มระดับของศักย์ไฟฟ้าในเซลล์ประสาทอย่างรวดเร็วตามด้วยการลดระดับที่เกิดขึ้นเป็นเวลายาว CSD จะเกิดขึ้นพร้อมกับอาการปวดหัวในผู้ป่วยไมเกรน และเป็นที่ยอมรับกันอย่างกว้างขวางว่า CSD มีความสัมพันธ์กับการเห็น แสงวาบ (aura) ในผู้ป่วยไมเกรน มีรายงานระดับคลินิกบ่งชี้ว่าการเกิด CSD ในผู้ป่วยไมเกรนนำไปสู่อาการหลงลืม

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของ CSD ต่อการสื่อประสาทและการเปลี่ยนแปลงของการสื่อประสาทในฮิปโปแคมปัส มีรายงานวิจัยบ่งชี้ว่า โรคไมเกรนออร่าเกี่ยวข้องกับอาการหลงลืม CSD ซึ่งเป็นปรากฏการณ์พื้นฐานสำหรับไมเกรนออร่า จึงมีความเป็นไปได้สูงที่จะมีผลต่ออาการต่าง ๆที่เกิดขึ้นในฮิปโปแคมปัส แต่ทว่ารายละเอียดเกี่ยวกับผลเหล่านี้ยังไม่มีการศึกษาที่อธิบายได้ชัดเจน

ในการศึกษานี้ ได้มีการแบ่งกลุ่มหนูแรทขาวออกเป็น 2 กลุ่ม ได้แก่ กลุ่ม CSD และกลุ่มคอนโทรล ปรากฏการณ์ CSD ถูกกระตุ้นให้เกิดซ้ำไปมาโดยการวางผลึกโพแทสเซียมคลอไรด์บนผิวสมองส่วนบนของหนูแรท และวัดความต่างศักย์ไฟฟ้าที่เกิดขึ้นบนผิวสมองส่วนหน้ารวมถึงในฮิปโปแคมปัส หลังจากนั้นนำเอาฮิปโปแคมปัสซีกที่ถูกกระตุ้นออกมาจัดทำเป็นแผ่นบาง ๆสำหรับการศึกษาการสื่อประสาทและการเปลี่ยนแปลงของการสื่อประสาทโดยวิธีทางไฟฟ้าสรีรวิทยา

ผลการทดลองแสดงให้เห็นว่า การวางผลึกโพแทสเซียมคลอไรด์สามารถกระตุ้นให้เกิด CSD แพร่กระจายไปตามผิวสมองและเข้าไปถึงในฮิปโปแคมปัส อีกทั้งมีผลต่อการสื่อประสาทในฮิปโปแคมปัส กล่าวคือ ปรากฏการณ์การเปลี่ยนแปลงการสื่อประสาทแบบเพิ่มขึ้นในระยะยาว (long-term potentiation:LTP) ในหนูแรทกลุ่ม CSD มีขนาดเล็กลงเมื่อเทียบกับหนูกลุ่มคอนโทรล บ่งชี้ว่าการตอบสนองของฝั่งตัวรับของจุดเชื่อมประสาทโดยเฉพาะตัวรับกลูตาเมทชนิด AMPA ถูกระงับโดย CSD

สรุปได้ว่า CSD มีผลต่อการสื่อประสาทในฮิปโปแคมปัสเนื่องจากระงับการทำงานของตัวรับกลูตาเมทชนิด AMPA ซึ่งปรากฏใน LTP ที่ลดลง องค์ความรู้ใหม่ที่ได้จากการศึกษานี้สามารถอธิบายอาการหลงลืมที่เกิดขึ้นระหว่างการปวดหัวไมเกรนได้

Key words: คอร์ติคาลสเปรดดิงดีเพรสชัน, ฮิปโปแคมปัส, การเปลี่ยนแปลงการสื่อประสาทแบบเพิ่มขึ้นในระยะยาว, การสื่อประสาทผ่านจุดเชื่อมประสาท

สรุปรายงานความก้าวหน้าของโครงการ

1. การดำเนินงาน ☑ ได้ดำเนินงานตามแผนที่วางไว้

☐ ได้ดำเนินงานล่าช้ากว่าแผนที่วางไว้

☐ ได้เปลี่ยนแปลงแผนงานที่วางไว้ดังนี้

2. รายละเอียดผลการดำเนินงานของโครงการ

2.1 กิจกรรมที่วางแผนไว้

- Study the effect of cortical spreading depression on input-output stimulation
- Study the effect of cortical spreading depression on paired-pulse stimulation
- Study the effect of cortical spreading depression on STP
- Data collection and analysis
- Writing articles

2.2 กิจกรรมที่ทำได้จริง

- Study the effect of cortical spreading depression on input-output stimulation
- Study the effect of cortical spreading depression on paired-pulse stimulation
- Study the effect of cortical spreading depression on STP
- Data collection and analysis
- Writing articles

2.3 ในกรณีที่ท่านมีความจำเป็นต้องเปลี่ยนแผนงาน ขอให้ระบุแผนการดำเนินงานที่จะทำใน 6 เดือนข้างหน้า พร้อมทั้งทำแผนกิจกรรมเดิม เปรียบเทียบกับแผนกิจกรรมใหม่ที่จะทำ รวมทั้งบอกเหตุผลในการเปลี่ยนแผนงาน

ผู้รับทุนขอเปลี่ยนแผนงานจาก “ผลของภาวะพร่องซีโรโตนินต่อการสื่อประสาทและการเปลี่ยนแปลงของการสื่อประสาทในฮิปโปแคมปัส” เป็น“ผลของคอร์ติคาลสเปรดดิงดีเพรสชันต่อการสื่อประสาทและการเปลี่ยนแปลงของการสื่อประสาทในฮิปโปแคมปัส” เนื่องจากผลการทดลองทางไฟฟ้าสรีรวิทยาในหนูแรทที่ก่อให้เกิดภาวะพร่องซีโรโตนิน ไม่แตกต่างอย่างมีนัยสำคัญทางสถิติ ตามในรายงานรอบ 18 เดือนนั้น ผู้วิจัยได้ดำเนินการวิจัยได้ครบตามแผนการที่วางใหม่ดังที่กล่าวไว้ในข้อ 2.1 และ 2.2

3. สรุปผลการดำเนินงานของโครงการโดยย่อ

ในงานวิจัยนี้ ผู้ทดลองแบ่งหนูเป็นสองกลุ่ม คือกลุ่มหนูที่เหนี่ยวนำให้เกิด CSD โดยการวาง KCl บนผิวสมอง และกลุ่มหนูปกติ ซึ่งวาง NaCl บนผิวสมองก่อนการทดลองเป็นเวลา 45 นาที ระหว่างการกระตุ้น CSD นี้ ผู้ทดลองได้บันทึกสัญญาณไฟฟ้าที่เกิดขึ้นในผิวสมองส่วนหน้าและฮิปโปแคมปัส ผลการทดลองสรุปได้ว่า การกระตุ้น CSD ก่อให้เกิดคลื่นไฟฟ้าแพร่กระจายไปทั้งใน 2 บริเวณ โดยที่คลื่นไฟฟ้าที่แพร่กระจายเข้าไปในฮิปโปแคมปัสมีความถี่น้อยกว่าและขนาดเล็กกว่าคลื่นไฟฟ้าที่แพร่กระจายในผิวสมองส่วนหน้าอย่างมีนัยสำคัญทางสถิติ

หลังจากนั้นหนูจะถูกทำให้สลบแล้วตัดหัว เพื่อนำสมองส่วนฮิปโปแคมปัสมาตัดให้เป็นชิ้นเนื้อสำหรับการศึกษาทางด้านสรีรวิทยาทางไฟฟ้า เมื่อค่า baseline ของ field excitatory synaptic potential (fEPSP) จากการกระตุ้น Schaffer collaterals คงที่เป็นเวลา 30 นาทีแล้ว ผู้ทดลองใช้การกระตุ้นความถี่สูง HFS (high-frequency stimulation) เพื่อเหนี่ยวนำให้เกิด LTP ในฮิปโปแคมปัสที่นำมาจากหนูกลุ่มปกติและหนูกลุ่ม CSD จากการทดลองพบว่าการกระตุ้นความถี่สูงเหนี่ยวนำให้เกิด LTP ในหนูทั้งสองกลุ่ม โดยหนูกลุ่ม CSD มีระดับการเกิด LTP (คำนวณจากค่าเฉลี่ย 50-60 นาที หลังจากการกระตุ้นความถี่สูง) น้อยกว่าหนูกลุ่มปกติอย่างมีนัยสำคัญทางสถิติ

ผู้ทดลองพบว่า ค่าความแรงไฟฟ้าที่กระตุ้นให้เกิด baseline ของ fEPSP ในหนูกลุ่ม CSD มีค่ามากกว่าหนูกลุ่มปกติ จึงตั้งสมมุติฐานว่า CSD น่าจะทำให้ประสิทธิภาพในการสื่อประสาทพื้นฐานของฮิปโปแคมปัสเปลี่ยนแปลงไป ซึ่งจะนำไปสู่การลดลงของ LTP เพื่อพิสูจน์สมมุติฐานนี้ผู้ทดลองดำเนินการวิจัยโดยเปรียบเทียบคุณสมบัติการสื่อประสาทพื้นฐานระหว่างหนูกลุ่มปกติและหนูกลุ่ม CSD ด้วยวิธีทางไฟฟ้าสรีรวิทยาต่อไปนี้

1) กระตุ้นแบบคู่ (Pair-pulse stimulation; PPS) --- อ้างอิงถึงปริมาณการหลั่งสารสื่อประสาทกลูตาเมต
2) กระตุ้นความถี่สูงในระยะสั้น (Short-term potentiation; STP) --- อ้างอิงถึงการทำงานที่เพิ่มขึ้นของตัวรับสารสื่อประสาทกลูตาเมตประเภท AMPA ที่ถูกกระตุ้นโดยตัวรับประเภท NMDA ระหว่างการเกิด LTP
3) Input/Output of AMPA receptor (I/O_{AMPA}) --- อ้างอิงถึงการตอบสนองตัวรับประเภท AMPA กล่าวคือ ความสะดวกของการผ่านไอออนเมื่อถูกกระตุ้นด้วยความแรงต่างๆ

จากผลทดลองการสื่อประสาทพื้นฐาน พบว่า ในหนูกลุ่ม CSD ค่า I/O_{AMPA} มีระดับลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูกลุ่มปกติ ในขณะที่ค่า PPS และ STP ไม่แตกต่างกัน

สรุปผลการทดลองทั้งหมดได้ว่า CSD มีผลยับยั้งการเกิด LTP ในฮิปโปแคมปัส โดยผ่านกลไกการระงับการตอบสนองของตัวรับกลูตาเมตประเภท AMPA ในฝั่งตัวรับของจุดเชื่อมประสาท

4. ความเห็นและข้อเสนอแนะ

ขอขอบคุณอาจารย์ เจ้าหน้าที่ และผู้เกี่ยวข้องทุกท่าน ทั้งที่จุฬาลงกรณ์มหาวิทยาลัยและที่สำนักงานกองทุนสนับสนุนการวิจัย ด้วยทุนวิจัยนี้ ผู้รับทุนสามารถดำเนินงานวิจัยได้อย่างราบรื่นและสรุปผลงานวิจัยได้สำเร็จตามเป้าหมายที่ตั้งไว้

ลงนาม.....

ผู้ช่วยศาสตราจารย์ ดร.ศักนัน พงศ์พันธุ์ผู้ภักดี

(หัวหน้าโครงการวิจัยผู้รับทุน)

ลงนาม.....

ศาสตราจารย์ นายแพทย์อนันต์ ศรีเกียรติขจร

EXECUTIVE SUMMARY

In the present study using the electrophysiological technique, the effect of repetitive CSDs on hippocampus functions was investigated. The following was the conclusions of our findings.

1. Topical solid KCl application on parietal cortex led to the repetitive negative potential shift characterized as CSD.
2. Topical solid KCl application on parietal cortex also led to the negative potential shift in hippocampus, suggesting the propagation of CSD into the hippocampus.
3. Repetitive CSD induction reduced hippocampal excitability, as evident by a decrease in postsynaptic AMPA receptor response.
4. Hippocampal synaptic plasticity was impaired following repetitive CSD induction, as indicated by reduction of LTP magnitude.

เนื้อหางานวิจัย

MATERIALS AND METHODS

Experimental Design

All male Wistar rats weighing 200-350 grams were divided into 2 groups, namely CSD and control, as described below. Direct current potential was recorded in vivo in CSD-induced rat or sham. After that, hippocampal slice was prepared for in vitro electrophysiological experiments.

1. CSD group

CSD was induced in vivo for 45 minutes by topical application of KCl crystal on exposed parietal cortex. Then, the rat was decapitated and hippocampal slices were prepared for electrophysiological studies. Field EPSPs were recorded in hippocampal CA1. The electrophysiological studies include LTP (n = 7) and basic synaptic transmission (n = 6).

2. Control group

Solid NaCl was topically applied on parietal cortex surface for 45 minutes. Then, the rat was decapitated and hippocampal slices were prepared for electrophysiological studies, including LTP (n = 7) and basic synaptic transmission (n = 6).

In the other preparation, direct current potential was recorded *in vivo* in hippocampus during CSD induction (n = 4).

Animals

Adult male Wistar rats (National Laboratory Animal Centre, Mahidol University, Thailand) weighing 200-350g were housed in stainless steel cages with free access to food (regular dry rat food) and water. The animals were maintained in a temperature-controlled room with 12-hour dark/light cycle. They were allowed to acclimate to the housing environment at least 7 days before experiments.

Animal Preparation

Each rat was anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and fitted with an intratracheal tube. Cannula was inserted into the femoral vein for later infusion of anesthetic. Additional doses of anesthetics were given as required to maintain surgical anesthesia based on response to tail pinch. Rat was placed in a stereotaxic apparatus. The skull above somatosensory and temporal cortices was exposed. For induction of CSD, a craniotomy of 2-mm in diameter was performed at right parietal bone (6 mm posterior to bregma and 2 mm lateral to midline). The bone was carefully drilled using slow speed, saline-cooled technique in order to minimize surgical irritation of the neurons. For CSD recording, a cranial window of 2 mm diameter was made at right frontal bone (3 mm anterior to bregma and 2 mm lateral to midline). A recording glass microelectrode for detecting negative direct current potential was inserted into frontal neocortex at the depth of 500 μm after careful removal of the dura mater. In another experiment, SD in hippocampus was recorded instead of CSD. For hippocampal SD recording, a cranial window of 2 mm diameter was made at right parietal bone (3 mm posterior to bregma and 2 mm lateral to midline). A recording glass microelectrode was inserted at the depth of 2.8 mm into the CA1 area of the hippocampus.

A Biopac amplifier (Biopac) with Acknowledge acquisition software was used for direct current recordings. Signals were amplified, low-pass filtered at 1 kHz, digitally sampled at 10 kHz, and stored for offline analysis.

Induction of CSD

Multiple waves of CSD were elicited by topical application of solid KCl (3 mg) on exposed cortex surface. In control group, NaCl crystal was used instead. Forty-five minutes after elicitation of CSD waves, the rat was decapitated and transverse hippocampal slices were prepared as described below.

Slice Preparation

The entire ipsilateral hippocampus was quickly removed from the brain. Coronal hippocampal brain slices (400 μm thickness) were cut using a Vibratome tissue slicer in ice-cold artificial cerebrospinal fluid (ACSF). The ACSF contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 MgSO4, 2.5 CaCl2, 1.0 NaH2PO4, 26.2 NaHCO3, 11 glucose, and .1 picrotoxin, a GABAA receptor antagonist. The ACSF was bubbled continuously with carbogen (95% O_2/5% CO_2). Fresh slices were placed in a humidified interface-type holding chamber and recovered for at least 1.5 h before electrophysiological experiments.

Electrophysiological Recording

A single slice was transferred into a submerge-type recording chamber, fixed with a nylon net, and submerged beneath the continuously perfusing ACSF (at a rate of 1.5–2.0 ml/min) saturated with carbogen. A cut was made to separate the CA3 region from the CA1 region to avoid epileptiform activity from the CA3 region. All experiments were performed at room temperature (25 °C).

A bipolar tungsten stimulating electrode was placed in the stratum radiatum at 200–300 μm from the recording site. Square-pulse stimuli of .2 ms duration at .1 Hz (1 pulse every 10 s) were delivered to the slice through the bipolar tungsten electrode to activate the Schaffer-collateral pathway projecting to CA1. Glass microelectrode with 2-8 MΩ resistance containing 3 M NaCl was positioned parallel to the stimulating electrode in stratum radiatum to record presynaptic fiber volleys followed by fEPSPs. An attempt was made to maintain similar orientation of the electrodes relative to the pyramidal cell layer and dentate gyrus to minimize changes in fEPSP properties attributable to electrode positioning.

For baseline recordings, stimulus intensity was adjusted to evoke fEPSPs of .15 to .20 mV/ms slopes. Only slice that gave a fEPSP amplitude of more than 1 mV and that was stable for at least 30 min was included in this study. All slices that failed to stabilize within 90 min were rejected.

An EPC-10 amplifier (HEKA) with Patchmaster software was used for electrophysiological recordings. Signals were amplified, low-pass filtered at 1 kHz, digitally sampled at 10 kHz, and stored for offline analysis.

Output ที่ได้จากโครงการ

RESULTS

CSD

After applying solid KCl (3 mg) on parietal cortex, a series of negative depolarization shifts characterized as spreading depression was recorded in frontal cortex surface (fig 1). The total numbers of CSD waves occurring within 45 min were 9.23 ± 1.74 waves. The amplitude, the duration, and the area under the curve of each wave were 34.62 ± 6.78 mV, 69.67 ± 19.60 s, and 712.35 ± 187.77 mV·s, respectively. The interval between each wave was 5.10 ± 1.49 min.

Fig. 1: The tracing showing the DC shift in frontal cortex surface induced by KCl application (Scale bar: 5 min; 10 mV)

Hippocampal SD

After applying solid KCl (3 mg) on parietal cortex, a series of negative depolarization shifts characterized as spreading depression was recorded in hippocampus (fig 2). The total numbers of CSD waves occurring within 45 min were 3.67 ± .58 waves. The amplitude, the duration, and the area under the curve of each wave were 24.79 ± 3.51 mV, 74.14 ± 28.93 s, and 810.46 ± 217.11 mV·s, respectively. The interval between each wave was 10.53 ± 2.27 min.

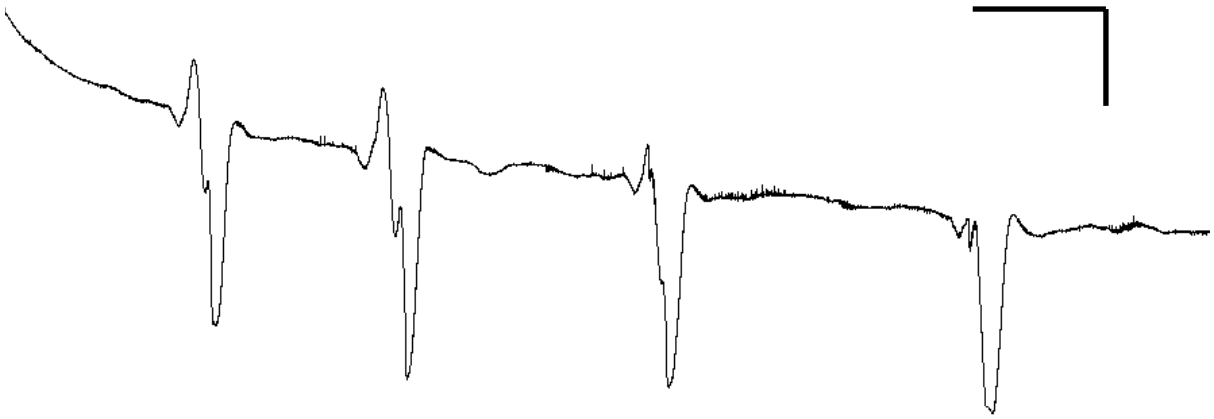

Fig. 2: The tracing showing the DC shift in hippocampus induced by KCl application (Scale bar: 5 min; 10 mV)

Investigation of cortical and hippocampal SD characters showed no difference in the duration, and the AUC. However, the total numbers of CSD waves and the amplitude in hippocampus were significantly lower than those in cortex. Moreover, the interval between each wave was significantly longer in hippocampus when compared to that in cortex.

	CSD	Hippocampal SD	*P*-value
Total numbers (waves/45 min)	9.23 ± 1.74	3.67 ± .58	0.004
Amplitude (mV)	34.62 ± 6.78	24.79 ± 3.51	0.04
Duration (s)	69.67 ± 19.60	74.14 ± 28.93	0.83
Area under the curve (mV·s)	712.35 ± 187.77	810.46 ± 217.11	0.56
Wave interval (min)	5.10 ± 1.49	10.53 ± 2.27	0.03

Table. 1 Comparing the electrophysiology variables related to SD between cortex and hippocampus

LTP

After recording stable baseline response for at least 30 min, a conditioning tetanic stimulus (100 Hz for 1 sec) was delivered to the Schaffer collateral fibers in hippocampal slices. In both group, tetanic stimulation produced a rapid, stable and lasting enhancement of the slope of the evoked potential in all slices (Fig. 3A). The potentiation rose within 1–2 min and stabilized within another 18–22 min after the stimuli. However, magnitude of LTP in the hippocampus was influenced by repetitive CSD induction. In CSD-induced rat, the magnitude of LTP was significantly decreased. (P = 0.04, Student's t-test; Fig.3B). The levels of LTP in CSD-induced and control groups were 119.8 ± 16.2 and 138.2 ± 9.8 percent, respectively (n = 7 each group).

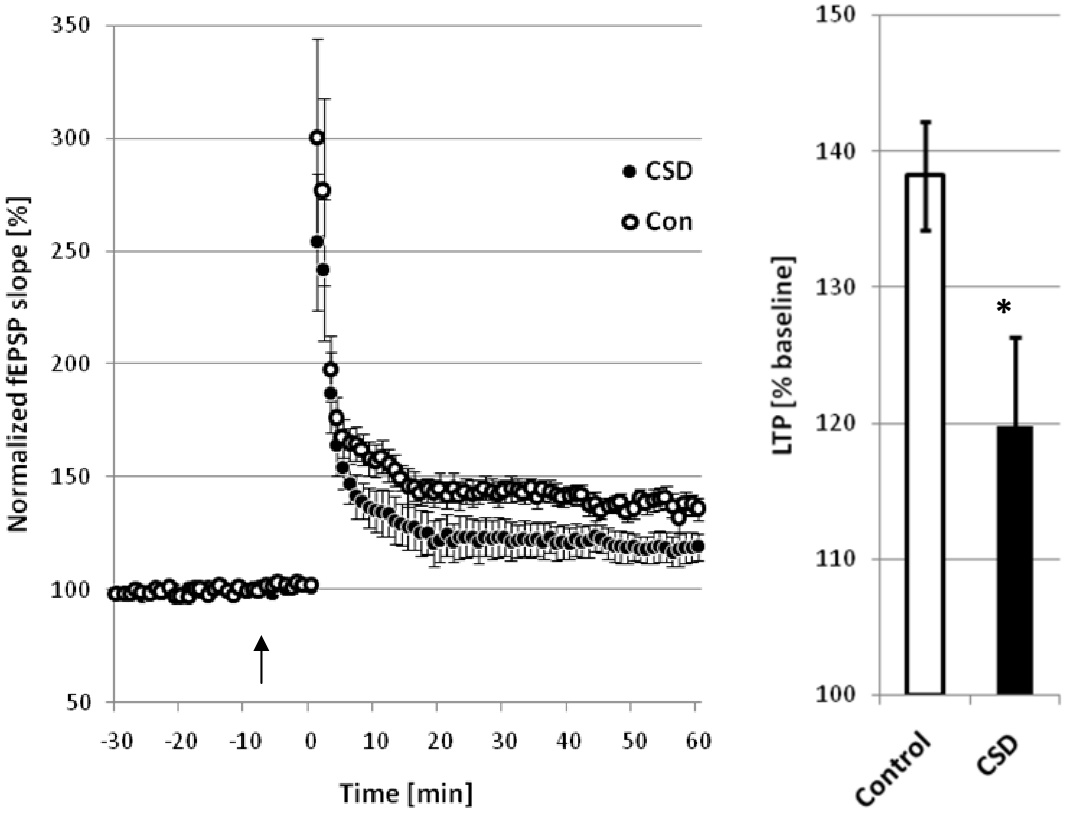

Fig. 3: LTP of fEPSP in the hippocampal slices obtained from CSD-induced and control rats. Tetanic stimulation (a train of 100 pulses at 100 Hz) produced a rapid and stable potentiation in the slope of the evoked field potentials. (A) Close circles and open circles showed the evoked fEPSP in hippocampal slices of CSD-induced and control rats, respectively. Arrow showed the time of tetanic stimulation. The time points given referred to LTP induction. (B) Bar graph showed magnitude of LTP, calculated as a percentage of baseline mean response slope. Note the significant reduction of LTP magnitude in CSD-induced rats (* P = .04, Student's t-test, n = 7 each group).

General neuronal excitability

In all electrophysiological protocols, stimulus intensity was adjusted to evoke fEPSPs of .15 to .20 mV/ms slopes as baseline response. Evoked fEPSP slopes obtained from baseline recording in the CSD slices were comparable with those recorded in the control slices (0.165 ± .029 and 0.156 ± 0.029 mV/ms, respectively; *P* = 0.534, n = 9 each group). However, the stimulation intensities needed to evoke the same fEPSP responses were higher in slices obtained from CSD-induced rats than those from controls (0.329 ± .047 and 0.282 ± .035 V, respectively; *P* = 0.031, n = 9 each group; Fig. 4). These data suggested that CSD had a significant effect on evoked neuronal excitability.

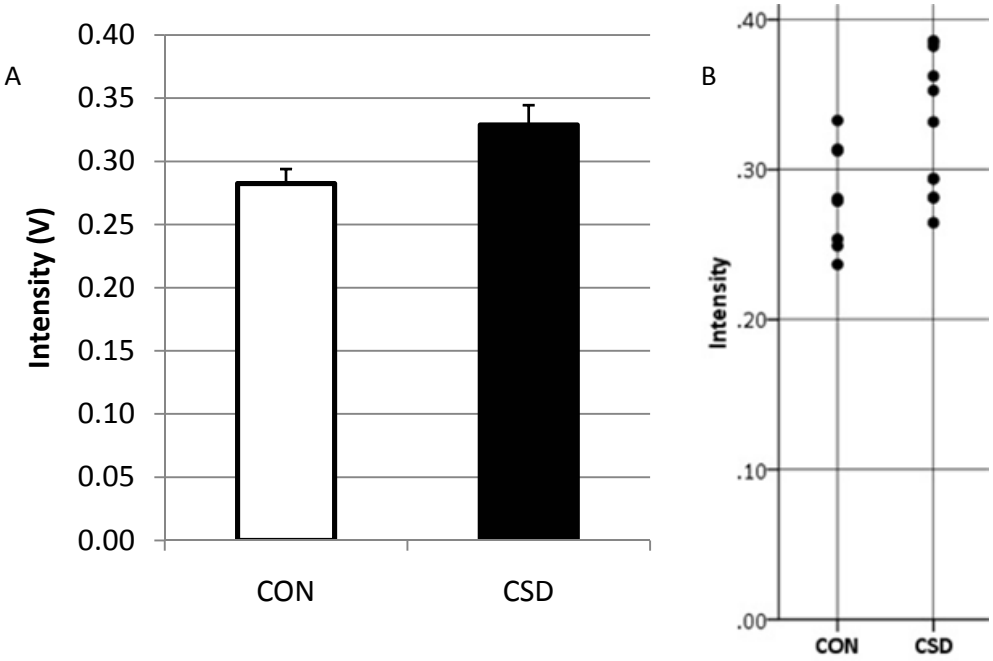

Fig. 4: CSD reduced evoked neuronal excitability of hippocampus as indicated by increased stimulation intensity. (A) Bar graph showed stimulation intensity evoking baseline fEPSP. (B) Dot-plot showed distribution of stimulation intensities in CSD and control slices.

In the next experiment, we performed a set of electrophysiological protocols to investigate any alteration in excitatory synaptic transmission at the Schaffer collateral-CA1 (SC-CA1) synapse in hippocampal slices obtained from CSD and control rats. Two of these protocols were paired-pulse facilitation and short-term potentiation (STP).

Paired-pulse facilitation (PPF)

PPF has been shown to be presynaptic in origin without any change in postsynaptic effectiveness. PPF protocol included 4 two-pulse pairs with decreasing inter-pulse interval (400, 200, 100, and 50 ms). Ratio of facilitation was calculated by dividing fEPSP slope elicited by the second pulse with fEPSP slope elicited by the first pulse.

In the experiment, we tested whether *in vivo* induction of CSD affected presynaptic release probability at SC-CA1 synapses in acute hippocampal slices by measuring the PPF index of fEPSPs. The result showed that no overall PPF profile change was observed between CSD and control slices (P = 0.551 by one-way repeated measures ANOVA, Fig. 5). This result indicated that presynaptic responses at SC-CA1 synapses were intact in CSD-induced rats.

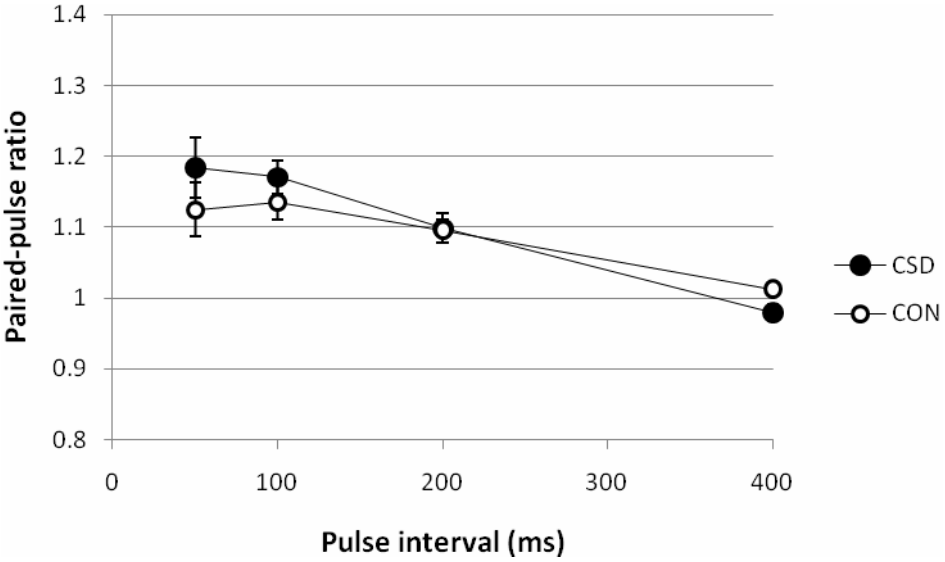

Fig. 5: PPF was not altered in CSD-induced rats compared with control rats (n = 10 each).

Short-term potentiation (STP)

In the STP experiment, a highly-selective NMDA receptor antagonist, APV, was applied by bath-application (25 μM) during tetanic stimulation (from -5 to 2 min). Tetanic stimulation induced a rapid and stable potentiation in the slope of the evoked field potentials. In contrast with LTP induction, fEPSP slopes decayed to baseline level in both CSD and control slices. There was no significant difference between fEPSP slope from both groups at each time point (Student's t-test, P > 0.05; n = 5 each group).

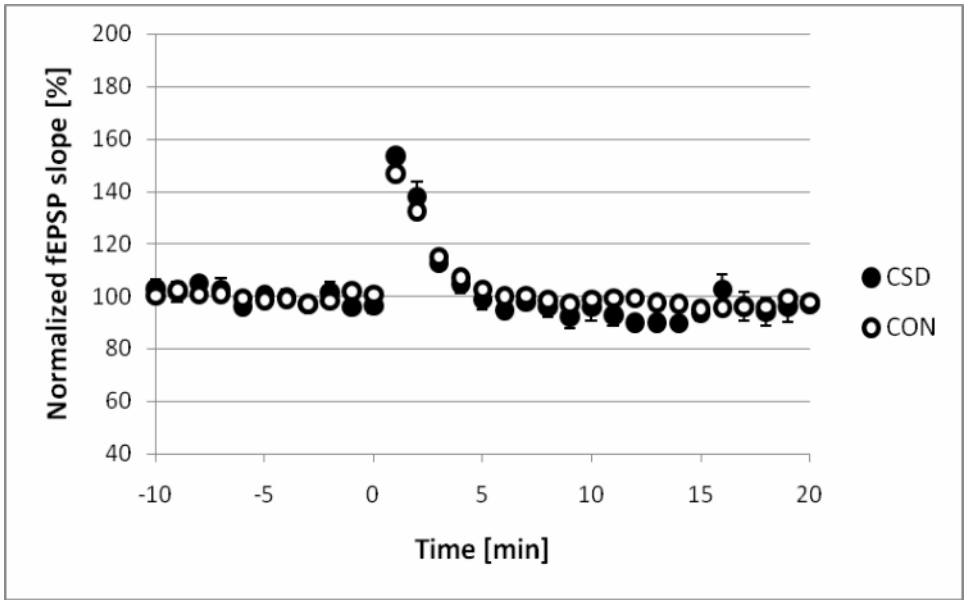

Fig 6: STP of fEPSP in the hippocampal slices obtained from CSD-induced and control rats. Tetanic stimulation (a train of 100 pulses at 100 Hz) produced a rapid and stable potentiation in the slope of the evoked field potentials. (n = 5 each group).

I/O of AMPA receptor

In this experiment, basal synaptic transmission at SC-CA1 synapse was examined using I/O response of AMPA receptor-mediated fEPSP. A highly-selective NMDA receptor antagonist, APV, was applied by bath-application (25 μM) to isolate AMPA receptor-mediated fEPSP. After recording stable baseline response for at least 15 min, an I/O response was recorded by stepping the stimulation intensity. The result showed that the overall I/O curve of AMPA receptor was significantly reduced in hippocampal slices obtained from CSD rats ($P = 0.032$, repeated-measures ANOVA, $n = 6$ each group; Fig. 7). The slice obtained from CSD-induced rat showed a significant reduction in evoked AMPA receptor-mediated fEPSPs across a range of stimulus intensities. This result indicated that AMPA receptor responses at SC-CA1 synapses were depressed in CSD-induced rats.

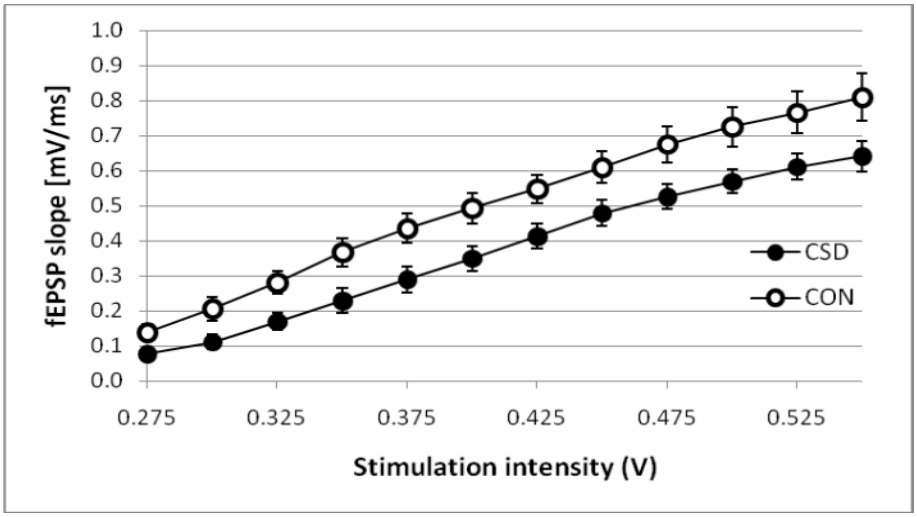

Fig. 7: I/O of AMPA receptor-mediated fEPSP in the hippocampal slices obtained from CSD-induced and control rats. Close circles and open circles showed the evoked fEPSP in hippocampal slices of CSD-induced and control rats, respectively. Note the significant reduction of AMPA receptor synaptic transmission in CSD-induced rats (P = .032, repeated-measures ANOVA, n = 6 each group).

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Nociceptin/Orphanin FQ modulates cortical activity and trigeminal nociception

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Background: Alterations in the levels of Nociceptin/orphanin FQ (N/OFQ) have been reported in patients with primary headaches, including migraines and cluster headaches. These clinical observations suggest that N/OFQ is involved in the pathogenesis of primary headaches.

Objectives: The present study was conducted to determine the role of N/OFQ in the control of trigeminal nociception and cortical excitation.

Methods: Cortical spreading depression (CSD) was elicited in Wistar rats by cortical application of potassium chloride, and electrocorticograms were recorded. N/OFQ was administered via an intracisternal injection. The presence of CSD-evoked trigeminal nociception was determined with Fos and transient receptor potential vanilloid 1 (TRPV1) immunoreactivity.

Results: N/OFQ produced a biphasic effect on CSD generation, characterized by an initial attenuation followed by delayed potentiation. The amplitude of CSD waves were lower in the initial period but became increased in the later period. The total number of CSD waves recorded in one hour was greater in the N/OFQ-treated group. Exposure to N/OFQ significantly increased the number of Fos-immunoreactive cells in the trigeminal nucleus caudalis and the number of TRPV1-immunoreactive cells in the trigeminal ganglia, indicating the enhancement of trigeminal nociception.

Conclusion: These results indicate that N/OFQ can lead biphasic effect characterized by an initial inhibition, and delay potentiation that eventually intensify CSD-evoked trigeminal nociception.

Keywords: migraine; nociceptin; N/OFQ; cortical spreading depression, trigeminal nociception

Abbreviations: N/OFQ = nociceptin/orphanin FQ; ORL1 = Orphan opioid receptor-like 1; CSD = cortical spreading depression; TRPV1 = transient receptor potential vanilloid 1; TG = trigeminal ganglia; Fos = protein encoded by the c-Fos immediate early gene; TNC = trigeminal nucleus caudalis

INTRODUCTION

Nociceptin/orphanin FQ (N/OFQ) is one of several neuropeptides that are expressed in the nociceptive system, including the trigeminal pathway. In the rat central nervous system (CNS), this peptide is similar to dynorphin A in both structure and distribution.[1] N/OFQ selectively binds to the N/OFQ receptor, a G-protein coupled receptor previously known as the opioid receptor-like receptor 1 (ORL1). This receptor belongs to the opioid receptor family and is expressed widely in both the CNS and peripheral nervous tissue.[2] In the CNS, the N/OFQ receptor is particularly abundant in the cerebral cortex, limbic system and several other areas involved in pain perception.[3]

N/OFQ immunoreactivity and ORL1 mRNA have been detected in human and cat trigeminal ganglia and have been shown to co-localize with the calcitonin gene related peptide (CGRP), substance P, nitric oxide synthase and pituitary adenylate cyclase activating peptide.[4] This anatomical colocalization suggests that N/OFQ plays a role in trigeminal sensory transmission and vascular regulation. Because these two processes are crucial in the control of trigeminovascular nociception, it is likely that N/OFQ is involved in the pathogenesis of vascular headaches. This hypothesis is supported by clinical observations; plasma levels of N/OFQ are decreased in patients with migraine without aura compared to non-headache controls, and these levels are further reduced during the initial period of migraine onset.[5] Reductions in the plasma concentration of this peptide are also observed during cluster headaches.[6] These suggest that N/OFQ levels during the migraine and cluster period may contribute to a defective regulation of trigeminal activity. The role of N/OFQ in the modulation of cortical activity and trigeminal nociception, however, remains unclear.

The objective of the present work was to determine the role of N/OFQ in the pathogenesis of migraines by investigating its effects on the development of cortical spreading depression (CSD) and the expression of c-Fos and transient receptor potential vanilloid 1 (TRPV1). Immunohistochemistry was used to identify the presence of c-Fos protein, an indicator of trigeminal nociceptive neuron activation, in the trigeminal nucleus caudalis (TNC). TRPV1 is a non-selective cation channel that is activated by a wide variety of exogenous and endogenous physical and chemical stimuli. TRPV1 may

play an important role in the pathogenesis of migraines because it integrates painful stimuli ranging from noxious heat to endovanilloids involved in inflammation.[7] We hypothesized that N/OFQ regulates cortical activity and trigeminal nociception. Using electrophysiological recordings of CSD development and immunohistochemical analysis of the trigeminal system, we investigated whether intracisternal administration of N/OFQ can modulate trigeminal nociception.

MATERIALS AND METHODS

Adult male Wistar rats weighing 200 to 300 g were obtained from the National Laboratory Animal Centre (Mahidol University, Thailand). The animals were housed five per cage in stainless steel bottom cages kept in a well-ventilated room. The room was equipped with an automated lighting timer, and the temperature was held at 25°C. The animals were provided *ad libitum* access to food and tap water. Protocols for this study were approved by the Faculty of Medicine Ethics Committee at Chulalongkorn University.

Chemicals

Pentobarbital sodium (Nembutal®) was purchased from Sanofi (Thailand). Normal saline was purchased from King Chulalongkorn Memorial Hospital Product Public (Thailand). Purified N/OFQ was purchased from Tocris (UK). Potassium chloride (KCl), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), sodium carbonate ($NaHCO_3$), hydrogen peroxide (H_2O_2) and ethanol were purchased from Merck (USA). Liquid DAB+ and Envision+ System-HRP (DAB) for use with Rabbit Primary Antibodies were purchased from Dako (Denmark). Paraformaldehyde (95%) was purchased from Sigma (USA). Rabbit anti-TRPV1 was purchased from Genetex (USA). Rabbit anti-Fos was purchased from Santa Cruz Biotechnology (USA). Normal goat serum was purchased from Dako (Denmark).

Experimental design

The rats were divided into control (n=8) and N/OFQ-treated groups (n=8). In both groups, CSD waves were induced by topical application of solid KCl (3 mg) to the parietal cortex. In the N/OFQ treated groups, N/OFQ (10 μM/100 μl) which is a

concentration that achieves maximum effect in the rat dorsal horn,[8] was administered intracisternally after completion of the third depolarization shift wave. The same volume of saline was administered to controls. Electrocorticograms were recorded continuously for one hour, and then the brain was removed for immunohistochemical analysis.

Animal preparation

The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). Additional doses (20 mg/kg) were given as needed to maintain surgical anesthesia based on the tail pinch reflex. A tracheotomy was performed to assist with ventilation. The left femoral vein was cannulated to allow for intravenous administration of anesthetic drugs and saline.

After the tracheotomy and cannulation were performed, the animals were placed on a surgical frame, and their heads were fixed on a stereotaxic frame. The right parietal bone was exposed by mobilizing the skin along either side of the midline incision. Two craniotomies were performed using a saline-cooled drill. The recording electrode was placed in the anterior opening that was located in the frontal bone 1 mm anterior and 1 mm lateral to the bregma. The other opening was placed in the parietal bone 7 mm posterior and 1 mm lateral to the bregma and used for the application of solid KCl for CSD initiation.

Electrocorticographic recording

Cortical depolarization was measured with a glass microelectrode (internal diameter 5 μm) prepared from a borosilicate glass capillary tube pulled with a microelectrode puller (P-30 Vertical Micropipette Puller, Sutter instrument, Novato, CA). The microelectrode was filled with 4 M NaCl solution, and then an Ag/AgCl wire was inserted. A hydraulic micromanipulator (Narishige, Scientific Instrument Lab, Tokyo, Japan) was used to insert the filled glass microelectrode perpendicular to the cortex at a depth of 500 μm from the cortical surface. Another Ag/AgCl wire was placed on the back of the animal and served as a reference point. The electrical signal was amplified with a microelectrode amplifier (MEZ-8301, Nihon Koden, Tokyo, Japan). Analog data were digitized with a data acquisition system (Biopac Physiograph

MP100A, Santa Barbara, CA).

The amplitude, duration, number of cycles, and area under the curve (AUC) of each CSD wave occurring within the 1-hour recording period were analyzed with AcqKnowlege version 3.7.3 (Biopac, Santa Barbara, CA). The amplitude was measured as the vertical length from the baseline to the peak of each depolarization shift. The duration was measured as the horizontal length (temporal difference) between the start- and endpoint of each depolarization shift. The number of cycles was defined as the total number of depolarization shifts that occurred within 1 hour of N/OFQ or saline administration. The AUC was the total area under the curve for each depolarization shift. All of the measured variables were converted to absolute values for analysis.

Immunohistochemical study

After the electrocorticographic recordings were completed, the rats were euthanized with an overdose of sodium pentobarbital, and thoracotomies and laparotomies were performed. A cannula was inserted into the apex of the heart and advanced distally into the aortic arch. The animals were perfused transcardially with 300 mL of 0.1 M phosphate-buffered saline (PBS), followed by 300 mL of 4% paraformaldehyde in 0.1 M PBS (pH = 7.4). The brain, cervical spinal cord and trigeminal ganglia were dissected and fixed overnight at 4°C in 0.01 M PBS.

The caudal medulla and cervical spinal cord (C1 and C2 region; approximately -1 to -6 mm from the obex) were cut into 5 mm blocks and immersed in a cryoprotectant solution (30% sucrose in 0.01 M PBS, pH = 7.4) for 24 hours at 4° C. The trigeminal ganglia were immersed in the cryoprotectant solution whole. Tissues were then placed on a stage and completely covered with an optimal cutting temperature (OCT) embedding medium. After freezing the OCT at -20° C, the samples were coronally sectioned (20 µm thick) with a cryostat. The sections were then washed 3 times and stored in cold 0.01 M PBS.

Immunohistochemistry was performed using the free floating technique. All of the sections were incubated at room temperature. The sections were initially rinsed 3 times with washing buffer (0.01 PBS). The sections were then incubated with 50% ethanol for 30 min followed by 3% hydrogen peroxide in 50% ethanol for 30 min to

minimize endogenous peroxidation. After another 3 rinse cycles with PBS, the brainstem and cervical spinal cord sections were incubated for 1 hour in PBS containing 3% normal goat serum and 1% bovine serum albumin. Sections from these regions were also incubated with anti-Fos polyclonal antibody (1:1000 dilution in PBS containing normal goat serum).

The trigeminal ganglia sections were incubated with rabbit anti-TRPV1 polyclonal antibody (1:500 dilution) for 20 hours at 4°C. These sections were then rinsed 3 times with PBS and incubated for 45 min with envision and rabbit anti-HRP. The levels of bound peroxidase were assessed by incubating the sections with liquid DAB for 10 min. The reaction was terminated with two successive rinses with distilled water. The sections were then mounted onto gelatin-coated slides, air dried overnight, and coverslipped with Permount.

c-Fos-immunoreactive (Fos-IR) cells were identified by a dark brown stain in the nucleus. Only cells located in the lamina I and II of the TNC with nuclei visible on the focal plane were included for analysis. Expression of Fos in the TNC was quantified by counting the number of Fos-IR neurons in lamina I and II of the TNC from 10 sections of the cervical spinal cord and 10 sections of the caudal medulla. The data are expressed as the mean number of Fos-IR cells per section.

TRPV1-immunoreactive (TRPV1-IR) cells were distinguished by their darkly stained cell bodies and processes. Expression of TRPV1 in the TG was determined by counting the number of TRPV1-IR cells from 500 randomly selected small to medium sized (diameter < 50 μm) ganglionic cells. The data are expressed as the percentage of TRPV1-IR cells per rat. Researchers were blinded to the treatment group during counting.

Data analysis

Data are expressed as the mean ± SD. The amplitude and onset time of the CSD waves were plotted. The differences between means were analyzed with a one way analysis of variance (one-way ANOVA) followed by a Student's *t*-test. Statistical significance was defined as $p < 0.05$.

RESULTS

Administration of KCl induced repeated CSD cycles (Figure 1), with the first CSD peak developing three minutes after KCl application. There were 12.2 ± 0.3 cycles during the 1 hour electrocorticographic recording period. In the control group, the CSD wave amplitude was stable throughout the recording period, with a mean value of 27.0 ± 1.5 mV. The average AUC duration was 63.3 ± 8.4 seconds, and each cycle lasted 17.3 ± 2.9 mV-seconds. Figure 1A shows a representative control electrocorticographic recording, and the electrophysiological data are presented in Table 1.

Effect of N/OFQ on CSD

N/OFQ exerted dual effects on CSD; there was an initial attenuation period lasting 10 to 15 minutes (Figure 1B) followed by a long lasting potentiation (Figure 2). Prior to N/OFQ treatment, the CSD amplitude was 29.7 ± 1.7 mV, but this value was reduced to 21.3 ± 1.1 mV during the attenuation period. There was a statistically significant difference ($p = 0.029$) between the initial waves of the N/OFQ-treated and control groups. The average amplitude of the delayed CSD waves in the N/OFQ-treated group was 33.3 ± 1.8 mV compared to 26.8 ± 1.1 mV for the controls ($p < 0.001$). The AUC of the delayed CSD wave was also significantly greater in the N/OFQ-treated group (N/OFQ-treated animals = 26.0 ± 2.9 mV-seconds; control group = 21.1 ± 3.2 mV-seconds; $p = 0.007$). Although here was no change in the duration of the CSD waves, the total number of CSD waves was greater in the N/OFQ-treated group (N/OFQ-treated group = 14.88 ± 0.29 waves; control group = 12.25 ± 0.31 waves; $p = 0.012$).

Effect of N/OFQ on CSD-evoked Fos expression in TNC

N/OFQ treatment significantly increased the number of Fos-IR cells in the TNC (Figure 3). The number of Fos-IR cells in the ipsilateral TNC was 34.7 ± 3.3 cells/slide for the N/OFQ-treated group and 24.6 ± 2.0 cells/slide for the control group ($p < 0.001$). A significant difference was also observed when we compared the number of Fos-IR cells in the contralateral TNC (Table 2), with 17.6 ± 2.1 cells/slide in the N/OFQ-treated group and 12.7 ± 2.5 cells/slide in the control group ($p < 0.001$). The observed increase in the number of Fos-IR cells in the N/OFQ-treated group suggests

that this peptide facilitates the process of trigeminal nociception.

Effect of N/OFQ on TRPV1 receptor expression in the TG

In addition to increasing the number Fos-IR cells in the TNC, N/OFQ administration also increased the expression of the TRPV1 receptor in the TG (Figure 4 and Table 2). The percentage of TRPV1-IR cells in the ipsilateral TG was 47.3 ± 5.5% for the N/OFQ-treated group and 20.2 ± 2.1% for the control group ($p < 0.001$). A lower number of TRPV1 cells were observed in the contralateral TG, with 40.2 ± 6.3% in the N/OFQ-treated group and 17.0 ± 3.0% in the control group ($p < 0.001$).

DISCUSSION

In the present study, we demonstrated that N/OFQ can alter CSD development and CSD-evoked trigeminal nociception. Electrophysiological variables indicative of CSD induction, namely amplitude, number of cycles and AUC, were enhanced by N/OFQ administration. We also observed that exposure to N/OFQ increased the expression of nociception-related proteins in the trigeminal system. Since several reports have indicated that intracestinal administration of N/OFQ inhibits nociceptive effect in spinal dorsal horn,[9,10,11] it is unlikely that N/OFQ can produce its direct effect in trigeminal nociception.

The present results show that N/OFQ has dual effects on cortical activity, characterized by an initial attenuation and followed by delayed potentiation of CSD development. This dual effect indicates that the role of N/OFQ in controlling cortical activity is complex. Several potential mechanisms could underlie these observations. It is known that N/OFQ can exert several cellular effects, including inhibition of adenylyl cyclase and Ca^{2+} channel currents.[12] N/OFQ can also increase conductance of the G-protein inwardly-rectifying potassium (GIRK) channel.[13] Studies have also indicated that N/OFQ-induced currents prolong hyperpolarization by 10 to 15 minutes.[14,15] These effects result in membrane hyperpolarization and cellular inhibition and may explain the initial attenuation that was seen in this experiment.

The delayed facilitation effect of N/OFQ is interesting. As mentioned above, N/OFQ usually exerts an inhibitory effect on post-synaptic cells. Therefore, the delayed

facilitation effect cannot be explained by the direct cellular action of this peptide. The most likely explanation is that N/OFQ causes cellular hyperexcitability by modulating the function of inhibitory interneurons. N/OFQ is known to modulate the release of several neurotransmitters, such as serotonin and norepinephrine, in the rostral ventromedial medulla and spinal cord.[16] In the cerebral cortex, the ORL1 receptor is expressed ubiquitously in presumptive GABAergic interneurons (Golgi type II cells) located in layer II.[1,17] The axons of these interneurons primarily terminate on projective glutamatergic neurons (Golgi type I cell) located in layer III. Because activation of ORL-1 receptors induces inhibitory modulation, intracisternal administration of N/OFQ may reduce GABA release from interneurons during CSD development, resulting in enhanced glutamate activation in adjacent projection neurons.

The effects of N/OFQ appear to result from the summation of two opposing actions. It has been suggested that N/OFQ produces analgesia that is readily antagonized by opioid antagonists,[18] thus functionally reversing the analgesic effects of a number of opioids.[19] At low concentrations, N/OFQ also shows bidirectional effects on monosynaptic transmission with GIRK activation.[20] Higher concentrations, however, reverse inhibition of the polysynaptic local circuit in the spinal cord.[21] Thus, our results suggest that N/OFQ modulates cortical activity and produces anti-nociception by initial attenuation. This initial anti-nociception is later reversed to nociception by delayed potentiation of CSD development.

In the present study, N/OFQ administration increased the number of TRPV1-IR cells in the TG. TRPV1 is predominantly expressed in trigeminal ganglion neurons that project to the trigeminal nucleus. Of the many stimuli that activate cation currents in primary sensory neurons, CSD is believed to be one of the physiological activators of TRPV1.[22] During CSD development, extracellular K^+ is elevated to 40 to 60 mM in the grey matter of the cortex.[23] Elevated K^+ may cause depolarization of primary sensory neurons in the cortex, which can contribute to elevated TRPV1 expression and prolonged migraine headaches that are associated with TRPV1 sensitization in the TG.[24] This result might explain the effect of TRPV1 on trigeminal nociception. This hypothesis is strengthened by our finding that N/OFQ increases TRPV1 expression in the TG. The N/OFQ receptor antagonist has also been shown to have anti-allodynic and

anti-hyperalgesic effects in rats after spinal nerve injury and inflammation.[25] This study also demonstrated increases in TRPV1 receptor expression in the TG. Up-regulation of this receptor may increase nociceptive sensitivity, resulting in increased pain perception.

The present study showed that N/OFQ administration led to an increase in the number of CSD-evoked Fos-IR cells in the TNC, indicating enhancement of CSD-evoked trigeminal nociception. In addition to the effect on the peripheral nociceptive pathway, N/OFQ has been found to modulate the central process of pain perception. For example, N/OFQ receptor blocker reduced Fos-IR that was induced by L5/L6 spinal nerve ligation, suggesting that N/OFQ system might be involved in the enhancement of allodynic and hyperalgesic effects in rats.[25] Therefore, the increase in CSD-evoked trigeminal nociception reported here is more likely to be explained by an increase in CSD leading to increased nociceptive activation and up-regulation of the TRPV1 receptor. It remains unclear whether changes in the central nociceptive process plays role in this facilitation.

In conclusion, the present study demonstrates that N/OFQ has a biphasic effect on cortical activity and CSD-induced trigeminal nociception. N/OFQ attenuates CSD in the early phase but enhances CSD protein expression in the trigeminal system in the late phase. The detailed mechanisms and physiological role of the biphasic modulating effects of N/OFQ on trigeminal nociception require further investigation and may provide a target for novel migraine therapeutics.

Acknowledgements

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Table 1 Electrophysiological CSD data for the control and N/OFQ-treated groups

Electrophysiological variables	Control (n = 8)	N/OFQ treated (n = 8)	P-value
Number of cycles	12.2 ± 0.3	14.9 ± 0.3	0.012
Amplitude (mV)			
– Pre-treatment	27.0 ± 1.5	29.7 ± 1.7	0.100
– Initial 2 waves	27.0 ± 1.9	21.3 ± 1.1	0.029
– Delayed waves	26.8 ± 1.1	33.3 ± 1.8	< 0.001
AUC (mV-second)			
– Pre-treatment	17.3 ± 2.9	20.0 ± 1.7	0.050
– Initial 2 waves	19.0 ± 1.6	17.8 ± 3.3	0.442
– Delayed waves	21.1 ± 3.2	26.0 ± 2.9	0.007
Duration (seconds)			
– Pre-treatment	63.3 ± 8.4	57.0 ± 5.3	0.130
– Initial 2 waves	65.3 ± 9.9	54.3 ± 5.3	0.038
– Delayed waves	65.4 ± 8.9	63.5 ± 8.9	0.959

Data are expressed as the mean ± SD.

Table 2 CSD-induced Fos-IR cells and TRPV1-IR cells in the trigeminal system

Measured variables	Control	N/OFQ treated	P-value
The number of Fos-IR (cells/slide)			
– Ipsilateral TNC	24.6 ± 2.0	34.7 ± 3.3	< 0.001
– Contralateral TNC	12.7 ± 2.5	17.6 ± 2.1	< 0.001
The percentage of TRPV1-IR cells (%)			
– Ipsilateral TG	20.2 ± 2.1	47.3 ± 5.5	< 0.001
– Contralateral TG	17.0 ± 3.0	40.2 ± 6.3	< 0.001

Data are expressed as the mean ± SD.

Figure 1 Representative traces showing the pattern of CSD in (A) control and (B) N/OFQ-treated rats. The second triangle indicates administration of normal saline or N/OFQ after the third CSD cycle. The effect of N/OFQ on CSD is characterized by a short initial period (approximately 10 min) of CSD attenuation followed by an extended period of CSD potentiation.

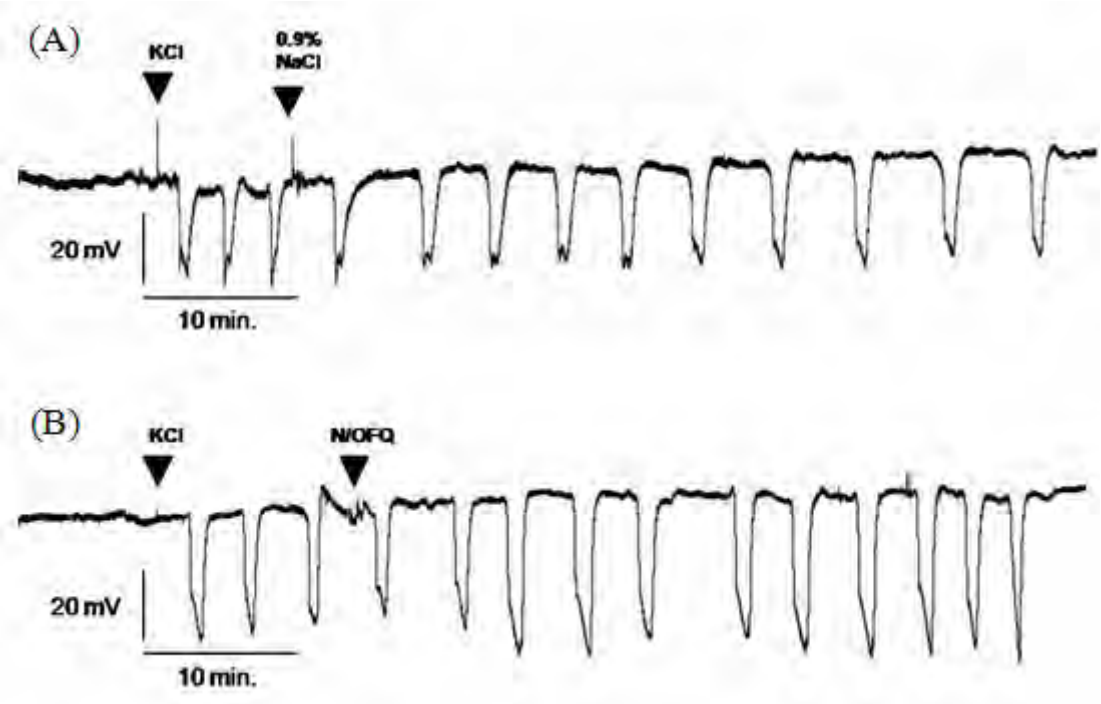


Figure 2 The effect of N/OFQ on CSD amplitude. N/OFQ administration transiently reduced the CSD amplitude. This attenuation was followed by a long lasting period of increased CSD wave amplitude and frequency.

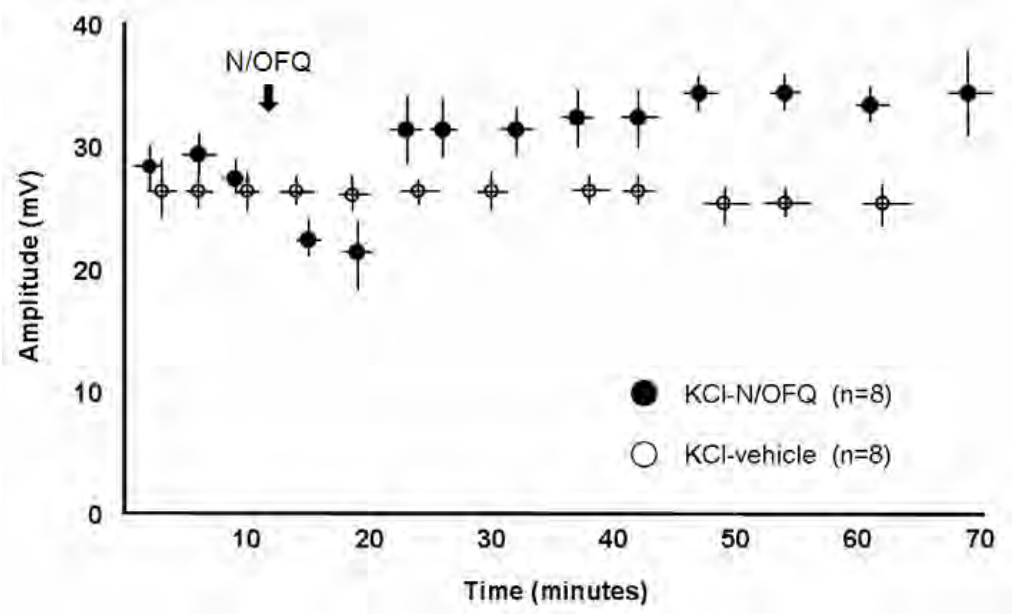


Figure 3 Representative micrographs showing Fos-IR cells in the TNC. An increased number of Fos-IR cells were observed in the N/OFQ-treated group. (A) Contralateral Control; (B) ipsilateral control; (C) contralateral N/OFQ treated; (D) ipsilateral N/OFQ treated. Scale bar = 250 μm.

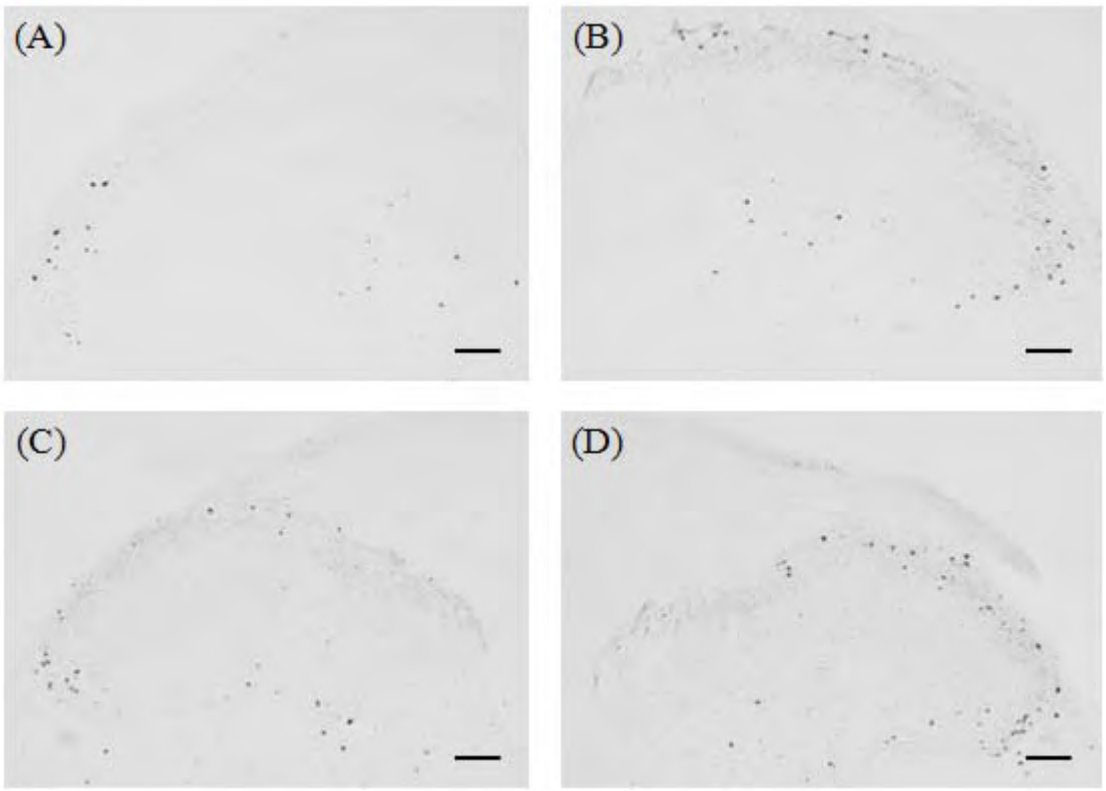
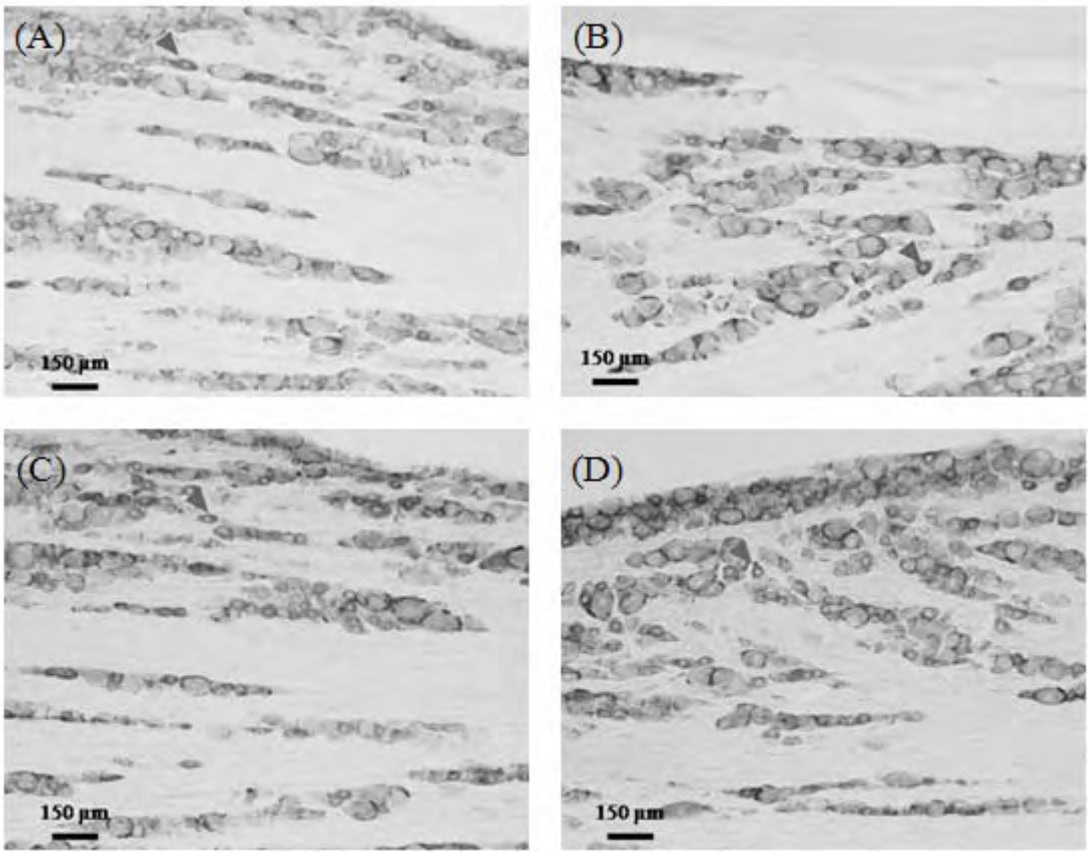


Figure 4 Representative micrographs showing TRPV1-IR cells in the TG. TRPV1 was expressed in small to medium sized ganglionic neurons (arrowhead). N/OFQ treatment increased the number of TRPV1-IR cells. (A) Contralateral Control; (B) ipsilateral control; (C) contralateral N/OFQ treated; (D) ipsilateral N/OFQ treated. Scale bar = 150 μm.



EFFECT OF CORTICAL SPREADING DEPRESSION ON HIPPOCAMPAL SYNAPTIC TRANSMISSION

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ABSTRACT

This study aimed to investigate the alteration of hippocampal long-term plasticity and basal synaptic transmission induced by repetitive cortical spreading depressions (CSDs). There is some relation between migraine aura and amnesic attack. CSD may be responsible for this hippocampus-related symptom. However, the precise role of CSD on hippocampus activity has not been studied.

In the present study, Wistar rats were divided into 2 groups, namely CSD and control. Repetitive CSDs were induced *in vivo* by topical application of solid KCl on parietal cortex. Direct current potential was recorded in frontal cortex. Forty-five minutes later, ipsilateral hippocampus was removed and prepared for transverse hippocampal slice. A series of *in vitro* electrophysiological studies was performed to investigate hippocampal synaptic transmission and plasticity. In the other preparation, direct current potential was recorded in hippocampus during CSD induction.

The results showed that solid KCl application evoked repetitive CSDs. These SDs propagated to hippocampus as well. These SDs traveling across cortical and hippocampal tissues had an influence on hippocampal synaptic efficacy. Magnitude of hippocampal long-term potentiation (LTP) decreased in slice obtained from CSD-induced rat. Furthermore, slice obtained from CSD-induced rat showed a reduction in the excitability as evident by a decrease in post-synaptic AMPA receptor output.

In conclusions, repetitive CSDs altered hippocampal transmission by suppressing postsynaptic AMPA receptor function. This modulation could participate in CSD-induced LTP impairment. The finding from this study may explain hippocampus-related symptoms occurring during migraine attack.

Key words: Cortical spreading depression, hippocampus, long-term potentiation, basic synaptic transmission

INTRODUCTION

Spreading depression (SD) is a slowly propagated wave of depolarization of neurons and glial cells, followed by a subsequent sustained suppression of spontaneous neuronal activity (Charles and Brennan 2009). Cortical SD (CSD) is originally linked to the aura phase of migraine (Leao and Morison 1945). However, some evidence also suggests the links between CSD and associated symptoms such as amnesia (Gorji 2001).

In particular, amnesic attacks are reported during aura period of migraine attack (Dalessio 1980; Sacquegna, Cortelli et al. 1986; Santoro, Casadei et al. 1988). Memory, attention, concentration and retention is usually poor during the migraine attacks (Dalessio 1980). Moreover, migraine is mentioned as a risk factor and a precipitating event for transient global amnesia (TGA), a particular type of memory disorder characterized by sudden episode of severe anterograde amnesia (Gorji 2001).

It is known that hippocampus plays a critical role in certain types of memory (Eichenbaum 2000). The underlying mechanisms of memory formation are believed to

depend on activity-dependent hippocampal synaptic plasticity such as long-term potentiation (LTP) (Bliss and Collingridge 1993). In addition, LTP has been regarded as a cellular model of memory formation (Lynch 2004).

Although CSD may relate to the memory disorder and the perturbation of hippocampal function, the effect of CSD on hippocampal plasticity is not fully investigated. Study from *in vitro* cortical-hippocampal combined slices shows that CSD could facilitate or depress hippocampal LTP depending on the propagation of SD into the hippocampus (Wernsmann, Pape et al. 2006). However, the underlying mechanism is still unknown. It is not clear whether SD induced in cortical tissue could propagate to hippocampus in *in vivo* preparation, even some study suggests the probability of the propagation (Henning, Meng et al. 2005).

This study aimed to investigate the effect of CSD on hippocampal long-term plasticity and basal synaptic transmission. In addition, the propagation of SD induced at parietal cortex along the cortical as well as hippocampal tissue was examined. The knowledge from this study may provide better understanding of mechanisms underlying the perturbation of hippocampal synaptic plasticity induced by CSD and pathogenesis of hippocampus-related symptoms occurring during migraine attacks.

LITERATURE REVIEW

Spreading Depression

Spreading depression (SD) is a transient reversible phenomenon which expresses as a self-propagating depolarization of neurons and glia, followed by a depression of the neuronal bioelectrical activity for a period of minutes, accompanied by complex and variable changes in vascular caliber, blood flow, and energy metabolism (Gorji 2001; Charles and Brennan 2009). A characteristic of SD is a propagating extracellular negative potential with an amplitude of 10-30 mV and a duration of more than .5-1 minute (Gorji 2001). During SD, there is a transient massive redistribution of ions between intracellular and extracellular compartments. K^+ and H^+ release from the cells, while Na^+, Ca^{2+} and Cl^- enter with water causing cells to swell and volume of the extracellular compartment to be reduced (Kraig and Nicholson 1978; Gorji 2001). Although SD has been most extensively studied in the cortex (i.e. cortical SD), the phenomenon could be induced in most grey matter regions including hippocampus and cerebellum of a variety of species (Bures, Buresova et al. 1974).

Cortical Spreading Depression

Cortical spreading depression (CSD) was first described in the rabbit, pigeon and cat cortex by Leao (1944). He reported a large negative shift in direct current potential lasting 1-2 min, followed by a positive deflection lasting 3–5 min before returning to baseline. Lashley (1941) described his own migraine aura and speculated that the disturbances started at visual field center and propagated to the temporal parts within 10-15 min, while function returned to normal within another 10-15 min. He estimated that a wavefront of intense excitation followed by a wave of complete inhibition of activity was spread across the visual cortex by a velocity of 3 mm/min. The similarity between the characteristics of CSD propagation and the

spread of the cortical representation of the migraine visual aura immediately give rise to the hypothesis that migraine aura is caused by CSD (Leao and Morison 1945).

Clinical Relevance of SD

CSD and Associated Migraine Symptoms

CSD was originally linked to the aura phase of migraine (Leao and Morison 1945). However, some evidence also suggests the links between CSD and migraine pain as well as associated signs and symptoms such as sexual arousal, yawning and drowsiness, nausea and vomiting, and amnesia (Gorji 2001). In particular, amnesic attacks and anomia are reported during aura (Dalessio 1980; Sacquegna, Cortelli et al. 1986; Santoro, Casadei et al. 1988). Memory, attention, concentration and retention is usually poor during the migraine attacks (Dalessio 1980).

SD and Transient Global Amnesia

Transient global amnesia (TGA) is a particular type of memory disorder characterized by sudden episode of severe anterograde amnesia lasting for several hours (Shekhar 2008). During a typical attack the patient has sudden onset of severe memory impairment for events of the present and the recent past coupled with ongoing anterograde amnesia which lasts for at least several hours and resolves gradually (Fisher and Adams 1964).

Many clinical studies indicate that hippocampus is implicated in TGA pathophysiology. Positron emission tomography study shows abnormalities in several regions such as hippocampus and amygdala (Eustache, Desgranges et al. 1997). Diffusion-weighted magnetic resonance imaging (MRI) showed transient disruption of blood flow in amygdala and hippocampus (Woolfenden, O'Brien et al. 1997).

Many studies report the relationship between TGA and migraine. Migraine is mentioned as a risk factor and a precipitating event for TGA. A higher incidence of TGA in subjects with migraine is reported (Gorji 2001). The amnesic attack is reported during the aura phase, concomitant with migraine attacks or follow a migraine status (Santoro, Casadei et al. 1988).

SD and Memory Dysfunction: Animal Studies

Although exact pathophysiology of TGA is not clear, SD in hippocampus may be relevant to TGA (Olesen and Jorgensen 1986). In experimental animals, SD in the hippocampus causes a temporary functional ablation lasting minutes to hours with full functional recovery (Avis and Carlton 1968). Bilateral hippocampal or cortical SD evoked immediately after acquisition of a passive avoidance reaction, elicits a partial amnesia (Bures, Buresova et al. 1974). In addition, CSD visualized using manganese-enhanced MRI following topical application of KCl to the exposed rat cortex reveals signal enhancement in CA1–3 areas, and the dentate gyrus of the hippocampus (Henning, Meng et al. 2005). However, the exact mechanisms of CSD-induced memory impairment remain to be elucidated.

Hippocampus and Memory

The mammalian hippocampus is a brain structure that is widely held to serve an important function in certain types of memory (Morris, Moser et al. 2003). It is essential for the formation of new episodic memories and might also have a role in their long-term storage (Neves, Cooke et al. 2008). In clinical studies, the most notable case was H.M., who underwent experimental surgery involving bilateral removal of the medial temporal lobe, including of both hippocampi. After the procedure, H.M. lost an ability to form new episodic memories (anterograde amnesia), coupled with a substantial, but not total, loss of old memories (retrograde amnesia) (Scoville and Milner 2000). Animal studies also reveal that lesions or pharmacological inactivation of hippocampus results in either a failure to learn or a loss of spatial memory (Morris, Anderson et al. 1986; Martin, De Hoz et al. 2005).

Basic Anatomy of the Hippocampus

The wiring diagram of the hippocampus is traditionally presented as a trisynaptic loop (Fig. 1). The major input is carried by axons of the perforant path, which convey polymodal sensory information from neurons in layer II of the entorhinal cortex to the dentate gyrus. Perforant path axons make excitatory synaptic contact with the dendrites of granule cells: axons from the lateral and medial entorhinal cortices innervate the outer and middle third of the dendritic tree, respectively. Granule cells project, through their axons (the mossy fibers), to the proximal apical dendrites of CA3 pyramidal cells which, in turn, project to ipsilateral CA1 pyramidal cells through Schaffer collaterals (Sch) and to contralateral CA3 and CA1 pyramidal cells through commissural connections. In addition to the sequential trisynaptic circuit, there is also a dense associative network interconnecting CA3 cells on the same side. CA3 pyramidal cells are also innervated by a direct input from layer II cells of the entorhinal cortex (not shown). The distal apical dendrites of CA1 pyramidal neurons receive a direct input from layer III cells of the entorhinal cortex. There is also substantial modulatory input to hippocampal neurons. The three major subfields have an elegant laminar organization in which the cell bodies are tightly packed in an interlocking C-shaped arrangement, with afferent fibers terminating on selective regions of the dendritic tree. The hippocampus is also home to a rich diversity of inhibitory neurons (not shown).

Hippocampal slices can be kept healthy for many hours if a steady flow of oxygen and artificial cerebrospinal fluid (ACSF) is supplied. The laminated organization of the hippocampus lends itself perfectly to extracellular recording techniques, allowing selective pathways to be stimulated and the evoked synaptic responses generated by a population of target neurons to be monitored for prolonged periods of time (Neves, Cooke et al. 2008).

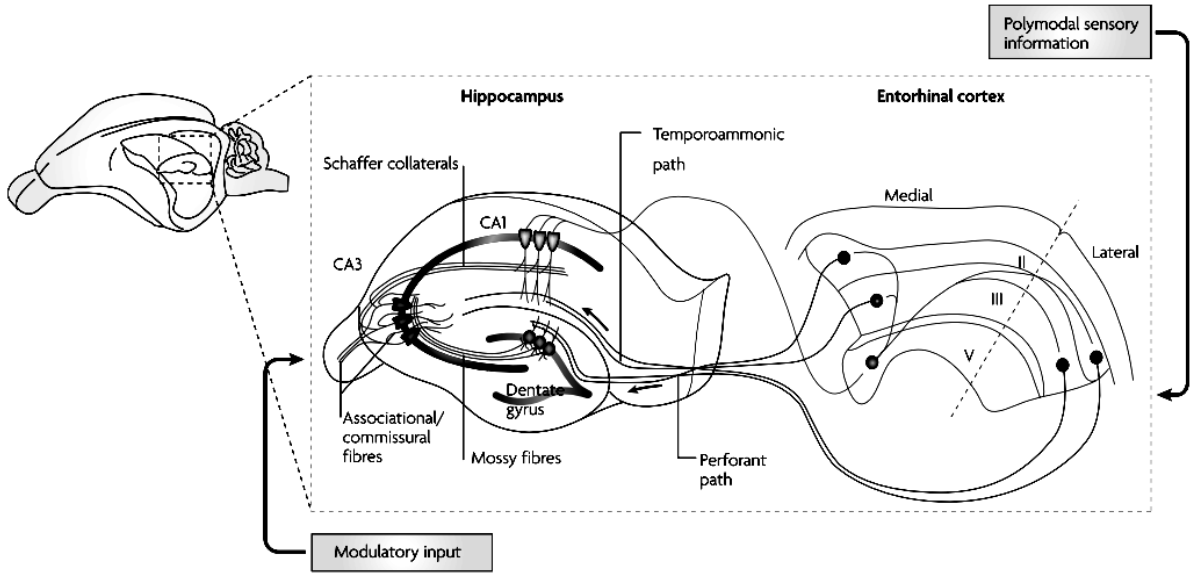

Fig. 1 Basic anatomy of the hippocampus. From Neves et al. (2008).

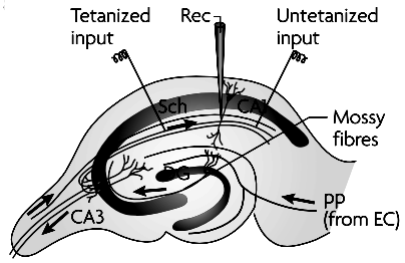

Fig. 2 *In vitro* extracellular recording of hippocampal synaptic events. From Neves et al. (2008).

Hippocampus and Synaptic Plasticity

The hippocampus has been a major experimental system for studies of synaptic plasticity in the context of putative information-storage mechanisms in the brain (Neves, Cooke et al. 2008). Its simple laminar pattern of neurons and neural pathways (Fig. 1) enables the use of extracellular recording techniques to record synaptic events (Fig. 2) (Andersen, Bliss et al. 1969). The much-studied model of hippocampal synaptic plasticity is long-term potentiation (LTP). Synaptic plasticity in the hippocampus, represented by LTP, has been regarded as a cellular model of memory formation (Lynch 2004). Furthermore, LTP models are also now used in order to test how a variety of neurological disorders might affect synaptic plasticity.

Hippocampal LTP

LTP was discovered in the rabbit's hippocampus using extracellular field potentials in unanaesthetized (Bliss and Gardner Medwin 1973) and anaesthetized (Bliss and Lomo 1973) animals. Since then, intense research has been conducted on LTP. Many studies have been conducted to gain insight into the molecular intracellular events that give rise to LTP, most of

which have used in vitro techniques, with brain slices or cultured neurons (Chaillan, Truchet et al. 2008).

LTP at all major hippocampal synapses share a common induction mechanism involving an initial rise in postsynaptic Ca^{2+} (Yeckel, Kapur et al. 1999). It is generally accepted that the rise in postsynaptic Ca^{2+} required for hippocampal CA1 LTP induction is due to the entry of Ca^{2+} via the N-methyl-d-aspartic receptor (NMDAR) complex (Huang and Malenka 1993). In LTP protocol, brief high-frequency stimulation (HFS; ~100 Hz) leads to a long-lasting increase in the strength of synaptic transmission. This single 100 Hz train (100 pulses over 1 s, at baseline stimulation intensity) has been used and is effective for inducing early NMDAR-dependent LTP (1–3 h), which is protein synthesis-independent (Vertes 2005; Albensi, Oliver et al. 2007).

OBJECTIVES

1. To examine whether CSD induced by KCl at parietal cortex could propagate and reach hippocampus.

2. To investigate the alteration of hippocampal synaptic transmission induced by CSD.

3. To investigate the effect of CSD on hippocampal synaptic plasticity.

MATERIALS AND METHODS

Materials

1. *Animals*
    a. Adult male Wistar rats
2. *Materials*
    a. Rat food
    b. Bedding
    c. Medical supplies (needles, syringes, gloves)

d. G
as mixture (95% O_2/5% CO_2)

e. C
apillary glass (for electrode fabrication)

3. *C*
hemicals

a. N
aCl (Sigma-Aldrich)

b. K
Cl (Sigma-Aldrich)

c. M
gSO_4 (Sigma-Aldrich)

d. C
aCl_2 (Sigma-Aldrich)

e. N
aH_2PO_4 (Sigma-Aldrich)

f. N
aHCO_3 (Sigma-Aldrich)

g. G
lucose (Sigma-Aldrich)

h. H
ydrochloric acid 1N (Merck)

i. P
icrotoxin (Sigma-Aldrich)

j. A
PV (Sigma-Aldrich)

Experimental Design

All male Wistar rats weighing 200-350 grams were divided into 2 groups, namely CSD and control, as described below. Direct current potential was recorded *in vivo* in CSD-induced rat or sham. After that, hippocampal slice was prepared for *in vitro* electrophysiological experiments.

1. CSD group

CSD was induced *in vivo* for 45 minutes by topical application of KCl crystal on exposed parietal cortex. Then, the rat was decapitated and hippocampal slices were prepared for electrophysiological studies. Field EPSPs were recorded in hippocampal CA1. The electrophysiological studies include LTP (n = 7) and basic synaptic transmission (n = 6).

2. Control group

Solid NaCl was topically applied on parietal cortex surface for 45 minutes. Then, the rat was decapitated and hippocampal slices were prepared for electrophysiological studies, including LTP (n = 7) and basic synaptic transmission (n = 6).

In the other preparation, direct current potential was recorded *in vivo* in hippocampus during CSD induction (n = 4).

Methods

Animals

Adult male Wistar rats (National Laboratory Animal Centre, Mahidol University, Thailand) weighing 200-350g were housed in stainless steel cages with free access to food (regular dry rat food) and water. The animals were maintained in a temperature-controlled room with 12-hour dark/light cycle. They were allowed to acclimate to the housing environment at least 7 days before experiments.

Animal Preparation

Each rat was anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and fitted with an intratracheal tube. Cannula was inserted into the femoral vein for later infusion of anesthetic. Additional doses of anesthetics were given as required to maintain surgical anesthesia based on response to tail pinch. Rat was placed in a stereotaxic apparatus. The skull above somatosensory and temporal cortices was exposed. For induction of CSD, a craniotomy of 2-mm in diameter was performed at right parietal bone (6 mm posterior to bregma and 2 mm lateral to midline). The bone was carefully drilled using slow speed, saline-cooled technique in order to minimize surgical irritation of the neurons. For CSD recording, a cranial window of 2 mm diameter was made at right frontal bone (3 mm anterior to bregma and 2 mm lateral to midline). A recording glass microelectrode for detecting negative direct current potential was inserted into frontal neocortex at the depth of 500 μm after careful removal of the dura mater. In another experiment, SD in hippocampus was recorded instead of CSD. For hippocampal SD recording, a cranial window of 2 mm diameter was made at right parietal bone (3 mm posterior to bregma and 2 mm lateral to midline). A recording glass microelectrode was inserted at the depth of 2.8 mm into the CA1 area of the hippocampus.

A Biopac amplifier (Biopac) with Acknowledge acquisition software was used for direct current recordings. Signals were amplified, low-pass filtered at 1 kHz, digitally sampled at 10 kHz, and stored for offline analysis.

Induction of CSD

Multiple waves of CSD were elicited by topical application of solid KCl (3 mg) on exposed cortex surface. In control group, NaCl crystal was used instead. Forty-five minutes after elicitation of CSD waves, the rat was decapitated and transverse hippocampal slices were prepared as described below.

Slice Preparation

The entire ipsilateral hippocampus was quickly removed from the brain. Coronal hippocampal brain slices (400 µm thickness) were cut using a Vibratome tissue slicer in ice-cold artificial cerebrospinal fluid (ACSF). The ACSF contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose, and .1 picrotoxin, a $GABA_A$ receptor antagonist. The ACSF was bubbled continuously with carbogen (95% O_2/5% CO_2). Fresh slices were placed in a humidified interface-type holding chamber and recovered for at least 1.5 h before electrophysiological experiments.

Electrophysiological Recording

A single slice was transferred into a submerge-type recording chamber, fixed with a nylon net, and submerged beneath the continuously perfusing ACSF (at a rate of 1.5–2.0 ml/min) saturated with carbogen. A cut was made to separate the CA3 region from the CA1 region to avoid epileptiform activity from the CA3 region. All experiments were performed at room temperature (25 °C).

A bipolar tungsten stimulating electrode was placed in the stratum radiatum at 200–300 µm from the recording site. Square-pulse stimuli of .2 ms duration at .1 Hz (1 pulse every 10 s) were delivered to the slice through the bipolar tungsten electrode to activate the Schaffer-collateral pathway projecting to CA1. Glass microelectrode with 2-8 MΩ resistance containing 3 M NaCl was positioned parallel to the stimulating electrode in stratum radiatum to record presynaptic fiber volleys followed by fEPSPs. An attempt was made to maintain similar orientation of the electrodes relative to the pyramidal cell layer and dentate gyrus to minimize changes in fEPSP properties attributable to electrode positioning. For baseline recordings, stimulus intensity was adjusted to evoke fEPSPs of .15 to .20 mV/ms slopes. Only slice that gave a fEPSP amplitude of more than 1 mV and that was stable for at least 30 min was included in this study. All slices that failed to stabilize within 90 min were rejected.

An EPC-10 amplifier (HEKA) with Patchmaster software was used for electrophysiological recordings. Signals were amplified, low-pass filtered at 1 kHz, digitally sampled at 10 kHz, and stored for offline analysis.

Electrophysiological Study Protocols

1. LTP

LTP protocol consisted of high-frequency stimulation (HFS; tetanus). After recording stable baseline fEPSPs for at least 30 min, LTP was elicited by applying tetanic stimulation of 100 Hz for 1 sec at the same stimulus intensity as for the baseline values. The post-tetanic responses were recorded for 60 min afterward. Each slice was treated with only one tetanic stimulation. Magnitude of LTP was calculated by dividing the average slope of 50–60 min post-induction responses with the average slope of 0–30 min pre-induction baseline responses.

2. Input-Output Stimulation (I/O) of AMPA receptor

I/O stimulation was used for investigating basal synaptic transmission and the possible change of postsynaptic response. I/O protocol consisted of 12-step stimulation intensities that were adjusted to evoke threshold to maximal fEPSP responses. After recording stable baseline fEPSPs for at least 15 min, I/O stimulation was delivered at .1 Hz to record 5 responses for each stimulation intensity. In this experiment, I/O was generated by

stepping the stimulation intensities from .275 to .550 V. During I/O recording, a highly-selective NMDA receptor antagonist, APV, was applied by bath-application (25 μM) to isolate AMPA receptor-mediated fEPSPs. Then, the slopes of AMPA receptor-mediated fEPSPs were plotted against the stimulus voltages to provide an indication of differences in the AMPA receptor-mediated responses to stimuli of a given intensity.

RESULTS

CSD

After applying solid KCl (3 mg) on parietal cortex, a series of negative depolarization shifts characterized as spreading depression was recorded in frontal cortex surface (fig 3). The total numbers of CSD waves occurring within 45 min were 9.23 ± 1.74 waves. The amplitude, the duration, and the area under the curve of each wave were 34.62 ± 6.78 mV, 69.67 ± 19.60 s, and 712.35 ± 187.77 mV·s, respectively. The interval between each wave was 5.10 ± 1.49 min.

Fig. 3 The tracing showing the DC shift in frontal cortex surface induced by KCl application (Scale bar: 5 min; 10 mV)

Hippocampal SD

After applying solid KCl (3 mg) on parietal cortex, a series of negative depolarization shifts characterized as spreading depression was recorded in hippocampus (fig 4). The total numbers of CSD waves occurring within 45 min were 3.67 ± .58 waves. The amplitude, the duration, and the area under the curve of each wave were 24.79 ± 3.51 mV, 74.14 ± 28.93 s, and 810.46 ± 217.11 mV·s, respectively. The interval between each wave was 10.53 ± 2.27 min.

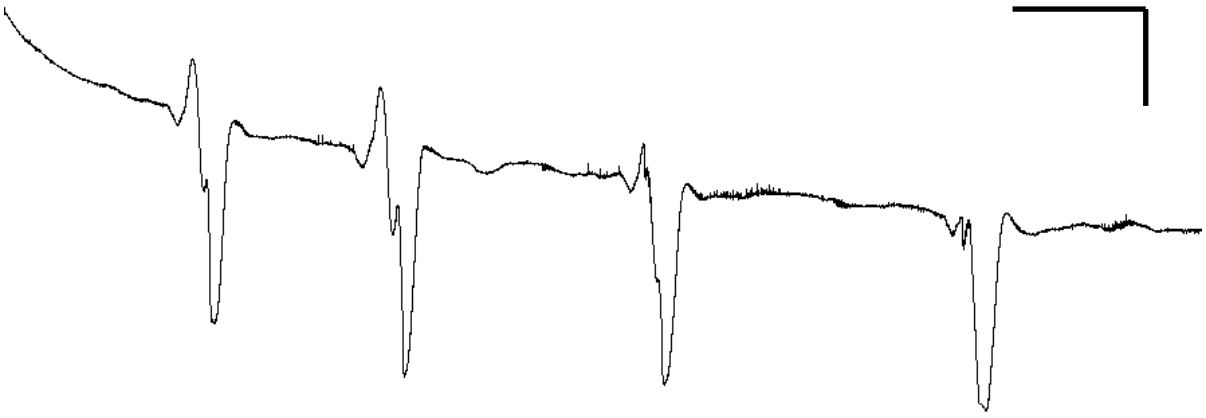

Fig. 4 The tracing showing the DC shift in hippocampus induced by KCl application (Scale bar: 5 min; 10 mV)

Investigation of cortical and hippocampal SD characters showed no difference in the duration, and the AUC. However, the total numbers of CSD waves and the amplitude in hippocampus were significantly lower than those in cortex. Moreover, the interval between each wave was significantly longer in hippocampus when compared to that in cortex.

	CSD	Hippocampal SD	*P*-value
Total numbers (waves/45 min)	9.23 ± 1.74	3.67 ± .58	0.004
Amplitude (mV)	34.62 ± 6.78	24.79 ± 3.51	0.04
Duration (s)	69.67 ± 19.60	74.14 ± 28.93	0.83
Area under the curve (mV·s)	712.35 ± 187.77	810.46 ± 217.11	0.56
Wave interval (min)	5.10 ± 1.49	10.53 ± 2.27	0.03

Table. 1 Comparing the electrophysiology variables related to SD between cortex and hippocampus

LTP

After recording stable baseline response for at least 30 min, a conditioning tetanic stimulus (100 Hz for 1 sec) was delivered to the Schaffer collateral fibers in hippocampal slices. In both group, tetanic stimulation produced a rapid, stable and lasting enhancement of the slope of the evoked potential in all slices (Fig. 5A). The potentiation rose within 1–2 min and stabilized within another 18–22 min after the stimuli. However, magnitude of LTP in the hippocampus was influenced by repetitive CSD induction. In CSD-induced rat, the magnitude of LTP was significantly decreased. (P = 0.04, Student's t-test; Fig.5B). The levels of LTP in CSD-induced and control groups were 119.8 ± 16.2 and 138.2 ± 9.8 percent, respectively (n = 7 each group).

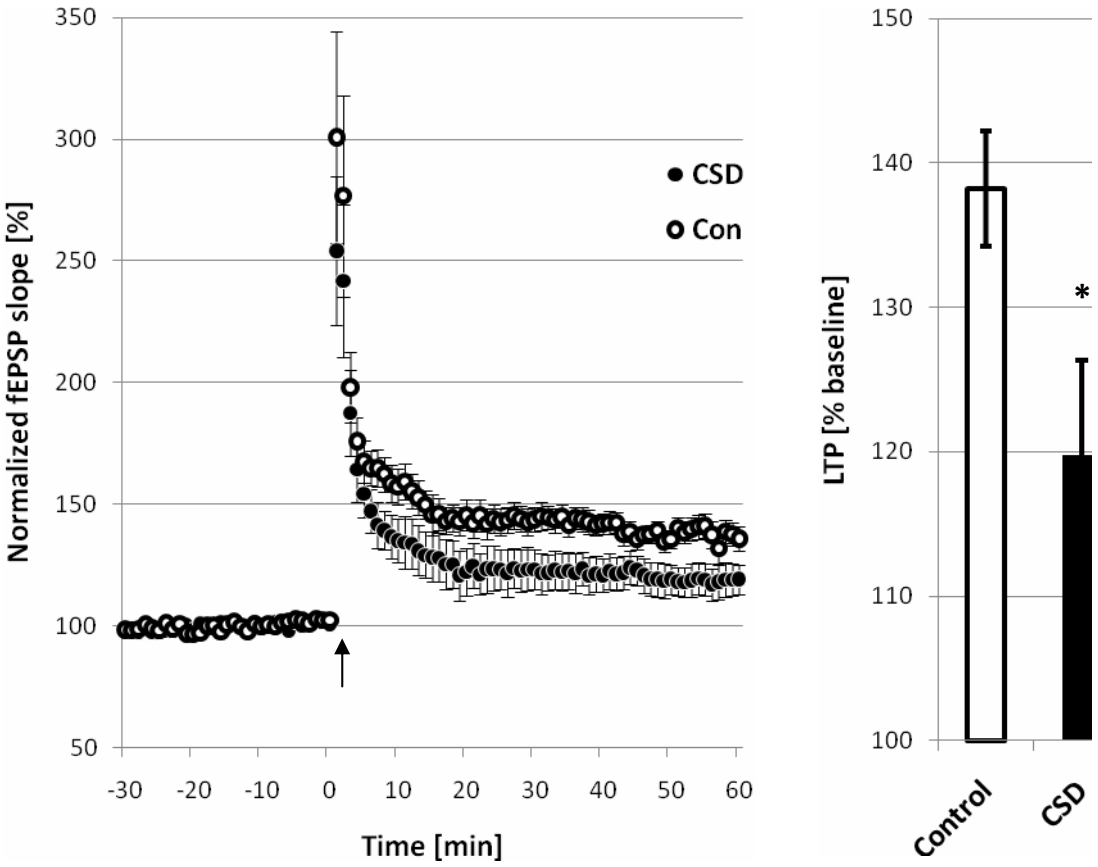

Fig. 5 LTP of fEPSP in the hippocampal slices obtained from CSD-induced and control rats. Tetanic stimulation (a train of 100 pulses at 100 Hz) produced a rapid and stable potentiation in the slope of the evoked field potentials. (A) Close circles and open circles showed the evoked fEPSP in hippocampal slices of CSD-induced and control rats, respectively. Arrow showed the time of tetanic stimulation. The time points given referred to LTP induction. (B) Bar graph showed magnitude of LTP, calculated as a percentage of baseline mean response slope. Note the significant reduction of LTP magnitude in CSD-induced rats (* P = .04, Student's t-test, n = 7 each group).

I/O of AMPA receptor

In this experiment, basal synaptic transmission at SC-CA1 synapse was examined using I/O response of AMPA receptor-mediated fEPSP. A highly-selective NMDA receptor antagonist, APV, was applied by bath-application (25 μM) to isolate AMPA receptor-mediated fEPSP. After recording stable baseline response for at least 15 min, an I/O response was recorded by stepping the stimulation intensity. The result showed that the overall I/O curve of AMPA receptor was significantly reduced in hippocampal slices obtained from CSD rats (P = .032, repeated-measures ANOVA, n = 6 each group; Fig. 6). The slice obtained from CSD-induced rat showed a significant reduction in evoked AMPA receptor-mediated fEPSPs across a range of stimulus intensities. This result indicated that AMPA receptor responses at SC-CA1 synapses were depressed in CSD-induced rats.

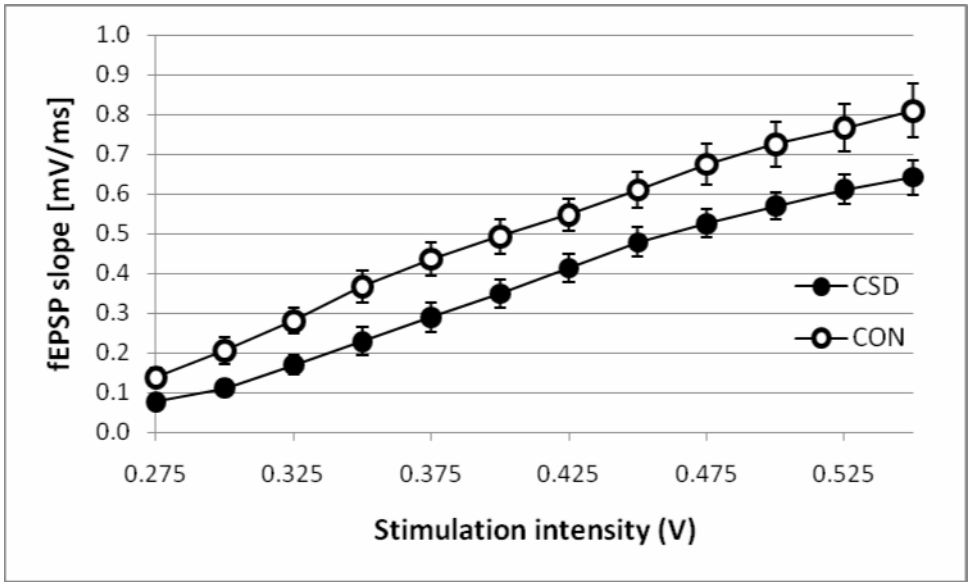

Fig. 6 I/O of AMPA receptor-mediated fEPSP in the hippocampal slices obtained from CSD-induced and control rats. Close circles and open circles showed the evoked fEPSP in hippocampal slices of CSD-induced and control rats, respectively. Note the significant reduction of AMPA receptor synaptic transmission in CSD-induced rats (P = .032, repeated-measures ANOVA, n = 6 each group).

DISCUSSION

The present study showed how CSD could affect hippocampal synaptic transmission and plasticity. Repetitive CSD induction reduced hippocampal excitability as well as LTP in CA1 area. In addition, DC recording in hippocampus showed that CSD may travel from neocortex and enter the hippocampus.

The electrophysiological study of cortical and hippocampal SD showed the possibility of SD propagation from cortical tissue to hippocampus. The possibility of CSD penetrating rat hippocampus in the present study is consistent with previous studies of CSD using manganese-enhanced MRI (MEMRI) (Henning, Meng et al. 2005). Topical application of KCl to the exposed rat cortex results in MRI signal increase in the ipsilateral cortex relative to the contralateral control region. In addition, an increase of MRI signal is also present in hippocampus, suggesting hippocampal SD. From our result, the lower number of SD waves in hippocampus compared to that of CSD may imply that only a portion of CSD waves propagated along the cortex and reached the hippocampus. Neocortex and hippocampus are anatomically connected through the parahippocampal region, comprised of the perirhinal (PRC), postrhinal (POR) and entorhinal (ERC) cortices (De Curtis and Pare 2004). The recent study shows that ERC is not only a passive relay station but also gate impulse traffic between neocortex and hippocampus. Impulse transferred from temporal neocortex to ERC and vice versa occurs with an extremely low probability, suggesting a powerful intrinsic inhibitory system (Pelletier, Apergis et al. 2004). Although the gating properties remain to be identified, it may act to abort SD propagation to hippocampus.

Alteration of synaptic efficacy could develop in both directions, depending on the characteristics and intensity of stimulation (Vilagi, Dobo et al. 2009). It has been shown that single CSD causes a hyperexcitability of neocortical tissue. Experiment in human neocortical slices demonstrates that CSD lead to a long-lasting increase of fEPSP. In addition, induction of LTP in the slice is enhanced following propagation of CSD (Berger, Speckmann et al. 2008). It has been shown that CSD affects not only the neocortical excitability but also the excitability of subcortical region, including hippocampus. Wernsmann and coworkers (2006) demonstrated that single CSD elicited in neocortical-entorhinal-hippocampal combined slice results in increased hippocampal excitability. The result shows an augmentation of hippocampal fEPSP in abortive CSD slice (i.e. a slice of which CSD does not propagate into the hippocampus). Furthermore, tetanus-induced LTP increases in CSD-induced slice. This increase of hippocampal excitability is consistent with another study. The analysis of receptor binding sites in the rat brain slice demonstrates a significant upregulation of AMPA receptor binding sites following abortive CSD (Haghir, Kovac et al. 2009). It is not clear how CSD alters AMPA receptor function, excitability, and synaptic plasticity of the hippocampus, even SD not reaches the hippocampus. However, this increase in AMPA receptor function may responsible for the occurrence of hyperexcitable state of hippocampal tissue induced by CSD.

Besides hyperexcitability, alteration of synaptic efficacy could occur in opposite direction. Our electrophysiological study showed a reduction of LTP magnitude after repetitive *in vivo* CSD induction. Extracellular recording of DC showed potential shifts in hippocampus, suggesting hippocampal SD, during CSD induction in temporal cortex. Thus, it was likely that CSD induced by topical application of KCl could propagate to hippocampus. These may imply that repetitive CSD or hippocampal SD could impair LTP. Study from Wernsmann and colleagues (2006) give the similar result. The reduction of hippocampal LTP is present in brain slice that CSD induced in the temporal cortex reaches the hippocampus. Interestingly, consistent result is obtained from different study model. Khaleghi Ghadiri and his team (2009) investigate the effect of periodic fasting on neuronal excitability in rat hippocampal tissues. In this experiment, water drinking elicits a negative DC potential shift, which is similar to SD, in parietal area in periodic fasting rat. This repeated DC potential shifts change the pattern of bioelectrical activities in hippocampus. Electrical stimulation of Schaffer collaterals in fasting rat induces a lower depolarization compared with control rat. Furthermore, the magnitude of LTP induced by tetanic stimulation is significantly decreased in hippocampal slices of the fasting rats. Taken together, these results suggest that repetitive cortical excitation results in an inhibitory tone on hippocampal plasticity. However, the exact mechanism has yet to be clarified.

Our electrophysiological study demonstrated that repeated CSD resulted in a significant decrease of the general excitability of the hippocampus together with a reduction in postsynaptic AMPA receptor response. These results are consistent with findings from various models of repeated neuronal activation. Electrophysiological study in *ex vivo* brain slices shows that chronic convulsions induced by multiple doses of 4-AP, a potassium channel blocker, result in a decreased cortical excitability. Quantitative immunohistoblot data from the same study also show that repeated seizures decrease GluR1–4 AMPA receptor subunit levels in all cortical layers (Vilagi, Dobo et al. 2009). Using quantitative immunoblot, Chazot and coworkers (Chazot, Godukhin et al. 2002) show that a 30% reduction in cortical AMPA GluR1 and GluR2 subunit immunoreactivities is observed 24 hours after 10 full consecutive recurrent CSDs induced *in vivo*.

The present electrophysiological study revealed a reduction of AMPA receptor function in hippocampus slice obtained from CSD-induced rat. It is not know whether this decrease in AMPA receptor response is responsible for LTP impairment or is an event that occurs simultaneously with LTP reduction. Aforementioned electrophysiological study and receptor binding analysis (Wernsmann, Pape et al. 2006; Haghir, Kovac et al. 2009) suggest some correlation between level of AMPA receptor function and hippocampal LTP. Furthermore, positive AMPA receptor modulators, CX516 and CX546, which prominently enhances synaptic transmission could facilitate LTP in hippocampal slice (Arai, Xia et al. 2004). These results suggest that alteration in AMPA receptor function is likely to have an effect on LTP induction. Thus, impairment of LTP induced by repetitive CSD in the present study may result from reduction of AMPA receptor response.

It is not know how SD could reduce AMPA receptor function. The most plausible explanation is that SD could induce AMPA receptor desensitization and internalization. During SD, glutamate is released from neuron and glia cells (Somjen 2001). High amount of glutamate released into the extracellular space may initiate AMPA receptor desensitization or internalization which might result in the decreased excitability (Greger and Esteban 2007; Vilagi, Dobo et al. 2009).

CONCLUSION

In the present study using the electrophysiological technique, the effect of repetitive CSDs on hippocampus functions was investigated. The following was the conclusions of our findings.

1. Topical solid KCl application on parietal cortex led to the repetitive negative potential shift characterized as CSD.

2. Topical solid KCl application on parietal cortex also led to the negative potential shift in hippocampus, suggesting the propagation of CSD into the hippocampus.

3. Repetitive CSD induction reduced hippocampal excitability, as evident by a decrease in postsynaptic AMPA receptor response.

4. Hippocampal synaptic plasticity was impaired following repetitive CSD induction, as indicated by reduction of LTP magnitude.

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Nociceptin, cortical spreading depression and long-term potentiation

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Nociceptin/orphanin FQ (N/OFQ) is a 17-amino acid neuropeptide with close similarity to the opioid peptides. N/OFQ has selective affinity for the orphan opioid receptor-like (ORL-1) G-protein coupled receptor. N/OFQ and ORL-1 receptor are widely distributed in the cortical nociceptive system, on the parity with limbic system. Such localization suggests their pathophysiological involvement in the mechanism of cortical spreading depression (CSD), and in the hippocampal long-term potentiation (LTP). Although immunohistochemical studies suggested that N/OFQ and the ORL-1 receptor may play a key role in CSD and LTP, their precise roles in synaptic transmission and plasticity are not well understood.

In this study, we demonstrate that LTP in the hippocampal CA1 region is inhibited by endogenous nociceptin in an activity-dependent manner. Our results indicate that endogenous nociceptin, either synaptically released or ambient, inhibits LTP induction and that the concentration of synaptically-released nociceptin is high enough to inhibit LTP.

Moreover, in order to study whether N/OFQ might be involved mediating the cortical excitability, we determined the effect of N/OFQ on electrophysiological waves of cortical spreading depression (CSD). We demonstrate that the peak amplitude and the number of CSD cycles were greater in N/OFQ-CSD compared with CSD group.

These results suggest that N/OFQ significantly inhibits LTP by decreasing hippocampal excitability. Interestingly, N/OFQ enhances the development of CSD by increasing cortical excitability. Its receptor, ORL-1 receptor, could be the target of pharmaceutical treatment of memory disorder, as well as migraine.

Keyword: Nociceptin/Orphanin FQ (N/OFQ), long-term potentiation (LTP), cortical spreading depression (CSD), electrophysiological study

Hippocampal long-term potentiation is inhibited by synaptically released endogenous nociceptin

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Summary

LTP (long-term potentiation) is a long-lasting enhancement in the efficiency of synaptic transmission between neurons, and is induced by repetitive and synchronous activation of presynaptic and postsynaptic cells. LTP of excitatory synaptic transmission in the hippocampus has been widely known as a cellular model of learning and memory. The induction of hippocampal LTP is regulated by many functional molecules at synapses, including the neuropeptide nociceptin identified as an endogenous ligand for the orphan opioid receptor. Mutant mice lacking the receptor exhibit enhanced LTP and hippocampus-dependent memory formation. However, little is known regarding their precise molecular and cellular mechanism. In this study, we demonstrate that LTP in the hippocampal CA1 region is inhibited by endogenous nociceptin in an activity-dependent manner. Thus, nociceptin is synaptically released from interneurons by high-frequency stimulation of afferent fibers. This endogenous nociceptin down-regulates the excitability of CA1 pyramidal cells by the hyperpolarizing current induced by the activation of K^+ channels, which are the common target shared with γ-aminobutyric acid type B ($GABA_B$) receptors although the mode of action is considerably different. Interestingly, the modulation of LTP by endogenous nociceptin is not observed when theta-burst stimulation is used in stead of high-frequency stimulation, suggesting that relatively longer high-frequency synaptic activation is required for the release of endogenous nociceptin. These results indicate that, in addition to GABA, nociceptin released from interneurons by their high-frequency activation is a novel endogenous neuromodulator that negatively regulates LTP induction in the hippocampus through direct modulation of pyramidal cells.

Enhancing effect of nociceptin in cortical spreading depression: electrophysiological study in animal model of migraine

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Summary

Migraine auras are important neurological disturbances appearing before the progress of migraine headache. A typical migraine aura is usually visual, apparently starting from the primary visual cortex producing a scintillation scotoma. Investigations in patients suggest that a visual migraine aura is the clinical manifestation of a Leao's cortical spreading depression (CSD); a condition caused by transient failure of brain ionic homeostasis. Elicitation of CSD in animals has been used as a model of migraine in last decades.

The control of cortical excitability is extremely complex and involves several chemical messengers, both small transmitter molecules and peptides.

Nociceptin/Orphanin FQ (N/OFQ) is a 17-amino acid neuropeptide. N/OFQ has a selective affinity for the ORL-1 receptor, a G-protein coupled receptor which is widely distributed in the nociceptive system. Previous studies revealed that intrathecal administration of N/OFQ has dual actions, hyperalgesia or antinociception. Regarding the pathogenesis of migraine, reduction in plasma N/OFQ levels has been reported during the attacks. However, the effects of N/OFQ in migraine attack are not well understood.

This study aims to determine the effect of nociceptin on the process of CSD development. Our results suggest that N/OFQ significantly enhances the development of CSD by increasing cortical excitability and activity of trigeminal system. Its receptor, ORL-1 receptor, could be the target of pharmaceutical treatment of migraine.

Nociceptin enhances the development of cortical spreading depression

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Background: The alternation in cortical activity is believed to represent initial manifestations of a migraine attack. While nociceptin (N/OFQ) is considered to involve in the cortical control, it is, however, still unclear as to what pathophysiological effect of N/OFQ include in migraine.

Objective: To study the effect of N/OFQ on development of cortical spreading depression (CSD), the animal model of migraine.

Methods: Wistar rats were divided into CSD, N/OFQ-CSD and control rats. N/OFQ was administered by intrathecal injection. CSD was induced by application of crystallized KCl on cerebral cortex.

Results: KCl application developed a train of depolarizing extracellular potential characterized as CSD. The development of CSD was enhanced by N/OFQ. The peak amplitude and the number of CSD waves within 1 hour were greater in N/OFQ-CSD compared with CSD rat, while area under curve and duration of each wave was not change.

Conclusion: Our results suggest that N/OFQ significantly enhances the progress of CSD by increasing cortical activity. Its receptor, ORL-1 receptor, could be the target of pharmaceutical treatment of migraine.

Effects of serotonin depletion in synaptic transmission and synaptic plasticity

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Abstract

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in the pathogenesis of many disease states such as depression, anxiety, and memory disorders.Anatomical and physiological studies indicate that serotonergic fibers densely innervate the hippocampus, suggesting a role for serotonin in learning and memory processes. Recent in vivo and in vitro studies have shown that LTP (long-term potentiation) in the hippocampus is reduced by depletion of serotonin. Therefore, we examined the effect of serotonin in hippocampal activity by electrophysiological experiments at the hippocampal acute slice level. Unfortunately, there was no significant difference between serotonin depletion group and control group in the experiment of hippocampal LTP. Additionally, serotonin depletion did not alter basic transmission, such as neurotransmitter release confirmed by paired-pulse stimulation (PPF). Nevertheless, it is interesting to understand the mechanism of memory disorder in migraine patient accompanied with aura which develops simultaneously with repetitive headache attacks relating to cortical spreading depression (CSD). CSD is electrophysiological phenomenon which occurs during migraine aura. Interestingly, it has been reported that CSD is enhanced in serotonin depletion. Then, we changed our approach to examine the relationship of CSD and hippocampal LTP, though our ultimate goal investigating the mechanism of memory disorder in migraine development is still unchanged.

Keywords: serotonin depletion, synaptic transmission, synaptic plasticity, long-term potentiation, cortical spreading depression

Outputs

1. Bongsebandhu-phubhakdi S, Phisonkulkasem T, Srikiatkhachorn A. *Enhancing effect of nociceptin in cortical spreading depression: electrophysiological study using an animal model of migraine.* Asian Biomedicine **2009**, 3: 325-329.
2. Bongsebandhu-phubhakdi S, Phisonkulkasem T, Srikiatkhachorn A. *Nociceptin/Orphanin FQ modulates cortical activity and trigeminal nociception.* Cephalalgia **2010**, submitted.

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Modulation of cortical spreading depression and trigeminal nociception by nociceptin/orphanin FQ

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Abstract

Activations of cerebral cortex and trigeminal nociceptive system are essential steps in pathogenesis of migraine. The control of these systems is extremely complex and involves several chemical messengers, both small molecule transmitters and peptides. Nociceptin/orphanin FQ (N/OFQ) is known as a neuropeptide with close similarity to the opioid peptides, while its effect in migraine is not fully understood. In this study, we investigated whether there is a modulation of N/OFQ in the trigeminal nociception induced by cortical spreading depression (CSD). CSD was elicited by topical application of solid potassium chloride on rat parietal cortex. Cortical activation was studied on the CSD development using electrophysiological methods. Trigeminal nociception was determined using Fos and TRPV1 (transient receptor potential vanilloid 1) immunoreactivity. Our results were revealed that intrathecal administration of N/OFQ produced biphasic effect which included initial attenuation and delayed potentiation of CSD development. Additionally, the number of Fos-immunoreactive neurons in the trigeminal nucleus caudalis (TNC) evoked by the CSD and the percentage of TRPV1 expression in the trigeminal ganglia (TG) after CSD were facilitated. N/OFQ modulation is likely to intensify the trigeminal nociception by increasing cortical activity. Orphan opioid receptor-like 1 (ORL1), the receptor of N/OFQ, may be a future target for pharmaceutical treatment of migraine.

Keywords: migraine; N/OFQ; ORL1; CSD; trigeminal nociception

Von: EHMTIC 2010 [mailto:ehmtic2010@abstractserver.com]

Gesendet: Montag, 10. Mai 2010 16:22

An: Saknan Bongsebandhu-phubhakdi

Betreff: EHMTIC 2010 - Your Abstract Submission

Dear Saknan Bongsebandhu-phubhakdi,

Thank you for submitting your abstract entitled [MODULATION OF CORTICAL SPREADING DEPRESSION AND TRIGEMINAL NOCICEPTION BY NOCICEPTIN/ORPHANIN FQ] to the 2nd European Headache and Migraine Trust International Congress- EHMTIC which will take place in Nice, France from October 28-31, 2010.

For future reference, please keep your abstract reference number:

A-242-0001-00063

Thank you again for your contribution.

Best regards,

The EHMTIC 2010 Abstract Team

Nociceptin/Orphanin FQ intensifies cortical activities and trigeminal nociception

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Abstract

Nociceptin/orphanin FQ (N/OFQ) is a 17-amino acid neuropeptide with close similarity to the opioid peptides. N/OFQ has selective affinity for the orphan opioid receptor-like (ORL-l) G-protein coupled receptor (GPCR). N/OFQ has been the subject of a large and growing number of studies intended to understand its physiological role in the nervous system. ORL-1 receptor is abundant expression widely in the central nervous system including the cerebral cortex, limbic system, brainstem and several areas involved in pain perception. Although, most of the studies have focused on the roles of N/OFQ in pain modulation, the effects of it remain controversial. A contentious issue is whether N/OFQ produces hyperalgesia (increasing pain) or analgesia (reducing pain). Distribution study suggests pathophysiological involvement of N/OFQ in the mechanism of cortical excitability and neurogenic headaches. It has been demonstrated that plasma N/OFQ level correlates with the frequency of migraine attacks. However, the precise role of N/OFQ in the involvement of the trigeminal nociceptive system, and the linkage between cellular action of N/OFQ and its behavioral effect are still unclear. The present study was designed to investigate the effect of N/OFQ on the cortical excitability and trigeminal nociceptive system. We indicated that N/OFQ significantly increased amplitude, number of peaks and area under the curve of CSD. Moreover, expression of transient receptor potential vanilloid 1 (TRPV1) in the trigeminal ganglia (TG) and number of Fos-immunoreactive neurons in the trigeminal nucleus caudalis (TNC) evoked by the CSD were facilitated. These results suggested that N/OFQ intensifies the trigeminal nociception by increasing cortical excitability.