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นายมนตรี มณีภาค

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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EFFECT OF CORTICAL SPREADING DEPRESSION ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS

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มนตรี มณีภาค : ผลของคอร์ติคัลสเปรดดิงดีเปรสชันต่อการส่งผ่านสัญญาณประสาทที่จุดประสานประสาทในฮิปโปแคมปัสของหนูแรท. (EFFECT OF CORTICAL SPREADING DEPRESSION ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศ. นพ. อนันต์ ศรีเกียรติขจร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. ดร. ศักนัน พงศ์พันธุ์ผู้ภักดี, ผศ. ดร. ศุภางค์ มณีศรี เลอกรองด์, 62 หน้า.

มีความสัมพันธ์ระหว่างการเกิดแสงวาบในผู้ป่วยไมเกรนและภาวะเสียความจำ คอร์ติคัลสเปรดดิงดีเปรสชันหรือซีเอสดีซึ่งคือปรากฏการณ์ที่ทำให้เกิดอาการปวดศีรษะไมเกรนอาจจะนำไปสู่อาการที่เกี่ยวเนื่องกับฮิปโปแคมปัส อย่างไรก็ตามบทบาทที่แน่ชัดของซีเอสดีต่อการทำงานของฮิปโปแคมปัสยังไม่ได้รับการตรวจสอบ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาการเปลี่ยนแปลงของสภาพพลาสติกของจุดประสานประสาทและการส่งผ่านสัญญาณประสาทพื้นฐานที่จุดประสานประสาทของฮิปโปแคมปัสเมื่อถูกเหนี่ยวนำด้วยซีเอสดี นอกจากนี้การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบว่าแคปไซซินสามารถปรับเปลี่ยนพลาสติกซิตีของฮิปโปแคมปัสซึ่งถูกทำให้เปลี่ยนแปลงโดยซีเอสดี หนูแรทสายพันธุ์วิสทาร์เพศผู้ถูกแบ่งออกเป็นกลุ่มซีเอสดีและกลุ่มควบคุม ซีเอสดีถูกเหนี่ยวนำซ้ำๆในกายสัตว์โดยการวางผลึกโพแทสเซียมคลอไรด์ สี่สิบห้านาทีต่อมาฮิปโปแคมปัสข้างเดียวกันกับการเหนี่ยวนำถูกนำออกมาและเตรียมเป็นแผ่นตัดฮิปโปแคมปัสสำหรับชุดของการศึกษาทางสรีรวิทยาไฟฟ้านอกกายสัตว์ หลังจากเหนี่ยวนำซีเอสดีพบว่าคลื่นสเปรดดิงดีเปรสชันปรากฏในฮิปโปแคมปัสเช่นกัน ซีเอสดีที่เหนี่ยวนำซ้ำๆนำไปสู่การลดลงในขนาดของลองเทอมโพเทนเทียชันหรือแอลทีพีในฮิปโปแคมปัส ซีเอสดียังลดประสิทธิภาพการส่งผ่านสัญญาณประสาทในฮิปโปแคมปัสดังที่แสดงโดยการลดลงของการสนองของตัวรับแอมพาหลังจุดประสานประสาท ในทางตรงกันข้ามการสนองของตัวรับเอ็นเอ็มดีเอหลังจุดประสานประสาทยังคงไม่เปลี่ยนแปลง อนึ่งยังไม่มีการเปลี่ยนแปลงในโพรไฟล์ของการกระตุ้นคู่ระหว่างกลุ่มทั้งสอง ซึ่งบ่งชี้ว่าซีเอสดีไม่ก่อให้เกิดการเปลี่ยนแปลงใดๆ ทางด้านก่อนจุดประสานประสาท แม้จะไม่มีการเปลี่ยนแปลงของการสนองของตัวรับเอ็นเอ็มดีเอ ซีเอสดีเพิ่มอัตราส่วนของหน่วยย่อยกลูเอ็นสองเอต่อหน่วยย่อยกลูเอ็นสองบีของตัวรับเอ็นเอ็มดีเอ นอกจากนี้การให้แคปไซซินสามารถฟื้นฟูแอลทีพีซึ่งถูกระงับโดยซีเอสดี การค้นพบนี้แสดงให้เห็นว่าการลดลงของการสนองของตัวรับแอมพาหลังจุดประสานประสาท และการเพิ่มขึ้นของอัตราส่วนของหน่วยย่อยกลูเอ็นสองเอต่อหน่วยย่อยกลูเอ็นสองบีเป็นกลไกที่เป็นมูลเหตุของแอลทีพีในฮิปโปแคมปัสที่บกพร่องซึ่งเกิดจากซีเอสดี แอลทีพีที่บกพร่องนี้สามารถทำให้กลับคืนได้โดยแคปไซซิน

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KEYWORDS : HIPPOCAMPUS / LONG-TERM POTENTIATION / CORTICAL SPREADING DEPRESSION / AMPA RECEPTOR / NMDA RECEPTOR

MONTREE MANEEPARK : EFFECT OF CORTICAL SPREADING DEPRESSION ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPUS. ADVISOR : PROF. ANAN SRIKIATKHACHORN, M.D., CO-ADVISOR : ASST. PROF. SAKNAN BONGSEBHANDHU-PHUBHAKDI, Ph.D., ASST. PROF. SUPANG MANEESRI LEGRAND, PH.D., 62 pp.

There is a relationship between migraine aura and amnesic attack. Cortical spreading depression (CSD), a phenomenon underlying migraine attack, may be responsible for hippocampus-related symptoms. However, the precise role of CSD on hippocampal activity has not been examined. The present study aimed to investigate the alteration of hippocampal synaptic plasticity and basal synaptic transmission induced by CSD. Furthermore, this study also aimed to examine whether capsaicin could modulate hippocampal plasticity that was altered by CSD. Male Wistar rats were divided into CSD and control groups. Repetitive CSDs were induced *in vivo* by topical application of solid KCl. Forty-five minutes later, the ipsilateral hippocampus was removed, and hippocampal slices were prepared for a series of *in vitro* electrophysiological studies. After CSD induction, SD waves also appeared in the hippocampus. Repetitive CSDs led to a decrease in the magnitude of long-term potentiation (LTP) in the hippocampus. CSD also reduced hippocampal synaptic efficacy, as shown by a reduction of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor responses. In contrast, postsynaptic *N*-methyl-D-aspartate (NMDA) receptor responses remained unchanged. Furthermore, there were no changes in paired-pulse profiles between the groups, indicating that CSD did not induce any presynaptic alterations. Despite unaltered NMDA receptor responses, CSD elevated the ratio of GluN2A to GluN2B subunit of NMDA receptor (Glu2A/B ratio). Moreover, capsaicin administration recovered LTP that was suppressed by CSD. These findings suggest that a reduction of postsynaptic AMPA receptor responses and an increase of Glu2A/B ratio are the mechanisms responsible for impaired hippocampal LTP induced by CSD. This impaired LTP could be improved by capsaicin.

Department : Physiology Student's Signature .....
Field of Study : Physiology Advisor's Signature .....
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CONTENTS

Page

Page

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ACSF	artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AUC	area under the curve
CaMKII	calcium/calmodulin-dependent protein kinase II
CSD	cortical spreading depression
DC	direct current
DMSO	dimethyl sulfoxide
EPSP	excitatory postsynaptic potential
ERC	entorhinal cortex
fEPSP	field excitatory postsynaptic potential
GluN2A/B	GluN2A/GluN2B
h	hour
HFS	high-frequency stimulation
i.p.	intraperitoneal
I/O	input-output
LTD	long-term depression
LTP	long-term potentiation
Min	minute
MRI	magnetic resonance imaging
N	number of rat
n	number of slice
NADA	N-arachidonyldopamine
NMDA	*N*-methyl-D-aspartate
POR	postrhinal cortex
PPF	paired-pulse facilitation
PRC	perirhinal cortex

PS	priming stimulation
S	second
SC	Schaffer-collateral
SD	spreading depression
θ_m	LTD-LTP crossover point
TGA	transient global amnesia
TRP	transient receptor potential
TRPV1	transient receptor potential vanilloid 1

CHAPTER I
INTRODUCTION

Background and Rationale

Spreading depression (SD) is a transient, reversible phenomenon, which is expressed as a self-propagating depolarization of neurons and glia, followed by a depression of neuronal bioelectrical activity for a period of minutes and accompanied by complex and variable changes in vascular caliber, blood flow, and energy metabolism (1,2). Although SD has been most extensively studied in the cortex (i.e., cortical SD - CSD), the phenomenon can be induced in most grey matter regions, including the hippocampus and the cerebellum, in a variety of species (3). CSD was originally linked to the aura phase of a migraine (4). However, some evidence also suggests a link between CSD and migraine pain as well as the associated signs and symptoms of migraine, such as sexual arousal, yawning and drowsiness, nausea and vomiting, and amnesia (1).

Transient global amnesia (TGA) is a memory disorder that is characterized by sudden episodes of severe anterograde and retrograde amnesia that last for several hours and then resolve fully and spontaneously within 24 hours (5,6). Many studies have reported a relationship between TGA and migraine. TGA attacks that are accompanied by migraine headache have been described in many case reports (7-9). In some cases, an amnesic attack occurs only during a migraine attack (10). Migraine is a risk factor and a precipitating event for TGA (11). Migraine and TGA also share common precipitants (7).

These associations between TGA and migraine support the hypothesis that both diseases might share a common pathophysiology like SD, as proposed by Olesen and Jorgensen (12). In the clinical study, diffusion-weighted magnetic resonance imaging (MRI) illustrated an increased signal intensity, which is compatible with SD, in the hippocampal region of TGA patients during attack (13). Bartsch and coworkers further demonstrated that an increased signal intensity during attack is selectively found in the hippocampal CA1 area of TGA patients (14). In animal study,

hippocampal injection of potassium chloride produced a deficit in retention of conditioned suppression learned 24 hours before injection (15). Bilateral hippocampal or cortical SD evoked immediately after acquisition of a passive avoidance reaction elicited a partial amnesia (3).

Although CSD may be related to memory disorder and perturbation of hippocampal function, the effect of CSD on hippocampal plasticity has not been fully investigated. A study of *in vitro* cortical-hippocampal combined slices showed that single CSD can, depending on the propagation of SD into the hippocampus, facilitate or depress hippocampal (long-term potentiation) LTP (16). However, the mechanism that underlies this phenomenon is unknown.

It has been well established that activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors is involved in the induction of LTP in the CA1 region of the hippocampus (17). An increase of intracellular Ca^{2+} trough activated NMDA receptor initiates an biochemical event required for LTP induction (18). Alteration of NMDA receptor subunit composition affects channel kinetic, which controls Ca^{2+} influx and synaptic plasticity thresholds (19,20). It has been hypothesized that the ratio of GluN2A to GluN2B subunit (GluN2A/B) of NMDA receptor affects the LTP induction threshold by altering Ca^{2+} entry (20).

In addition to NMDA receptors, "α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid" (AMPA) receptors play a pivotal role in LTP induction. Jia and coworkers showed that knockout of AMPA receptor subunits affected LTP induction (21). Furthermore, pharmacological modulation of AMPA receptors influenced synaptic plasticity in the hippocampus (22).

Capsaicin, "8-methyl-N-vanillyl-6-nonenamide" is a substance that gives hot and spicy sensation and is found in chili (23). Capsaicin could activate the "transient receptor potential vanilloid 1" (TRPV1) receptor, which is a cation channel permeable to Ca^{2+} (24). TRPV1 receptor is also an important receptor that mediates hippocampal synaptic plasticity (23,25). Study in TRPV1-deficient mice shows that LTP in the hippocampal slices is impaired (23). Thus, this study will use capsaicin as a promising treatment to facilitate LTP which may be impaired by CSD.

From the above literature, it is tempting us to study the effect of CSD on hippocampal plasticity and mechanisms underlying the CSD-induced plasticity alteration.

Research Questions

How does CSD affect hippocampal plasticity? Is this plasticity change mediated by altering basal synaptic transmission or GluN2A/B subunit ratio? Does capsaicin treatment recover CSD-induced LTP impairment?

Hypothesis

CSD impairs hippocampal LTP by altering basal synaptic transmission and ratio of GluN2A/B subunit. This LTP impairment could be restored by capsaicin supplementation.

Research Objectives

1. To examine whether CSD could propagate to hippocampus
2. To study the effect of CSD on hippocampal synaptic plasticity.
3. To investigate the mechanisms that underlie an alteration of hippocampal plasticity induced by CSD
4. To examine the effect of capsaicin on the modulation of hippocampal plasticity.

Expected Benefit and Application

The knowledge obtained from this study provides a better understanding of the mechanisms that underlie the perturbation of hippocampal synaptic plasticity induced by CSD, as well as the pathogenesis of hippocampus-related symptoms that occur during a migraine attack.

CHAPTER II
LITERATURE REVIEWS

Spreading Depression

SD is a transient, reversible phenomenon that 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 (1,2). SD is a propagating wave of negative potential (1). During SD, ions form intracellular compartment, such as K^+ and H^+, are released. Ions from extracellular compartment, such as Na^+, Ca^{2+} and Cl^-, flux into cells. Cells swell and volume of extracellular compartment is reduced because water enters cells with ion influx. The amplitude and duration of SD potential are 10 to 30 mV and more than 0.5 to 1 min, respectively (1,26). 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 (3).

Cortical Spreading Depression

CSD was first described in the rabbit, pigeon and cat cortex by Leao (27). He reported a large negative shift in direct current (DC) potential lasting 1 - 2 min, followed by a positive deflection lasting 3 - 5 min before returning to baseline. Lashley (28) described his own migraine aura. He hypothesized that the perturbation of neural function originated at the center of visual field and spread to the temporal area within 10 to 15 min. This perturbed function become normal within 10 to 15 min. He considered that this wave of excitation followed by suppression of neuronal activity traveled along cortex surface of visual area at a rate of 3 mm per min. The similar characteristics between the spread of the cortical representation of the migraine visual aura and CSD propagation immediately give rise to the hypothesis that migraine aura is caused by CSD (4).

Clinical Relevance of SD

A. CSD and Associated Migraine Symptoms

CSD was originally linked to the aura phase of migraine (4). 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 (1). During aura, anomia and amnesic attacks are described in particular (10,29,30). In addition, retention, concentration, attention, and memory are typically weak during the migraine episodes (30).

B. SD and Transient Global Amnesia

TGA is a memory disorder characterized by sudden episode of severe anterograde amnesia lasting for several hours (5). During an episode, the patient shows a sign of abrupt and severe impairment of memory for the recent past and present events (retrograde amnesia) coupled with anterograde amnesia. This memory dysfunction lasts for several hours before it becomes recovered gradually (31).

Many clinical studies indicate that the hippocampus is implicated in TGA pathophysiology. “Positron emission tomography” study demonstrated abnormalities in several brain areas including the amygdala and hippocampus (32). “Diffusion-weighted MRI” showed transient disruption of blood flow in the amygdala and hippocampus (33).

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 (1). The amnesic attack is reported during the aura phase, concomitant with migraine attacks or follow a migraine status (10).

SD and Memory Dysfunction: Animal Studies

Although exact pathophysiology of TGA is not clear, SD in the hippocampus may be relevant to TGA (12). In experimental animals, hippocampal SD induced by KCl injection caused a temporary memory deficit that lasted minutes to hours with complete functional recovery (15). Bilateral hippocampal or cortical SD evoked immediately after acquisition of a passive avoidance reaction, elicited a partial amnesia (3). In addition, “manganese-enhanced MRI” illustrated an elevated signal in

the dentate gyrus as well as CA1–3 areas of the hippocampus after CSD induction by topical KCl application on the exposed rat cortex (34). However, the exact mechanisms of CSD-induced memory impairment remain to be elucidated.

Hippocampus and Memory

The mammalian hippocampus is a brain structure that has a crucial function related to certain types of memory (35). It plays a role in acquiring of episodic memory, and may be also implicated in consolidation of long-term memory (36). In clinical studies, the most notable case was H.M., whose medial temporal lobes, including both hippocampi, was excised during experimental neurosurgery. After the surgery, he lost an extensive amount of past memory (retrograde amnesia), coupled with an inability to gain a novel episodic memory (anterograde amnesia) (37). Studies in animal also demonstrated that pharmacological inactivation or lesions of hippocampus results in either a learning failure or a loss of spatial memory (38,39).

Basic Anatomy of the Hippocampus

Traditionally, the diagram illustrating hippocampal wiring is shown as a "trisynaptic loop" (Fig. 1). Polymodal sensory information is conducted by the perforant path axons from lamina II of the entorhinal cortex (ERC). These axons provide the prime input to granule cells of the dentate gyrus. Perforant path axons from the medial and lateral entorhinal cortices synapse with the middle third and outer of the granule cells' dendrite, respectively. The proximal apical dendrites of CA3 pyramidal cells receive an input via the mossy fibers, which are granule cells' axons. CA3 pyramidal cells send their axons, Schaffer-collateral (SC), to ipsilateral CA1 pyramidal cells and project other axons, commissural fibers, to contralateral CA1 and CA3 pyramidal cells. The distal apical dendrites of CA1 pyramidal neurons also directly obtain an input from the lamina III neurons of the ERC. The cell bodies of these three principal subfields (dentate gyrus, CA3, and CA1) are compactly bundled in an elegant laminar organization ("interlocking C-shaped arrangement") with their afferent fibers projecting to definite regions of the dendrite. In addition to the sequential "trisynaptic loop", ipsilateral CA3 pyramidal cells also inter-connect by a dense associative network. CA3 pyramidal cells are also directly received an input

from neurons in lamina II of the ERC. In addition, a diversity of inhibitory neurons as well as extensive modulatory input to hippocampal neurons also present in the hippocampus (not shown in the figure).

Hippocampal slices are suitable for prolonged extracellular recording experiments due to many reasons. First, hippocampal slices could be maintained in a good condition for many hours with a supply of artificial cerebrospinal fluid (ACSF) and oxygen. Second, the laminar organization of cells in the hippocampus allows stimulation of selective pathways and a recording of the postsynaptic responses evoked by a group of target neurons (36).

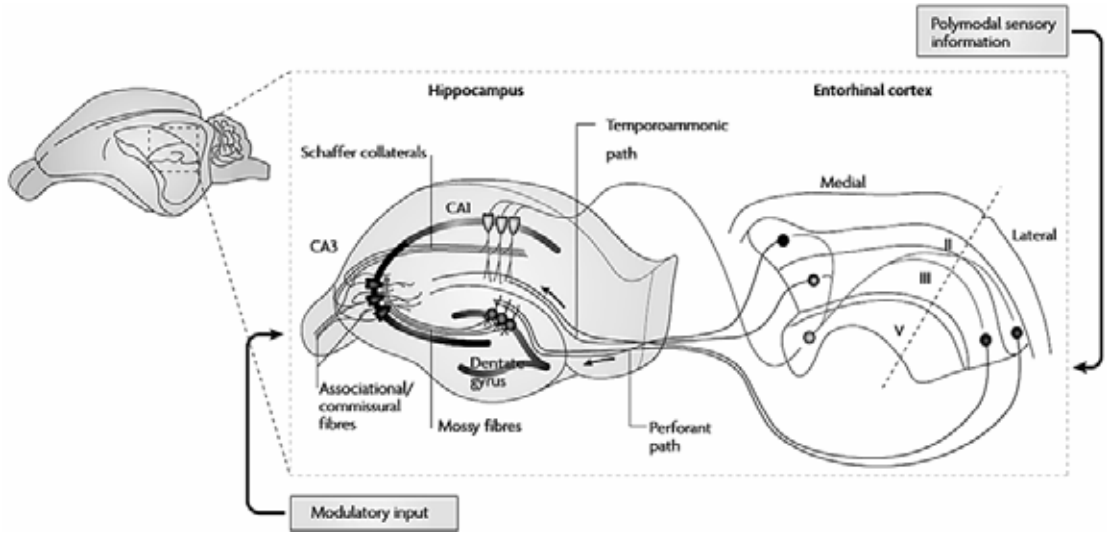

Figure 1 "Basic anatomy of the hippocampus" From Neves et al. (36).

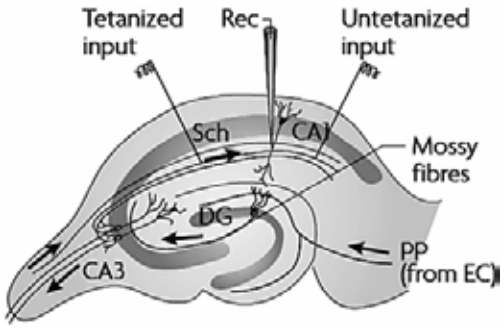

Figure 2 *In vitro* extracellular recording of hippocampal synaptic events. From Neves et al. (36).

Hippocampus and Synaptic Plasticity

The mechanisms of synaptic plasticity related to memory formation in the brain have been studied using the hippocampus as a prime experimental model. (36). Synaptic events could be monitored in the hippocampus using extracellular recording due to its simple laminar organization of synaptic pathways (Fig. 2) (40). LTP is a model of hippocampal synaptic plasticity that has been extensively studied. LTP, representing synaptic plasticity in the hippocampus, has been considered as a model of memory formation at cellular level (41). Furthermore, LTP models are wildly employed in many studies to investigate the synaptic plasticity that might be altered in various neurological diseases (42).

Hippocampal LTP

Using extracellular field potentials recording, LTP was first discovered in the hippocampus of anaesthetized (43) and unanaesthetized (44) rabbits. After that, numerous studies have been conducted on LTP in order to shade the light on a mechanism of LTP induction at cellular and molecular levels, most of which have employed *in vitro* recording in brain slices or cultured neurons preparation (45).

A first rise in intracellular Ca^{2+} in postsynaptic cells is a shared mechanism of LTP induction at all key hippocampal synapses (46). It is well known that influx of Ca^{2+} trough NMDA receptor complex and subsequent elevation of postsynaptic Ca^{2+} level are required for LTP induction in the hippocampal CA1 (47). In LTP induction protocol, brief high-frequency stimulation (HFS; ∼100 Hz) leads to a prolonged enhancement of the synaptic strength. This single 100 Hz train (100 pulses at baseline stimulation intensity, over 1 s) has been used as an effective protocol for inducing early LTP (lasting for 1 - 3 h), which is NMDA receptor-dependent and protein synthesis-independent (42,48).

Basic Synaptic Transmission

To examine basal synaptic transmission, two experimental protocols, including input-output (I/O) relationship and paired-pulse facilitation (PPF), are conducted. PPF are used to determine whether the presynaptic mechanism is altered or not. The I/O

relationship is used for measures synaptic efficacy. It could indicate both presynaptic and postsynaptic alteration.

PPF is an enhancement of the synaptic response to the second of two closely spaced stimulating pulses (49). PPF is known to be modified when the Ca^{2+}-dependent release of glutamate from presynaptic terminal is diminished, but not when excitatory postsynaptic potential (EPSP) amplitudes are diminished by postsynaptic mechanisms. Manipulations that lessen Ca^{2+}-mediated glutamate release from presynaptic terminal of SC fibers could increase PPF (50). In contrast, PPF does not altered by manipulating EPSPs of CA1 neuron via postsynaptic actions (51).

Metaplasticity

Many studies indicate that preceding synaptic event influences the induction of synaptic plasticity. Modulation of subsequent synaptic plasticity that depends on prior synaptic activity is typically referred to as metaplasticity or priming (52).

Nowadays, the metaplasticity is phenomenologically described as the simultaneous shifts in different directions in the levels of integrated postsynaptic response needed for inducing subsequent LTP and long-term depression (LTD). Strong synaptic activation can increase the threshold for the following induction of LTP and, concomitantly, reduce that for LTD. Conversely, weak prior synaptic stimulation lessens the threshold for the ensuing induction of LTP and, concurrently, elevates that for LTD (53).

Mechanisms of Metaplasticity

The underlying mechanisms of metaplasticity have not yet been fully understood. However, possible mechanisms have been proposed. It is postulated that prior changes in Ca^{2+} regulation account for the reversal shifts in the magnitudes of integrated postsynaptic response required for eliciting LTP and LTD (19).

In this literature review, two receptors that may mediate metaplasticity are described. The first receptor is NMDA receptor, of which functional alteration is apparent probable mechanism for controlling Ca^{2+} entry and thresholds of synaptic plasticity (19). The second receptor is TRPV1, of which activation increases intracellular Ca^{2+} and modulates synaptic plasticity (54).

NMDA receptor

NMDA receptor is a ligand-gated ionotropic receptor that is highly permeable to monovalent ions and calcium (55). Native NMDA receptor is composed of GluN1, GluN2 (A, B, C, and D) or GluN3 (A and B) subunits. Functional NMDA receptor channels require a combination of GluN1, a necessary channel-forming subunit, and at least one of the GluN2 subunits (56). Using quantitative immunoprecipitation techniques, Al-Hallaq *et al.* (57) have recently reported that the majority of native NMDA receptor in the rat hippocampus is di-heteromeric receptors containing either GluN1/2A or GluN1/2B subunits. It has been shown that the type of GluN2 subunit contained in heteromeric GluN1/2 NMDA receptor channels defined biophysical and pharmacological properties of the channels. These properties include kinetics of desensitization and offset decay, sensitivity to magnesium block, affinity for agonists and antagonists, susceptibility to modulation by glycine, reducing agents, polyamines, and phosphorylation (58,59). The different properties of GluN2A- and GluN2B-containing NMDA receptors are listed in table 1.

Table 1 Comparison between properties of GluN2A- and GluN2B-containing NMDAR receptors

Properties	GluN2A	GluN2B	References
Open probability	High	Low	(60)
Deactivation	Fast	Slow	(61)
Peak current	High	Low	(61)
Rise time	Fast	Slow	(60)
Decay time	Fast	Slow	(60)
Charge transfer	Low	High	(61)
Ca^{2+}/EPSC	Low	High	(62)
CaMKII binding	Weak	Strong	(63)

Ratio of GluN2A/GluN2B and Metaplasticity

Several studies investigate the function of NMDA receptor subunit component in synaptic plasticity. At synapses, the GluN2A/B ratio is not constant, but it could be

modified with synaptic plasticity (64). These modifications might assist the optimization of the threshold for inducing LTP and LTD. Given that GluN2B-containing NMDA receptors reveal longer currents (65), carry more Ca^{2+} per unit of current (62), and interact more preferentially with "calcium/calmodulin-dependent protein kinase II" (CaMKII) than GluN2A-containing NMDA receptors (66), it has been speculated that GluN2B subunits are more prone to prefer the LTP induction compared to GluN2A subunits.

This hypothesis has been supported by numerous pieces of evidence. Firstly, ifenprodil, a GluN2B-specific antagonist, entirely obstructs pairing protocol-induced LTP in immature hippocampal slice cultures. This result suggests that GluN2B subunits are critically required for the LTP induction (67). Secondly, genetic deletion of GluN2A unsuccessfully suppresses LTP in hippocampus of P28 mice. This suggests that GluN1/GluN2B di-heteromic NMDA receptors are adequate to provoke hippocampal LTP (68,69). Thirdly, overexpression of GluN2B by genetic manipulation elevates LTP in hippocampus of 4 - 6 month-old mice (70).

Although many studies support the hypothesis, direct evidence that could confirm the link between prior activity-dependent alteration of GluN2A/B ratio and the bidirectional metaplastic modulation of LTP/LTD threshold is scarce. Recently, Xu and coworkers (71) show that bidirectional metaplastic alteration and the GluN2A/B ratio could be modulated by "priming stimulations" (PS), which is a preceding activity given by applying a stimulation with frequencies of a different range (1 - 100 Hz). The 1 and 5 Hz low-frequency PS lead to a reduction of GluN2A/B ratio. On the contrary, 50 and 100 Hz high-frequency PS lead to an increased GluN2A/B ratio. The altered GluN2A/B ratio governs the threshold for subsequent LTP/LTD. The magnitudes of LTP decrease as the level of GluN2A/B ratios increase, and vice versa. These data reveal that activity-dependent alterations in the GluN2A/B ratio are likely to be critical factors to govern the metaplastic LTP/LTD threshold (Fig. 3).

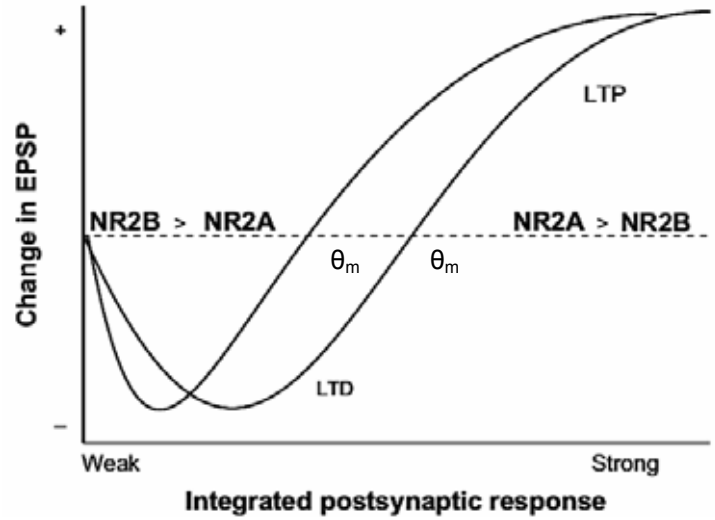

Figure 3 Schematic depicting how NMDA receptor subtype modulates the characteristics of synaptic plasticity. The x-axis is the magnitude of the "integrated postsynaptic response", which is corresponded to the stimulating frequency and synaptic depolarization. The y-axis is the potentiation (+) or depression (-) of synaptic strength. θ_m represents the LTP/LTD crossover point. If the GluN2A/B ratio is high, θ_m should slide to the right. LTD is favored over LTP. On the other hand, if the GluN2A/B ratio is low, θ_m should slide to the left. The probability that LTP will be provoked is reduced (20).

TRPV1 Activation and Synaptic Plasticity

TRPV1 is a channel receptor that is belonged to the "vanilloid TRP" family (72). The TRPV1 channel comprises four subunits that form tetramer. Each subunit contains six membrane-spanning domains. Opening of TRPV1 receptor channels are controlled by endovanilloids, proton, or heat. Endovanilloids are endogenous ligands, which include "long-chain, linear fatty acid – dopamides" (e.g., "N-arachidonyldopamine": NADA) and "anandamide", which is "lipoxygenase derivatives" of "arachidonic acid" (24).

TRPV1 is expressed throughout most unmyelinated small- to medium-size neurons in various ganglia, including nodose sensory, trigeminal, and dorsal root ganglia. In spinal dorsal horn, TRPV1 receptor is expressed in laminae I and II (73). In central nervous system, this receptor is also expressed in many brain areas, including hippocampus, despite the fact that its function is not fully characterized

(74). Several studies report the mRNA and protein expression of the receptor in pyramidal neurons of mouse (75) and rat (76) hippocampus.

Many studies report that TRPV1 may play a major role in modulation of hippocampal synaptic plasticity. Firstly, Marsch and co-workers (23) demonstrated the impairment of both fear memory related to hippocampus and CA1 LTP in hippocampal slices in TRPV1-knockout mice. Secondly, Gibson and his team (25) studied synaptic plasticity at the excitatory synapses onto hippocampal inhibitory interneurons and found that activation of TRPV1 is necessary and adequate to elicit LTD. Excitability of innervated pyramidal cells is supposed to enhance by that selective depression of the inhibitory interneurons.

TRPV1 is also activated by exogenous ligand called capsaicin. Capsaicin, "8-methyl-N-vanillyl-6-nonenamide" is a substance found in chili, which gives hot and spicy sensation (23). Capsaicin has been used in many researches related to pain, hyperalgesia, and inflammation due to its dramatic biological effects (76). In the context of synaptic plasticity, Li and colleague (54) showed that TRPV1 activation by capsaicin facilitates LTP and suppresses LTD in the hippocampus. Baseline synaptic transmission is not modified by capsaicin. Capsaicin greatly shifts the threshold of LTP induction towards lower frequency. As a result, stimulation with low frequency could produce LTP instead of LTD. Thus, our study will use capsaicin supplementation as a treatment for facilitating LTP which is supposed to be impaired by CSD.

Conceptual Framework

Considering the above literature review, it might be said that TGA and migraine may share a common pathophysiology, such as SD. This tempted us to speculate that CSD might propagate and reach to the hippocampus, and affect its function. The possibility of SD propagation into the hippocampus had been investigated in the experiment part A. It has been shown that hippocampal SD could disrupt synaptic plasticity, but the underlying mechanism has not yet been determined. Changes of a function at presynaptic and postsynaptic site, including modification of AMPA and NMDA receptor responses and of GluN2A/B ratio, could contribute to an impairment of synaptic plasticity. Therefore, in the experiment part B. we employed a battery of

in vitro electrophysiological protocols to investigate the effect of CSD on hippocampal synaptic plasticity and transmission. Capsaicin has been shown to facilitate LTP induction in the hippocampus. Hence, we examined whether capsaicin could restore LTP that was suppressed by CSD in the experiment part C. The diagram illustrating conceptual framework is shown in Fig. 4.

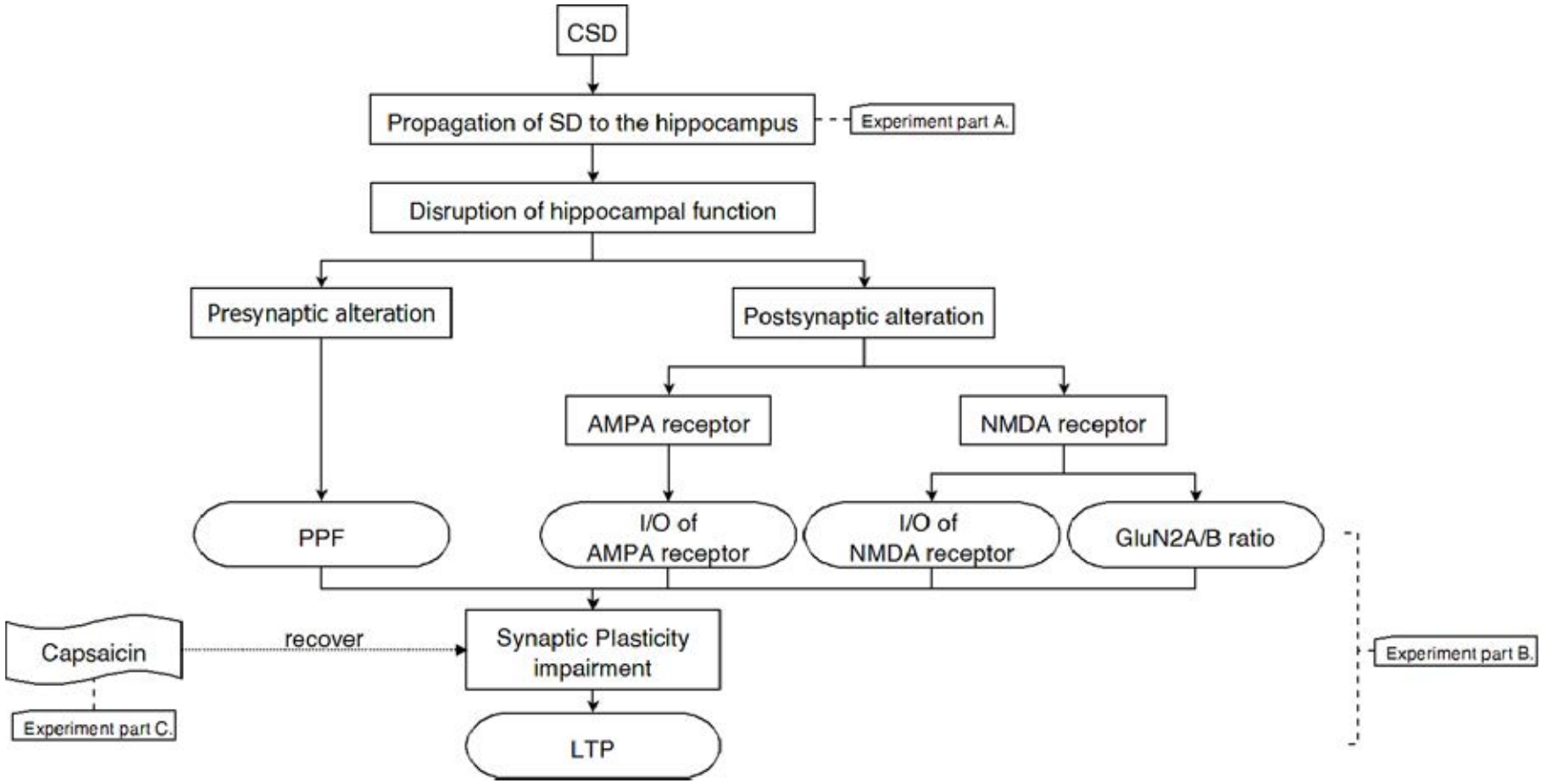

Figure 4 Conceptual framework

CHAPTER III
MATERIALS AND METHODS

MATERIALS

A. Chemicals and Biochemicals

Dimethyl sulfoxide (DMSO; "Sigma-Aldrich, MO, USA")

Potassium chloride (KCl; "Sigma-Aldrich, MO, USA")

Sodium chloride (NaCl; "Merck, Germany")

Magnesium sulfate ($MgSO_4$; "Sigma-Aldrich, MO, USA")

Calcium chloride ($CaCl_2$; "Merck, Germany")

Sodium phosphate (NaH_2PO_4; "Sigma-Aldrich, MO, USA")

Sodium bicarbonate ($NaHCO_3$; "Sigma-Aldrich, MO, USA")

Glucose ("Sigma-Aldrich, MO, USA")

Hydrochloric acid 1N ("Merck, Germany")

B. Drugs

Sodium pentobarbital ("Ceva Sante Animale, France")

Picrotoxin ("Sigma-Aldrich, MO, USA")

APV ("Sigma-Aldrich, MO, USA")

CNQX ("Tocris Bioscience, UK")

Capsaicin ("Tocris Bioscience, UK")

Ifenprodil ("Tocris Bioscience, UK")

C. Materials

Intra-tracheal tube

Medical supplies (needles, syringes, gloves)

Gas mixture (95% O_2/ 5% CO_2)

Capillary glass ("Sutter Instrument, CA, USA")

D. Research Instruments

Stereotaxic apparatus (“Narishige, Japan”)

Dental drill (“NSK, Japan”)

Hydraulic micromanipulator (“Narishige, Japan”)

Motorized micromanipulator (“World Precision instruments, FL, USA”)

Micromanipulator (“Narishige, Japan”)

Amplifier (“Nihon Kohden, Japan”)

MP100 data acquisition system (“Biopac Systems Inc., CA, USA”)

Vibratome tissue slicer (“Vibratome, IL, USA”)

Microelectrode puller (“Sutter Instrument, CA, USA”)

Light microscope (“Olympus Corporation, Japan”)

Stereo microscope (“Olympus Corporation, Japan”)

Peristaltic pump (“Rainin Instrument Company Inc., MA, USA”)

Vacuum pump (“Gast Manufacturing Incorporated, MI, USA”)

EPC10 Amplifier (“HEKA Instruments Inc., NY, USA”)

EXPERIMENTAL DESIGN

In order to investigate the effect of CSD on hippocampal electrophysiology and the underlying mechanisms, the experiments were divided into 3 parts as described.

A. The Study of Properties of *in vivo* Hippocampal Spreading Depression

The aims of this study were to investigate whether CSD could propagate into hippocampus or not and to characterize SD in the hippocampus. In this study, *in vivo* CSD was induced in parietal cortex. DC potential was recorded in the frontal cortex (4 rats; N = 4) or hippocampal CA1 (N = 4). The variables of SD in both frontal cortex and hippocampus were measured and compared.

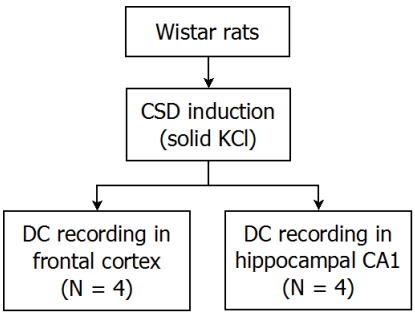

Figure 5 Diagram illustrating the experimental design of part A.

B. The Study of Alterations of Hippocampal Synaptic Transmission and Plasticity Induced by CSD

The aim of this study was to examine the effect of CSD on hippocampal LTP and basal synaptic transmission. In this study, the animals were divided into CSD and control groups. In the CSD group, repetitive waves of CSD were induced *in vivo* by topical application of solid KCl onto the parietal cortex for 45 minutes. In the controls, solid NaCl was applied topically on the cortex instead of KCl crystal. After 45 minutes of CSD induction, the rat was sacrificed, and ipsilateral hippocampus was removed for preparing hippocampal slices. After being recovered for at least 1.5 h in a humidified chamber, the slice was submerged under perfusing ACSF in a recording chamber. Then, *in vitro* electrophysiological study of hippocampal SC-CA1 synapses was performed. The electrophysiological protocols included LTP (7 slices per group; n = 7 per group), PPF (n = 10 per group), I/O relationship of AMPA receptor (n = 7 per group), I/O relationship of NMDA receptor (n = 7 per group), and GluN2A/B ratio (n = 6 per group). In one rat, up to 3 slices were used in the experiment. In all experiment, hippocampal slice obtained from the same rat was not subjected for studying the same protocol (i.e., number of slice (n) was equal to number of rat (N) in each protocol).

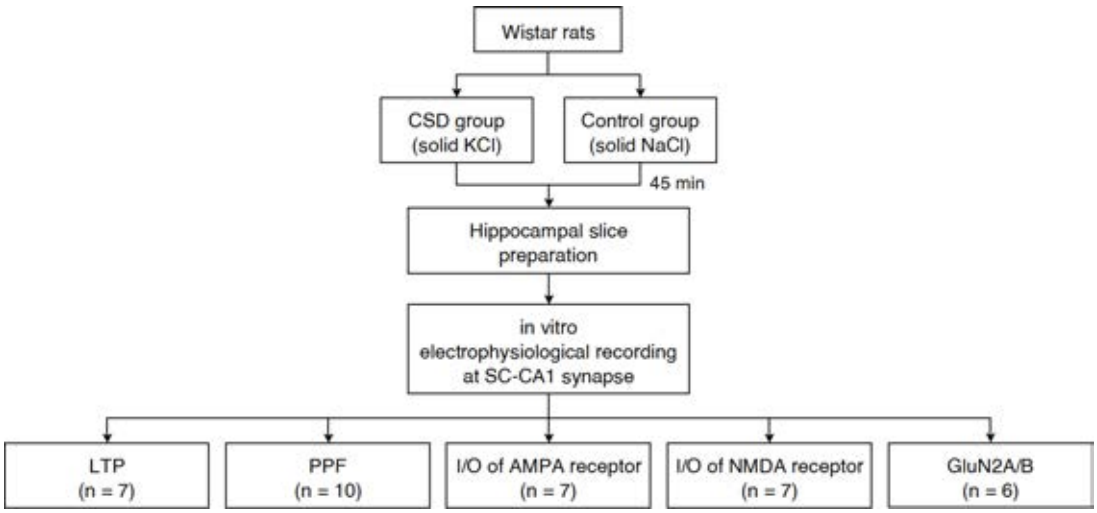

Figure 6 Diagram illustrating the experimental design of part B.

C. The Study of the Effect of Capsaicin on Hippocampal LTP

The objective of this study was to examine the effect of capsaicin on hippocampal LTP in normal and CSD-induced conditions. We postulate that capsaicin may recover LTP that was impaired by CSD. In this study, the animals were divided into 2 groups, namely CSD and control. In the CSD group, repetitive waves of CSD were induced *in vivo* by topical application of solid KCl onto the parietal cortex for 45 minutes. In the control group, solid NaCl was applied topically on the cortex instead of KCl crystal. After 45 minutes of CSD induction, the rat was sacrificed, and ipsilateral hippocampus was removed for preparing hippocampal slices. After being recovered for at least 1.5 h in a humidified chamber, the slice was submerged under perfusing ACSF in a recording chamber. Then, *in vitro* electrophysiological study of hippocampal SC-CA1 synapses was performed. In each group, LTP was induced in 2 conditions, with capsaicin or vehicle (n = 6 per condition per group).

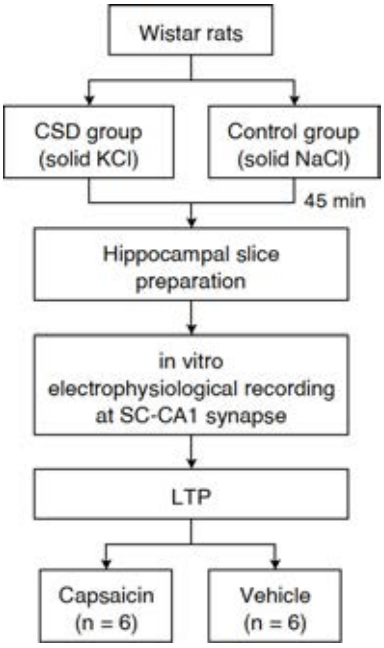

Figure 7 Diagram illustrating the experimental design of part C.

METHODS

A. Animals

Adult male Wistar rats were purchased from "National Laboratory Animal Center" ("Mahidol University", Thailand). The Animals weighing 200-350 g were maintained in stainless steel cages (8 rats per cage) with free access to food (regular dry rat food) and water. The animals were housed in a temperature-controlled facility with (12-h light/12-h dark cycle). They were acclimatized to the housing facility for at least 7 days before experiments.

The experiments were conducted according to "the principles of humane experimental technique (77)" ("the principles of 3Rs") briefly described as follow. Firstly, the author had considered alternative methods of substituting conscious living higher animals of insentient material (replacement). Secondly, the author reduced the numbers of animals used to obtain information of a given amount and precision (reduction). Thirdly, the author decreased the incidence or severity of "inhumane" procedures applied to those animals that still have to be used (refinement). The study was also conducted according to the guideline for experimental animals suggested by the "National Research Council of Thailand" (1999). The study protocol was

approved by the “Ethics Committee of the Faculty of Medicine”, “Chulalongkorn University” (No. 012/2010).

B. Animal Preparation and CSD Induction

Each rat was anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and fitted with an intratracheal tube. Additional doses of anesthetics were given as required to maintain surgical anesthesia based on a response to tail pinch. Rat was placed in a stereotaxic apparatus (Narishige, Japan). A surgical incision was made on the midline, and the skin and soft tissue overlying the skull were removed. For induction of CSD, a 2-mm diameter craniotomy was made in the right parietal bone (6 mm posterior to the bregma and 2 mm lateral to the midline). The bone was drilled carefully with a dental drill (NSK, Japan) using a slow speed, saline-cooled technique to minimize surgical irritation of the brain. The dura mater was opened using a microneedle. Multiple waves of CSD were elicited for 45 minutes by topical application of solid KCl (3 mg) onto the exposed surface of the parietal cortex. In the control group, solid NaCl (3 mg) was used instead of KCl.

C. *in vivo* Cortical DC Recording

For DC recording, a 2-mm diameter craniotomy was created in the right frontal bone (3 mm anterior to the bregma and 2 mm lateral to the midline). After careful removal of the dura mater, a recording glass microelectrode for detecting DC potential was inserted into the frontal neocortex using a hydraulic micromanipulator (Narishige) to a depth of 500 μm. A Ag/AgCl reference electrode was placed on the skin at the back of the rat. The electrical signal was amplified using an amplifier (Nihon Kohden, Japan). Analog data were converted into a digital format using an MP100 data acquisition system (Biopac Systems Inc., CA, USA). The converted data were then analyzed using the AcqKnowledge acquisition software (Biopac Systems Inc.). The variables that were measured included the area under the curve (AUC) and the amplitude of each CSD wave as well as the number of CSD waves that occurred within a 45-minute period.

D. *in vivo* Hippocampal DC Recording

In a separated set of experiment, DC potential was recorded in the CA1 region of the hippocampus instead of the neocortex. For DC recording, a 2-mm diameter craniotomy was made in the right parietal bone (4 mm posterior to the bregma and 2 mm lateral to the midline). A recording glass microelectrode was inserted into the CA1 area of the hippocampus to a depth of 2.2 mm. The variables of hippocampal SD were measured and analyzed as those of CSD.

E. Hippocampal Slice Preparation

Forty five minutes after elicitation of CSD waves, each rat was decapitated, and the entire ipsilateral hippocampus was quickly removed from the brain. Transverse hippocampal slices (400 μm thickness) were cut using a Vibratome tissue slicer (Vibratome, IL, USA) in ice-cold ACSF. The composition of ACSF was 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 0.1 picrotoxin, a $GABA_A$ receptor antagonist. The ACSF was bubbled continuously with carbogen (95% O_2/ 5% CO_2). Fresh slices were transferred to a humidified holding chamber (interface-type) and recovered for at least 1.5 h before electrophysiological experiments were conducted.

F. *in vitro* Electrophysiological Recording in Hippocampal Slices

An individual slice was transferred into a recording chamber, fixed with a nylon net, and submerged in carbogen-saturated ACSF. ACSF was continuously perfused into a chamber at a rate of 1.5-2.0 mL/min using a peristaltic pump (Rainin Instrument Company Inc., MA, USA), and was drawn out of the chamber using a vacuum pump (Gast Manufacturing Incorporated, MI, USA). All experiments were performed at room temperature (25 °C).

To avoid epileptiform activity from the CA3 region, a cut was made to split the CA1 region from the CA3 region. A bipolar stimulating electrode made of tungsten was positioned in the stratum radiatum using a micromanipulator (Narishige). To evoke a postsynaptic response, square-pulse stimulus of 0.2 ms duration at 0.1 Hz (1 pulse every 10 s) was delivered to a slice through the bipolar tungsten electrode to activate the SC pathway that projected to CA1. A glass microelectrode with 2-8 MΩ resistance containing 3 M NaCl was positioned parallel to the stimulating electrode in

the stratum radiatum, 200-300 μm from the stimulating site, using a motorized micromanipulator (World Precision instruments, FL, USA) to record presynaptic fiber volleys that were followed by field excitatory postsynaptic potentials (fEPSPs). The setup of electrophysiological recording is illustrated in Fig. 8. To avoid changes in fEPSP properties that could be attributed to electrode positioning, an attempt was made to maintain a similar orientation of the electrodes relative to the cell layers of CA3 and dentate gyrus. For baseline recording, stimulus strength was adjusted to evoke fEPSP with slope of 0.12 to 0.20 mV/ms. Only slice that produced fEPSP amplitude of more than 1 mV and 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 with Patchmaster software (HEKA Instruments Inc., NY, USA) was used for all *in vitro* electrophysiological recordings. The electrical signals were amplified, digitally sampled at 10 kHz, low-pass filtered at 1 kHz, and stored for offline analysis.

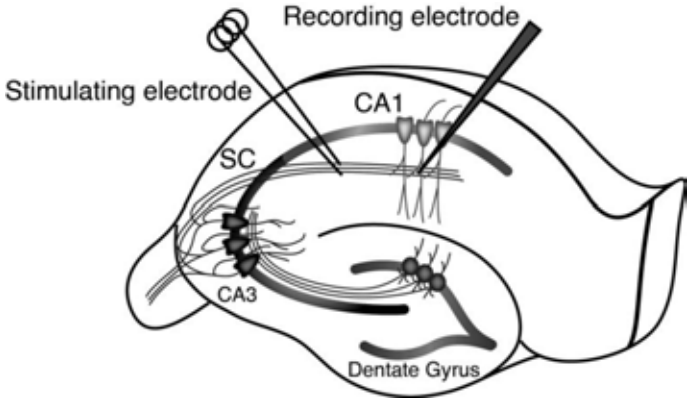

Figure 8 The diagram illustrating *in vitro* electrophysiological setup for extracellular recording of evoked hippocampal CA1 potentials.

G. Electrophysiological Study Protocols

1. LTP

The LTP protocol consisted of an induction of hippocampal LTP using tetanic stimulation (HFS). After stable baseline fEPSPs were recorded for at least 30 min, LTP was elicited by applying tetanic stimulation of 100 Hz for 1 sec at the same stimulus intensity as that used for the baseline fEPSPs. Post-tetanic fEPSPs were recorded for 60 min afterwards. The magnitude of LTP was calculated by dividing the

average slope of the 50 - 60 min post-induction responses by the average slope of the 0 - 30 min pre-induction baseline responses. In capsaicin experiment, capsaicin (5 μM in 0.1% DMSO in ACSF) was applied by bath application for 10 min before LTP induction and 10 min afterwards. In vehicle-treated slice, 0.1% DMSO in ACSF was applied instead.

2. PPF

PPF has been shown to be presynaptic in origin and does not induce any changes in postsynaptic effectiveness. The PPF protocol included 4 two-pulse pairs with decreasing inter-pulse intervals (400, 200, 100, and 50 ms). After stable baseline fEPSPs were recorded for at least 15 min, PPF stimulation was delivered at 0.1 Hz. Ten responses for each inter-pulse interval were recorded. The ratio of facilitation was computed by dividing the fEPSP slope evoked by the second pulse with the fEPSP slope evoked by the first pulse.

3. I/O Relationship of AMPA Receptor

The I/O protocol consisted of stimulation with multi-step intensities that were adjusted to evoke threshold to maximal fEPSP responses. After stable baseline fEPSPs were recorded for at least 15 min, AMPA receptor-mediated fEPSPs were isolated by bath application of a highly selective NMDA receptor antagonist, APV (25 μM), for 10 min before performing I/O protocol. Then, Input stimulation was delivered at 0.1 Hz (1 pulse every 10 s), and 5 output responses were recorded at each intensity. To provide an indication of differences in receptor-mediated responses to stimuli of a given intensity, the slopes of the AMPA receptor-mediated fEPSPs were plotted against the stimulus voltages.

4. I/O Relationship of NMDA Receptor

The I/O protocol consisted of stimulation with multi-step intensities that were adjusted to evoke threshold to maximal fEPSP responses. After stable baseline fEPSPs were recorded for at least 15 min, NMDA receptor-mediated fEPSPs were isolated by bath application of a potent AMPA receptor antagonist, CNQX (10 μM in 0.1% DMSO), in modified ACSF (3 mM $CaCl_2$, and 0.1 mM $MgSO_4$). Ten minutes after application of the drug, I/O protocol was performed. Input stimulation was delivered at 0.1 Hz (1 pulse every 10 s), and 5 output responses were recorded at each

intensity. To provide an indication of differences in receptor-mediated responses to stimuli of a given intensity, the areas under the NMDA receptor-mediated fEPSPs were plotted against the stimulus voltages.

5.) Ratio of GluN2A/B

After steady baseline fEPSPs were recorded for at least 15 min, NMDA receptor-mediated fEPSPs were isolated by bath application of a potent AMPA receptor antagonist, CNQX (10 μM in 0.1% DMSO), in modified ACSF (3 mM $CaCl_2$, and 0.1 mM $MgSO_4$). Ten minutes after application of the drug, input stimulation was delivered at 0.033 Hz (1 pulse every 30 s) and its intensity was adjusted to evoke stable NMDA receptor-mediated fEPSPs. Ten minutes after, GluN2A component of NMDA receptor-mediated fEPSPs was isolated using CNQX (10 μM; bath application) and GluN2B subunit-selective NMDA receptor antagonist, ifenprodil (3 μM in 0.1% DMSO in modified ACSF) for 1 h.

Total component of NMDA receptor responses was the averaged AUC of the NMDA receptor-mediated fEPSPs during 10 min before ifenprodil application. GluN2A component of NMDA receptor responses was the averaged AUC of the NMDA receptor-mediated fEPSPs during 50 - 60 min after ifenprodil application (i.e., “ifenprodil-insensitive component”). GluN2B component (i.e., “ifenprodil-sensitive component”) was total component of NMDA receptor responses subtracted with GluN2A component. GluN2A/B ratio was calculated by dividing GluN2A component with GluN2B component.

H. Statistical Analysis

All data were shown as the mean ± SEM. Statistical analysis was performed using SPSS version 13.0. Student’s t-test was used for comparing means between 2 groups. Repeated-measures ANOVA was used for comparing means of a parameter subjected to repeated measurements such as PPF and I/O relationship. Probability values of less than 0.05 were considered to be statistically significant.

CHAPTER IV
RESULTS

The Study of Properties of *in vivo* Hippocampal Spreading Depression

In the first experiment, DC potential was recorded in the neocortex or hippocampal CA1 during *in vivo* CSD induction in order to examine whether repetitive CSDs could propagate and reach the CA1 area and to characterize SD properties. In the experiment, DC potential was recorded in the frontal cortex (4 rats, N = 4) or hippocampal CA1 (N = 4) during CSD induction.

In cortical DC recording, a glass recording electrode was inserted into frontal neocortex (from bregma: anterior-posterior, +3 mm; lateral, 2 mm; and dorsal-ventral, 0.5 mm). Then, CSD was elicited by applying solid KCl on the parietal cortex for 45 min. The result showed that multiple shifts of negative DC characterized as SD were detected in frontal neocortex in all rats (N = 4), which indicate that solid KCl application consistently induced multiple waves of CSD (Fig. 9).

In hippocampal DC recording, a glass recording electrode was inserted into CA1 area of the hippocampus (from bregma: anterior-posterior, -4 mm; lateral, 2 mm; and dorsal-ventral, 2.2 mm). After that, CSD was elicited by applying solid KCl on the parietal cortex for 45 min. The result illustrated that a series of negative DC potentials characterized as SD was detected in hippocampal CA1 in all rats (N = 4; Fig. 10). This data indicate that SD could propagate and reach CA1 area of the hippocampus.

To characterize CSD and hippocampal SD properties, several of parameters, including total numbers of SD waves, amplitude, duration, AUC, and wave interval, were measured and shown in table 2. Comparison between variables of cortical and hippocampal SD revealed some differences in SD properties. Total numbers of SD waves and amplitude in the hippocampus were significantly lower than those in the cortex. Moreover, the interval between each wave was significantly longer in the hippocampus when compared to that in the cortex. However, there was no difference in duration and AUC.

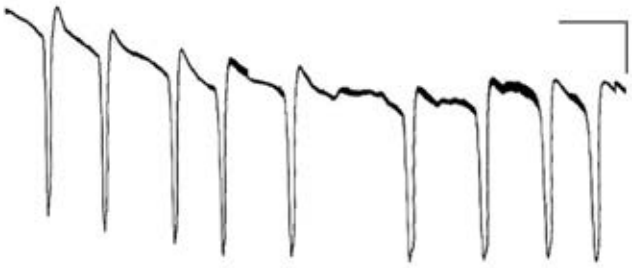

Figure 9 The representative tracing showing the DC shift in frontal cortex surface induced by KCl application (Scale bar: 5 min; 10 mV)

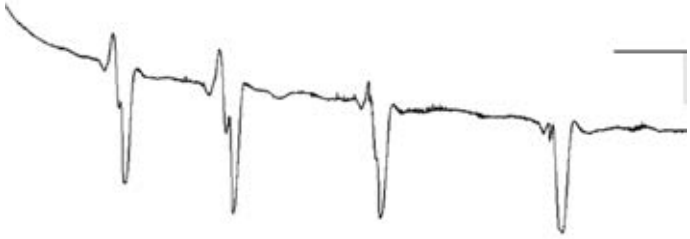

Figure 10 The representative tracing showing the DC shift in hippocampus induced by KCl application (Scale bar: 5 min; 10 mV)

Table 2 Comparison of the electrophysiological variables related to SD between cortex and hippocampus (Student's t-test, N = 4 each group).

	CSD	Hippocampal SD	*P*-value
Total numbers (waves/45 min)	9.23 ± 1.74	3.67 ± 0.58	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
AUC (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

The Study of Alterations of Hippocampal Synaptic Transmission and Plasticity Induced by CSD

In this experiment, several *in vitro* electrophysiological studies were conducted in hippocampal slices obtained from CSD and control rats in order to investigate the effect of *in vivo* CSD on an alteration of hippocampal synaptic plasticity and

transmission. Briefly, CSD was elicited by applying solid KCl for 45 min in CSD group. Solid NaCl was applied instead in the control group. Representative traces of DC recording in CSD and control rats were shown in Fig. 11. Then, the rat was sacrificed, and hippocampal slices were prepared for *in vitro* electrophysiological experiments, which include LTP, PPF, I/O relationship of AMPA receptor and NMDA receptor, and GluN2A/B ratio.

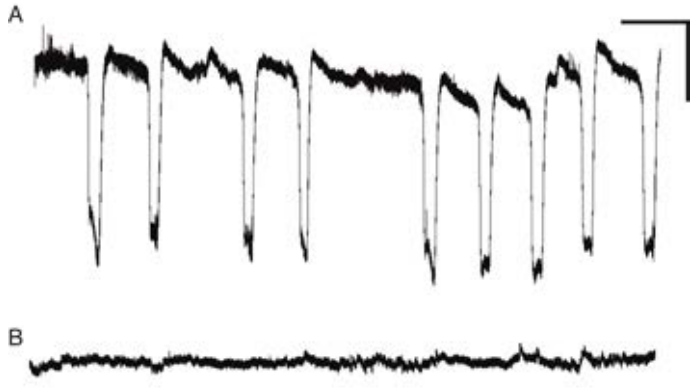

Figure 11 Representative trace recorded in cortex during **A)** solid KCl or **B)** solid NaCl application. (Scale bar: 5 min; 10 mV)

A. Effect of CSD on Hippocampal Excitability

In all electrophysiological protocols, stimulus intensity was adjusted to evoke fEPSPs of 0.12 to 0.20 mV/ms slopes as the baseline responses. Evoked fEPSP slopes obtained from baseline recordings in the CSD slices were comparable with those recorded in the control slices (0.159 ± 0.007 and 0.157 ± 0.005 mV/ms, respectively; $P = 0.828$, n = 10 each group; Fig. 12A). However, the stimulation intensities needed to evoke the same fEPSP responses were higher in slices obtained from the CSD rats than those from the control rats (0.327 ± 0.012 and 0.264 ± 0.014 V, respectively; $P = 0.003$; n = 10 each group; Fig. 12B). These data suggest that CSD has a significant effect on evoked neuronal in the hippocampus.

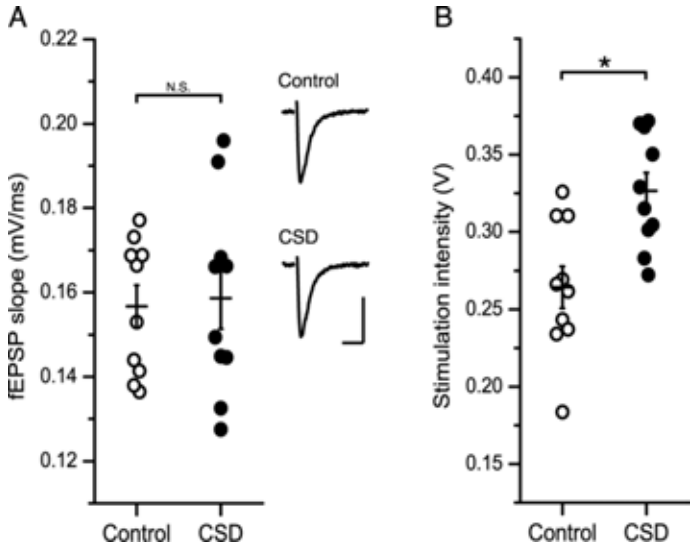

Figure 12 Neuronal excitability in the hippocampus was reduced by CSD induction. **A)** Evoked baseline fEPSPs were not significantly different between the CSD and control groups. Inset: representative traces of fEPSPs. Calibration: 20 ms, 0.2 mV. N.S.: not significant. **B)** Stimulation intensities required to evoke baseline fEPSPs were higher in the CSD group. n = 10 each group. * $P < 0.05$, Student's t-test. Bar and whisker plots indicate the mean ± SEM.

B. LTP

LTP is a model of activity-dependent synaptic plasticity proposed to mediate memory and learning (78). In the experiment, LTP was elicited by HFS (100Hz for 1 s) after stable baseline was recorded for 30 min. A conditioning tetanic stimulation induced a rapid, stable and lasting enhancement of the evoked potentials in both CSD and control groups (Fig. 13A). The potentiation rose within 1 - 2 min and stabilized within another 18 - 22 min after the stimulation. The increased fEPSPs remained stable for at least 60 min. However, LTP magnitude in the hippocampus was influenced by repetitive CSD induction. In the CSD group, the LTP magnitude significantly decreased when compared to the control group ($P = 0.009$, Student's t-test; Fig. 13B). The levels of LTP in the CSD and control groups were 122.7 ± 4.2 and 140.8 ± 4.1 %, respectively (n = 7 each group). This data indicate that hippocampal synaptic plasticity was impaired by CSD induction.

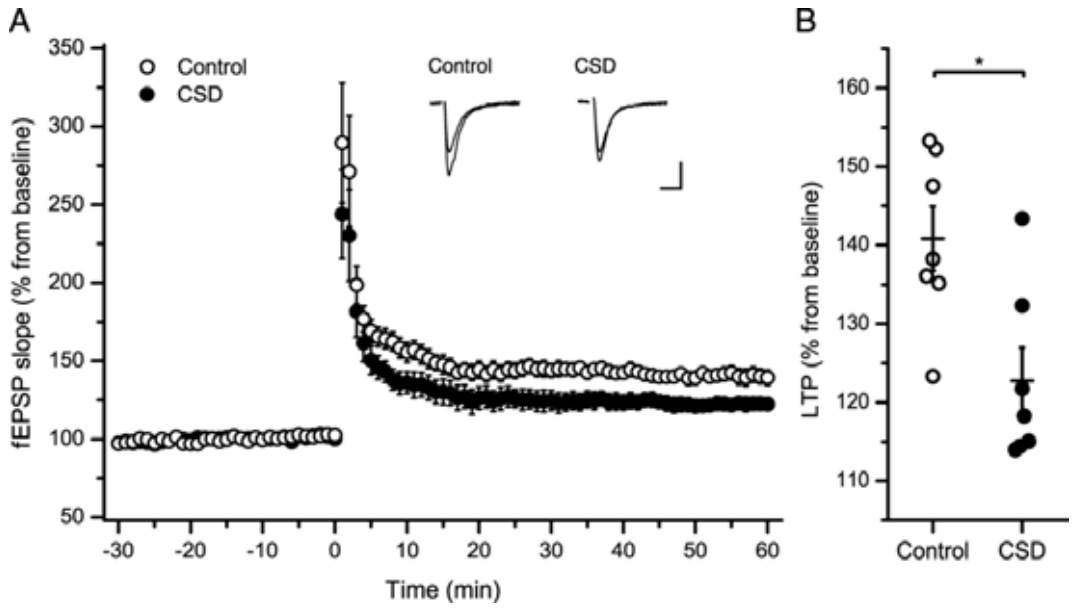

Figure 13 CSD induction affected LTP in hippocampal slices. **A)** Hippocampal LTP in the control and CSD groups. LTP was induced by tetanic stimulation (100 Hz for 1 s) applied at time 0. Inset: Representative traces recorded immediately prior to LTP induction and 60 min after LTP induction. Calibration: 20 ms, 0.2 mV. **B)** Data plot of the LTP magnitude. The magnitude of potentiation was calculated by dividing the averaged fEPSP slope values from 50 to 60 min with those from -30 to 0 min. LTP was significantly reduced in hippocampal slices obtained from the CSD rats. n = 7 each group. * $P < 0.05$ Student's t-test. Bar and whisker plots indicate the mean ± SEM.

C. PPF

PPF is a form of plasticity in which the second of two pulses, delivered within 25-250 ms of one another, evokes a larger synaptic response than the first pulse. PPF is thought to be presynaptic in origin and attributable to a residual increase in the concentration of internal calcium within the presynaptic terminal (79). In the experiment, the effects of CSD on hippocampal presynaptic modifications were studied by examining PPF. The results showed that stimulation of 2 consecutive pulses with 50, 100, and 200 ms interpulse interval facilitated the response of the second pulse in both groups. However, no overall PPF profile change was observed between the CSD and control slices (P = 0.651 by one-way repeated measures

ANOVA, n = 10 each group; Fig. 14). This result indicates that presynaptic responses at hippocampal SC-CA1 synapses were not altered in the rats that received CSD.

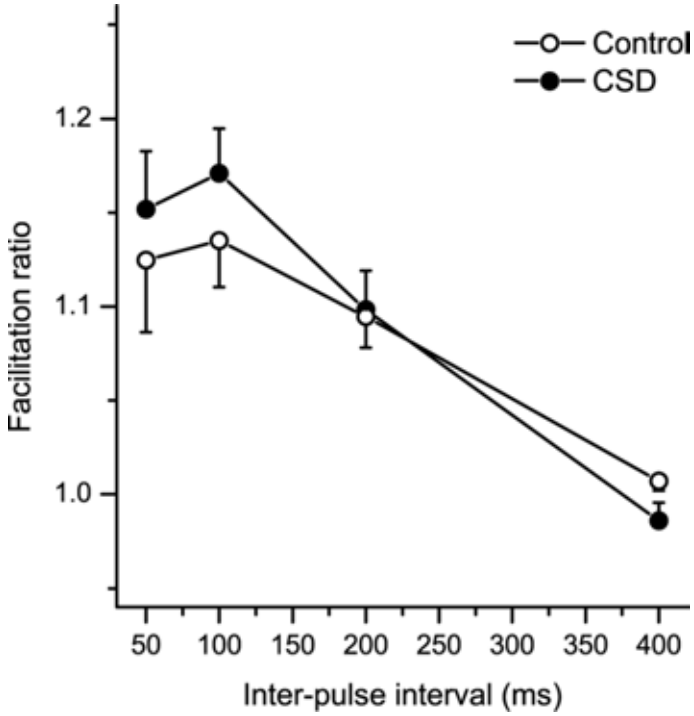

Figure 14 Paired-pulse profiles of the hippocampal CA1 synapses were not affected by CSD. Closed circles (CSD) and open circles (control) showed the facilitation ratios of the two groups. n = 10 each group. No significant differences in PPF profiles were observed.

D. I/O Relationship of AMPA Receptor

The effects of CSD on hippocampal postsynaptic AMPA receptor responses were studied by examining I/O profile of AMPA receptor-mediated fEPSPs. The results showed that increasing of stimulating intensity enhanced AMPA receptor-mediated fEPSP responses in both groups. The overall I/O curve of the AMPA receptor responses was significantly reduced in hippocampal slices obtained from the CSD rats ($P = 0.012$, one-way repeated measures ANOVA, n = 7 each group; Fig. 15). When responses were compared at each stimulating intensity, the slices obtained from the CSD rats showed a significant reduction in evoked AMPA receptor-mediated fEPSPs across a range of stimuli intensities ($P < 0.05$, Student's t-test). This result indicates that AMPA receptor responses at SC-CA1 synapses were depressed in CSD-induced rats.

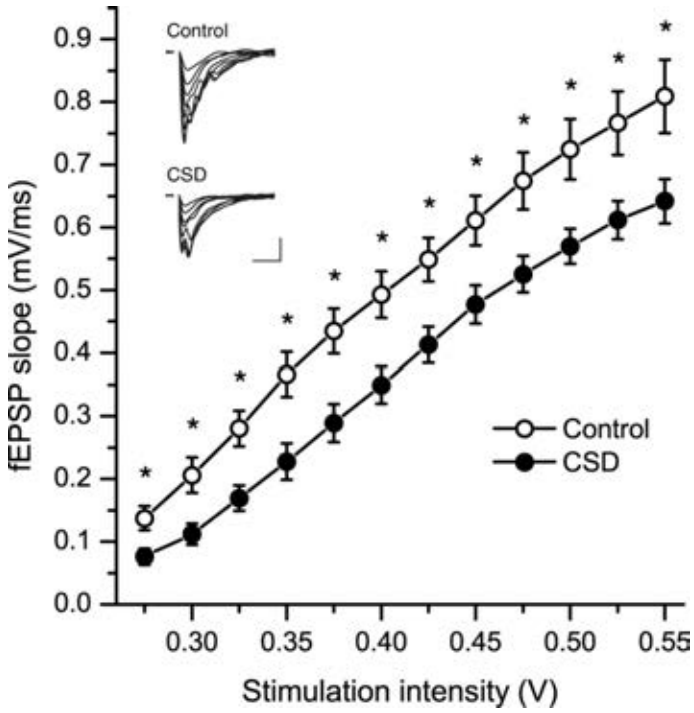

Figure 15 I/O relationship of AMPA receptor-mediated fEPSPs in the hippocampal slices obtained from CSD and control rats. CSD significantly suppressed the AMPA receptor component of fEPSP (P = 0.012, repeated-measures ANOVA). Closed circles (CSD) and open circles (control) showed evoked AMPA receptor-mediated fEPSPs in hippocampal slices. Representative traces showed AMPA receptor responses to stimuli at 0.275, 0.300, 0.325, 0.350, 0.400, 0.450, 0.500, and 0.550 V. Calibration: 20 ms, 0.4 mV. n = 7 each group. * $P < 0.05$, Student's t-test.

E. I/O Relationship of NMDA Receptor

The effects of CSD on hippocampal postsynaptic NMDA receptor responses were studied by examining I/O profile of NMDA receptor-mediated fEPSPs. The results showed that increasing of stimulating intensity enhanced NMDA receptor-mediated fEPSP responses in both groups. These increased responses seem to be saturated at stimulating intensity of 0.7 V. Examination of the I/O relationship of NMDA receptor-mediated fEPSPs showed no significant differences in the overall response between CSD and control slices ($P = 0.256$, one-way repeated measures ANOVA, n = 7 each group; Fig. 16). This result indicates that repetitive CSDs did not alter NMDA receptor function at SC-CA1 synapses.

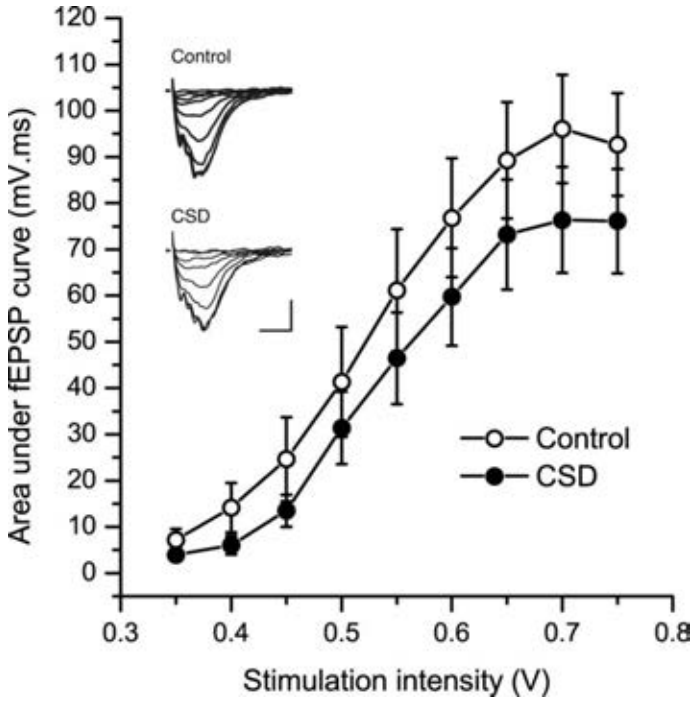

Figure 16 I/O relationship of NMDA receptor-mediated fEPSPs in hippocampal slices obtained from CSD and control rats. CSD did not significantly alter the NMDA receptor component of fEPSP (P = 0.256, repeated-measures ANOVA). Closed circles (CSD) and open circles (control) showed evoked NMDA receptor-mediated fEPSPs in hippocampal slices. Representative traces showed NMDA receptor responses to given stimuli. Calibration: 50 ms, 0.4 mV. n = 7 each group.

F. Ratio of GluN2A/B

It has been proposed that the ratio of GluN2A/B contribute to the threshold modification of synaptic plasticity (80). In the experiment, GluN2A component was isolated by application of 3 μM ifenprodil, a GluN2B receptor antagonist. After application of ifenprodil, NMDA receptor-mediated fEPSP was reduced in both control and CSD slices (Fig. 17A). The reduction of NMDA receptor-mediated fEPSP was stabilized within 40 - 60 min after ifenprodil application. In control slices, ifenprodil decreased NMDA receptor-mediated fEPSPs by a degree comparable to previous studies on the hippocampal CA1 (81). The extent of reduction was not significantly different between CSD and control groups (P = 0.092, Student's t-test). The reduction magnitudes of NMDA receptor-mediated fEPSPs in CSD and control slices were 23.5 ± 2.1 and 34.4 ± 5.5 %, respectively (n = 6 each group). However,

analysis of GluN2A/B ratio revealed that the ratio of the CSD group significantly increased when compared to that of the control group ($P = 0.018$, Student's t-test; Fig. 17B). The GluN2A/B ratios in CSD and control groups were 3.38 ± 0.36 and 1.97 ± 0.35, respectively. After treatment with ifenprodil, the remaining fEPSPs were abolished by the NMDA receptor antagonist, APV (25 μM), which verifies that the isolated fEPSPs were mediated by NMDA receptor. This result indicates that CSD altered the composition of NMDA receptors in hippocampal CA1 toward a greater GluN2A/B ratio.

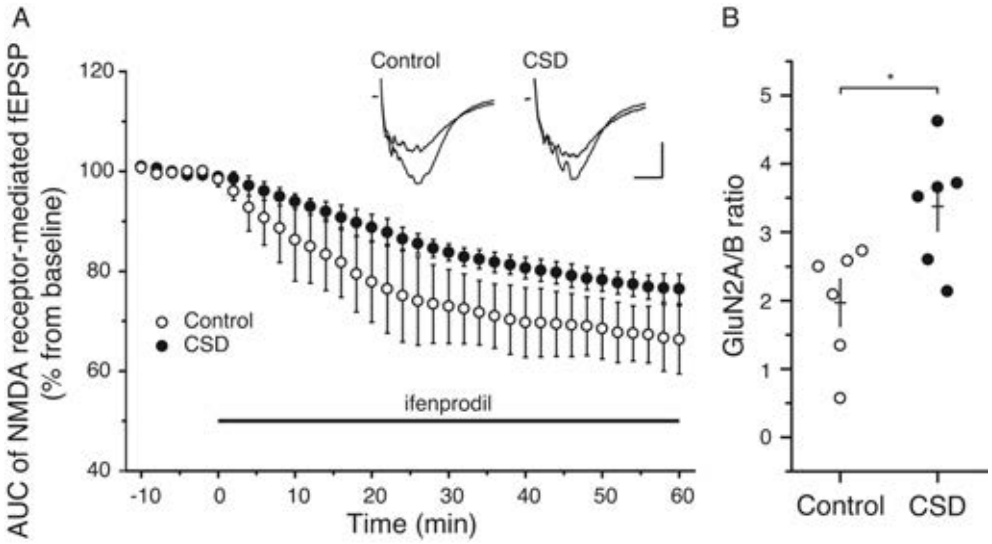

Figure 17 CSD induction affected functional GluN2A/B ratio in hippocampal slices. **A)** Isolation of GluN2A component from NMDA receptor-mediated fEPSPs in the control and CSD groups. GluN2A component was isolated by bath application of 3 μM ifenprodil, a GluN2B receptor antagonist, at time 0 to 60. Inset: Representative traces recorded immediately prior to ifenprodil application and 60 min after ifenprodil application. Calibration: 50 ms, 0.4 mV. **B)** Data plot of the GluN2A/B ratio. The ratio of GluN2A/B was calculated by dividing the averaged AUC values of ifenprodil-insensitive component with those of ifenprodil-sensitive component at time 50 to 60. The GluN2A/B ratio significantly elevated in hippocampal slices obtained from the CSD rats. n = 6 each group. * $P < 0.05$ Student's t-test. Bar and whisker plots indicate the mean ± SEM.

The Study of the Effect of Capsaicin on Hippocampal LTP

In this experiment, capsaicin, which is TRPV1 receptor agonist, was used as a supplement, of which the effect is supposed to reinforce LTP by increasing Ca^{2+} influx through its channel. Firstly, the effect of capsaicin on evoked fEPSP was examined by bath application of capsaicin onto the slices. After a stable baseline was recorded for 20 minutes, capsaicin or 0.1% DMSO vehicle dissolved in ACSF was perfused onto the slice for 20 minutes. The result showed that capsaicin reduced evoked fEPSP in a dose-dependent manner (Fig. 18). The effect of capsaicin doses on fEPSP were compared using normalized fEPSP slopes recorded during capsaicin application (time 31 to 40). The result showed that there was no significant difference in normalized fEPSP slopes during capsaicin application (P = 0.027, one-way ANOVA, n = 3 each dose). However, a profound reduction of fEPSP slopes was observed when a high dose of capsaicin (10, 50, and 100 μM) was applied. These doses of capsaicin tended to diminish evoked fEPSPs, but the effects did not reach the statistic significance when compared to vehicle (P = 0.972, P = 0.271, P = 0.068, respectively; Tukey post hoc). In contrast, fEPSP slopes during perfusion of a low dose of capsaicin (1 and 5 μM) remained comparable with control vehicle (P = 1 and P = 1, respectively; Tukey post hoc). The normalized fEPSPs during vehicle, 1, 5, 10, 50, and 100 μM capsaicin perfusion were 99.7 ± 0.7, 100.7 ± 0.7, 95.1 ± 4.1, 84.6 ± 5.2, and 78.6 ± 9.7, respectively (Fig. 18B). In the following experiment, 5 μM of capsaicin was used since it was the highest dose that did not affect fEPSP responses.

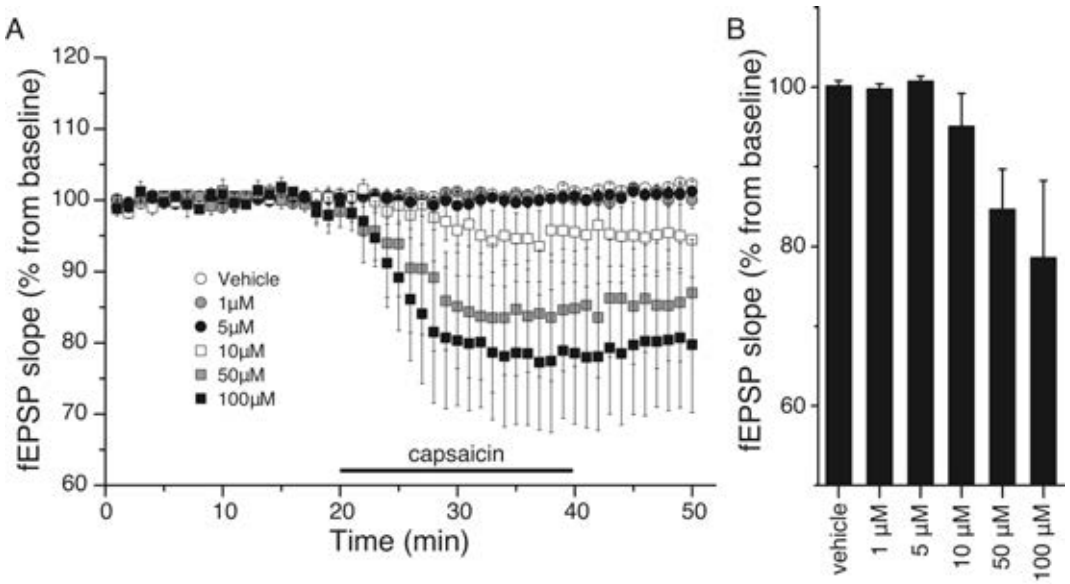

Figure 18 High doses of capsaicin affect fEPSP responses. **A)** Capsaicin reduced an evoked fEPSP in a dose-dependent manner. Capsaicin was applied at time 21 to 40 min (horizontal bar). **B)** A bar graph showed a normalized fEPSP during capsaicin application (averaged at time 31 to 40 min). No statistically significant difference in fEPSPs was observed (one-way ANOVA, n = 3 each group).

Next, effect of capsaicin on tetanus-induced LTP in CSD-induced and control slices was investigated. In this experiment, 5 μM capsaicin or 0.1% DMSO vehicle were perfused onto a slice for 20 minutes during LTP induction. After stable baseline was recorded for 30 min, HFS (100Hz for 1 s) was applied to elicit LTP. The HFS stimulation rapidly induced a stable enhancement of the evoked potentials in all groups (Fig. 19A). The fEPSP augmentation rose within 1-2 min and stabilized within another 18-22 min after the stimulation. The increased fEPSPs remained stable for at least 60 min. However, LTP magnitude in the hippocampus was affected by CSD induction with capsaicin administration (P = 0.001, one-way ANOVA). CSD induction significantly suppressed the level of LTP (P = 0.010, Tukey post hoc) as shown in our preceding study. In the control group, LTP magnitude of capsaicin-treated slice tended to increase when compared to that of vehicle-treated slice, but the level of significance was not reached (P = 0.446, Tukey post hoc). Moreover, capsaicin supplementation also tended to increase the LTP level in CSD slice, albeit statistical significant was not reached (P = 0.159, Tukey post hoc). In addition, LTP

magnitude of CSD slice with capsaicin treatment remained comparable to that of control-vehicle slice (P = 0.553, Tukey post hoc). This data indicate that capsaicin could recover LTP that was weakened by CSD induction. The magnitudes of LTP in the control-vehicle, control-capsaicin, CSD-vehicle, and CSD-capsaicin were 138.4 ± 1.9, 146.5 ± 4.9, 119.7 ± 2.5, and 131.4 ± 4.8, respectively (Fig. 19B).

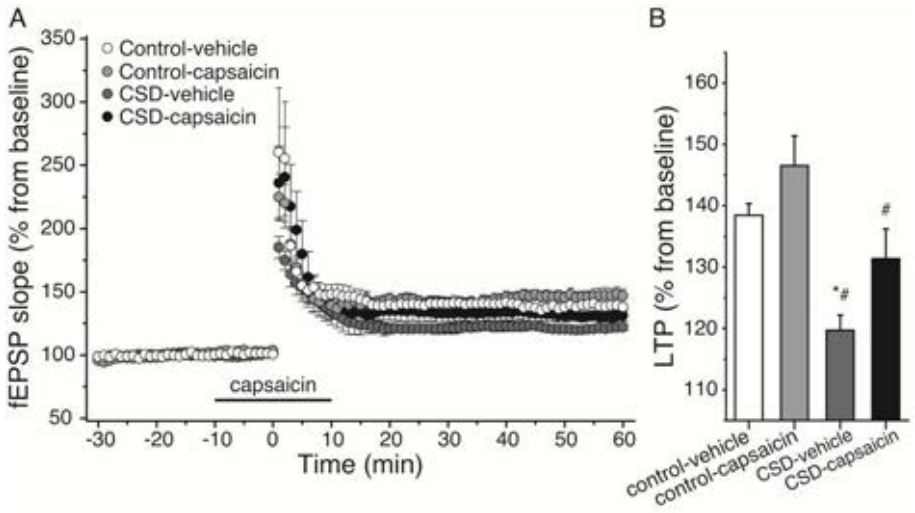

Figure 19 Capsaicin supplementation recovered impaired LTP by CSD induction. **A)** Hippocampal LTP in the control and CSD slices with or without capsaicin treatment. Capsaicin was applied at time -10 to 10 min. Tetanic stimulation (100 Hz for 1 s) was applied at time 0. **B)** Bar graph of the LTP magnitude. The magnitude of potentiation was calculated by dividing the averaged fEPSP slope values from 50 to 60 min with those from -30 to 0 min. LTP was significantly diminished in hippocampal slices obtained from the CSD rats. n = 6 each group. * P < 0.05 compared to control-vehicle and $^{\#}$ P < 0.05 compared to control-capsaicin, Tukey post hoc test.

CHAPTER V
DISCUSSION

The Study of Properties of *in vivo* Hippocampal Spreading Depression

In the present study, shifts of DC potential were detected in CA1 area of the hippocampus during CSD induction, suggesting that SD may travel from neocortex and enter the hippocampus. The possibility of SD propagation from cortical tissue to the hippocampus in our study was consistent with previous studies of *in vivo* CSD induction using "manganese-enhanced MRI" (34). Applying potassium chloride onto cortex surface of the rat resulted in an elevation of MRI signal in the ipsilateral cortex compared to the contralateral control region. In addition, an increase of MRI signal was 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, which comprised the "perirhinal cortex" (PRC), "postrhinal cortex" (POR) and "entorhinal cortex" (ERC) (82). ERC was not only a passive relay station but also an active controlling station that gated impulse travelling between neocortex and hippocampus. Impulse transferred from temporal neocortex to ERC and vice versa occurred with a tremendously low probability, suggesting a powerful intrinsic inhibitory system (83). This inhibitory system may act to abort SD propagation to hippocampus. However, whether the transsynaptic inhibitory system could gate non-transsynaptic propagation, like SD, or not remains to be identified.

The Study of Alterations of Hippocampal Synaptic Transmission and Plasticity Induced by CSD

This study demonstrated that CSD could affect hippocampal synaptic transmission and plasticity. Repetitive CSD induction resulted in a reduction of synaptic efficacy as well as a reduction of LTP in the CA1 region of the hippocampus. Our results were in

contrast to those of previous studies (84-86). Experiments in human neocortical slices demonstrated that single CSD led to a long-lasting increase of fEPSP and an enhancement of neocortical LTP (84). Furthermore, experiments in rat neocortex also showed an enhancement of fEPSP after propagation of single CSD (85). In addition, single CSD that propagated to lateral amygdale increased amygdala LTP in rat amygdale-hippocampus-cortex slices (86). It seems that single CSD promoted hyperexcitability of cortex and amygdala. In contrast to single CSD, repetitive induction of CSD led to a reduction of CSD susceptibility, indicating a reduction of excitability of cortical tissue (87). The mechanisms underlying these phenomena are not clear but may involve an alteration of AMPA receptor function. It has been illustrated that repetitive CSDs (10 waves) could suppress the expression of GluA1 and GluA2 protein subunits of the AMPA receptor (88).

The above observations suggest that alteration of synaptic efficacy could develop in both directions, depending on the characteristics and intensity of stimulation (89). In our *in vivo* model, topical application of solid KCl induced repetitive CSDs. In our preceding experiment, DC shifts were also present in the hippocampus during CSD induction. Thus, our study suggests that repetitive CSDs induced by solid KCl application propagated to the hippocampus, which suppressed hippocampal synaptic efficacy and caused a reduction in tetanus-induced LTP. This observation is consistent with the study of Wernsmann and coworkers (16). Their study showed that single CSD that propagated and reached hippocampus diminished the hippocampal LTP and fEPSP.

According to the results from our study, the changes in synaptic response and plasticity induced by repetitive CSDs were likely to be postsynaptic in origin. Our study showed no alteration of PPF (which is sensitive to changes in presynaptic release probability) in CSD rats. These data suggest that neurotransmitter release was likely to be normal in the CSD rats. This finding was consistent with the data from Hsu and coworkers (90) who showed that exposure of a hippocampal slice to low extracellular magnesium, a condition that induces seizure-like activity and SD (91), impaired hippocampal plasticity without any significant changes in PPF and postsynaptic NMDA receptor responses. Our results suggest that both the reduction of

synaptic efficacy and LTP suppression by repetitive CSDs are mediated via a postsynaptic process rather than by a presynaptic process.

Our present study showed that CSD impaired AMPA receptor responsiveness as examined by the I/O relationship. The mechanism by which CSD decreases AMPA receptor function remains unclear. Reduction of AMPA receptor transcription and translation after SD episodes may be responsible for fewer available AMPA receptors and a decrease in AMPA receptor response. Evidence for this hypothesis has been shown in an ischemic model (92), which shares similar phenomena with our model (e.g., excessive glutamate release) and also induces SD from the ischemic core out toward the margins of the ischemic zone (93). The another mechanism could be mediated by a change in single channel properties of AMPA receptors or by a decrease in AMPA receptor trafficking to synapses, which has also been demonstrated in ischemic model (94). Our results and these data indicate that AMPA receptor transmission is impaired after an SD episode, possibly via these mechanisms.

The present electrophysiological study revealed a reduction of AMPA receptor function as well as LTP suppression in hippocampal slices obtained from rats that received CSD. It is unknown whether the decrease in AMPA receptor response was responsible for LTP impairment. It has been well established that mechanisms of LTP induction involve AMPA receptor trafficking and potentiated receptor response by phosphorylation (95,96). Thus, any changes in the properties of AMPA receptors are likely to affect LTP. These data suggest that the impairment of LTP induced by repetitive CSDs in the present study may result from a reduction of AMPA receptor response.

We have shown that NMDA receptor function remained intact in rats that received CSD. However, our results could not exclude a role of NMDA receptors in LTP suppression. It is possible that the excessive glutamate release and overstimulation of NMDA receptors that occur during an SD episode affect the subsequent induction of LTP. Much evidence supports the hypothesis by which prior NMDA receptor activation suppresses LTP induction (90,91,94,97,98). These data suggest that NMDA receptors may be involved in disrupting LTP induction even though SD does not affect their overall responses.

In spite of unaltered NMDA receptor responses, our study demonstrated an elevated ratio of functional GluN2A/B in CSD slices. Albeit the mechanism of relative reduction of GluN2B is still unknown, this down regulation may result from hyperexcitability state, such as during SD. It has been shown in hippocampal CA1 of rat in benzodiazepine-withdrawal model that reduction of GluN2B-containing NMDA may be secondary to AMPA receptor hyperexcitability (99). Benzodiazepine withdrawal elevated AMPA receptor-mediated current and reduced GluN2B-mediated currents. In addition, preincubation of slices obtained from 1-day benzodiazepine withdrawn rat with AMPA elicited a reduction of GluN2B-mediated currents. Those results suggest that depression of GluN2B-containing NMDA receptor responses is probably secondary to overstimulation of AMPA receptor. According to our study, excessive glutamate release and subsequent overstimulation of AMPA receptors that occur during an SD may be responsible for relative reduction of GluN2B responses.

In the present study, LTP suppression concurrently occurred with an increase of GluN2A/B ratio in CSD group. Our results conform to the hypothesis stating that GluN2B subunit is more prone to produce LTP than GluN2A subunit. Many studies (71,100), including ours, demonstrated the coexisting of impaired LTP and increased GluN2A/B ratio. The alteration of GluN2A/B ratio influences the CaMKII function. It has been determined that activation of the CaMKII is necessary for induction of LTP and memory formation (101). Ca^{2+} influx through the NMDA receptor mediates CaMKII translocation, its direct binding to GluN2B subunit, and ensuing regulation of synaptic strength. Accumulating evidence enunciated the role of GluN2B and its interaction with CaMKII in synaptic plasticity (102). Recently, Halt and coworkers (103) showed that the point mutation that obstructed GluN2B binding to CaMKII could attenuate LTP by 50%. They illustrated that GluA1 subunit of the AMPA receptor phosphorylation at the synaptic site by CaMKII, which enhances AMPA receptor conductance, was also impaired. In the present study, CSD led to a relative reduction of GluN2B. Accordingly, interaction of GluN2B with CaMKII at the synapse may be declined, resulting in a decreased of LTP magnitude.

In consideration to clinical implication, it has been hypothesized that SD may be responsible for amnesic attack (12). An increased MRI signal intensity, which is compatible with SD, was demonstrated in TGA patients during attack (14). TGA

attack could be triggered by migraine attack in some migraineurs (9). During amnesic attack, patients lose an ability to acquire a new memory (5). LTP is a type of activity-dependent synaptic plasticity proposed to mediate memory and learning, and has been considered as a model of memory acquisition (41,78). Thus, it is intriguing to speculate that the suppression of hippocampal LTP induced by CSD may relate to impaired ability to obtain new information during TGA attack concomitant with headache in some migraine patients.

Collectively, the present study demonstrated that repetitive CSDs could alter hippocampal synaptic plasticity, as shown by a reduction in LTP magnitude. The results indicate that the underlying mechanism may involve a reduction of postsynaptic AMPA receptor responses and an increase of GluN2A/B ratio of NMDA receptor, while postsynaptic NMDA receptor responses and presynaptic processing remain unchanged.

The Study of the Effect of Capsaicin on Hippocampal LTP

This study demonstrated that capsaicin affected hippocampal synaptic transmission since it reduced evoked fEPSPs. Unexpectedly, our data were in contrast with other study, which showed that capsaicin at 10-100 μM did not alter fEPSP of CA1 pyramidal cell (104,105). However, It has been demonstrated that 10 μM capsaicin could diminish evoked EPSCs at SC-CA1 synapse (106) as found in our study. They also showed that suppressed EPSCs by capsaicin could be antagonized by capsazepine (a TRPV1 receptor antagonist), which suggested that this effect was specifically mediated by TRPV1 receptor. In hippocampus, TRPV1 is expressed in CA1 pyramidal cell (25,75,76) and in presynaptic site of CA3-GABAergic interneuron synapse that forward inhibitory input onto CA1 pyramidal cell (25). Thus, the mechanism of fEPSP reduction should involve either TRPV1 receptor expressed in CA3-interneuron or pyramidal cell synapse. Since the difference between recent experimental protocol and theirs is unclear, the discrepancy of the result between these experiments could not be explained.

The present study showed that capsaicin supplementation could enhance hippocampal CA1 LTP in a slice obtained from normal rats. Our finding confirms previous reports of the implication of TRPV1 receptor in hippocampal synaptic plasticity and the facilitation effect of capsaicin on hippocampal LTP (54,104). Activation of TRPV1 receptor, which is a Ca^{2+}-permeable cation channel, results in a higher intracellular Ca^{2+} level during HFS. Higher level of intracellular Ca^{2+} leads to an elevated activation of CaMKII, which is an essential protein required for LTP induction, resulting in facilitation of LTP. It has been demonstrated that CaMKII activation could be triggered by TRPV1 receptor stimulation (107). Besides direct elevation of intracellular Ca^{2+} level trough TRPV1 receptor on CA1 pyramidal cell, LTP enchantment may be indirectly mediated by disinhibition of CA1 pyramidal cell. It was demonstrated that TRPV1 receptor mediated a new type of LTD in CA1 GABAergic inhibitory interneurons, which make a synapse onto CA1 pyramidal cell (25). It has been postulated that this inhibitory interneuron LTD could disinhibit CA1 pyramidal cells, leading to a promotion of CA1 LTP (104). Theoretically, if LTD of GABAergic interneuron is involved in a facilitation of CA1 LTP, then picrotoxin,

$GABA_A$ receptor antagonist, should alter the increase of CA1 LTP induced by capsaicin. However, all recordings of our experiment were made in the presence of 100 μM picrotoxin to obstruct $GABA_A$ receptor-mediated potentials. This might complicate the interpretation of the finding. Our result was in accordance with that of Li and colleague (54), which illustrated that LTP could be facilitated by capsaicin or resiniferatoxin, a TRPV1 receptor agonist, even in the presence of picrotoxin. Data from our study and Li's team suggest that capsaicin-induced enhancement of CA1 LTP was unlikely involved GABAergic interneuron system.

Our study also demonstrated that capsaicin supplementation could improve an impaired LTP induced by CSD. Retrieval of synaptic plasticity by capsaicin has been reported in acute stress model (54). Bath application of capsaicin recovered hippocampal LTP that was diminished by acute stress. Our previous finding that CSD increased the ratio of NR2A/B in postsynaptic CA1 suggested that CaMKII activation was presumably weaker during HFS compared to that of control slice, leading to lower LTP magnitude. Activation of TRPV1 receptor by capsaicin may increase intracellular Ca^{2+} level and strengthen CaMKII activity during LTP induction, resulting in a recovery of LTP magnitude.

Collectively, these data demonstrated that TRPV1 receptor activation by capsaicin could modulate synaptic plasticity. Capsaicin treatment, at least in *in vitro* model, could facilitate LTP induction in both normal and impaired states.

Summary

In consideration to this study, detection of DC shift in the hippocampus during CSD induction suggested that CSD could propagate into the hippocampus, which disturbed the hippocampal synaptic plasticity and transmission. Our results showed no alteration of PPF, indicating that presynaptic function remained intact. In contrast, an I/O relationship of AMPA receptor showed a reduction of postsynaptic AMPA receptor responses. Albeit examining an I/O relationship of AMPA receptor revealed unaltered postsynaptic NMDA receptor responses, monitoring an isolated response of NMDA receptor subunit demonstrated an increase of GluN2A/B ratio. These disturbed synaptic transmissions concurrently occurred with an impairment of synaptic plasticity, as indicated by a suppression of LTP. Our data pointed out that a

decrease of AMPA receptor responses and a relative decrease of GluN2B responses may involve a disruption of hippocampal plasticity induced by CSD. In addition, capsaicin could improve LTP induction that was impeded by CSD. The diagram illustrating these findings is shown in Fig. 20.

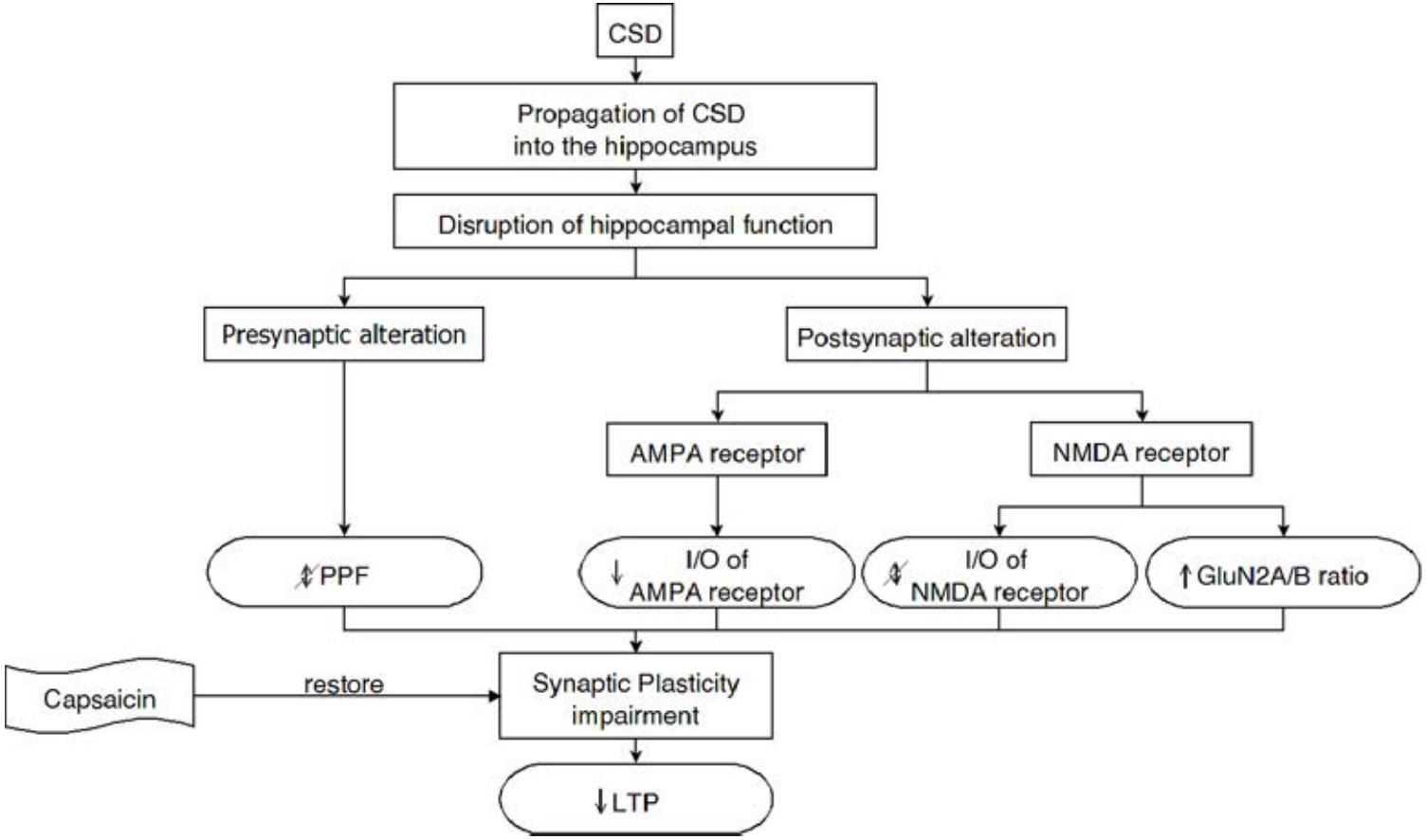

Figure 20 The diagram showing the findings from this study.

Proposed Mechanisms

SD induced at cortical surface could propagate and reach to the hippocampus. During SD traveling in the hippocampus, excessive glutamate releases from presynaptic terminal, and activates NMDA receptor. Sustained activation of NMDA receptor induces Ca^{2+} influx into the dendrite of pyramidal CA1 cell. This Ca^{2+} influx trigger signal transduction cascades, which finally result in a reduction of functional AMPA receptor response and an increase of functional GluN2A/B ratio. Any alteration of receptor kinetics, transcription, translation, forward trafficking, and backward trafficking could contribute to the functional reduction of AMPA receptor response. Evidence from Chazot and colleagues (88) suggests that AMPA receptor internalization may be responsible for a decreased AMPA receptor response after CSD episodes. Endocytosis of AMPA receptor may involve high-affinity calcium-dependent protein phosphatases, including calcineurin and protein phosphatase I (108). Functional increase of GluN2A/B ratio could result from modification of either NMDA receptor biogenesis or trafficking. It has been shown that GluN2B-containing NMDA receptor is more prone to be removed from synapse than GluN2A-containing NMDA receptor (109). Thus, internalization of GluN2B-containing NMDA receptor might serve as a molecular basis for the elevation of functional GluN2A/B ratio. This postulation has been supported by the study of Shen and Tietz (99), which demonstrates that synaptic GluN2B expression decreases after neural hyperexcitability. Endocytosis of GluN2B-containing NMDA receptor is mediated by clathrin. Phosphorylation of GluN2B subunit at tyrosine 1472 in YEKL is required to hinder clathrin-dependent endocytosis (110). Hence, de-phosphorylation of GluN2B by unidentified phosphatase might promote internalization of GluN2B-containing NMDA receptor (20). Both reduction of functional AMPA receptor response and increase of functional GluN2A/B ratio could contribute to LTP impairment, as discussed in above section.

Further Study

Our study employed electrophysiological technique to investigate the alteration of hippocampal plasticity and transmission induced by CSD. The molecular basis underlying functional electrophysiological data was lack. Thus, further investigation

should be emphasized on a molecular cascade that may be responsible for the observed electrophysiological phenomena.

During SD, trafficking of AMPA receptor and GluN2A- or GluN2B-containing NMDA receptor may be investigated using fluorescent tags. Synaptic expression of those receptors after SD episode may be quantified using immunoprecipitation with immunoblotting. Measuring activity of CaMKII in CSD and control slices during LTP induction is made possible by using Camui activity sensor and two-photon fluorescence lifetime imaging. Interaction of CaMKII and synaptic NMDA receptor may be examined using immunoprecipitation with immunoblotting. The extent that down-regulation of AMPA receptor and GluN2B-containing NMDA receptor expression induced by CSD might affect LTP could be investigated using genetic manipulation that prevent receptor internalization.

CHAPTER VI
CONCLUSIONS

In the present study, the mechanisms underlying CSD-induced alteration of hippocampal synaptic plasticity and transmission have been investigated. The following was the conclusions of our study.

Solid potassium chloride topically applied on the exposed rat cortex evoked multiple waves of DC shift characterized as CSD.

1. During CSD induction, repetitive DC shifts were also presented in the hippocampus.
2. Hippocampal synaptic plasticity was impaired in CSD-induced rats, as indicated by a lower magnitude of HFS-induced LTP when compared to that in controls.
3. Presynaptic function remained unaffected by CSD since PPFs were comparable between CSD and control groups
4. I/O relationship of AMPA receptor demonstrated that AMPA receptor responses were weakened in CSD rats as compared to controls.
5. I/O relationship of NMDA receptor showed that overall NMDA receptor responses were similar in both CSD and control group.
6. Pharmacologically isolated responses of NMDA receptor subunit revealed an elevated ratio of GluN2A/B in CSD-induced rats when compared to that in controls.
7. Capsaicin at doses over 5 μM (10, 50, 100 μM) decreased evoked fEPSP responses.
8. Capsaicin at 5 μM tended to facilitate LTP in both CSD and control group, and improved LTP induction that was impaired by CSD.

Collectively, we would like to suggest the mechanism underlying CSD-induced perturbation of hippocampal synaptic plasticity as follows. During CSD induction, CSD propagates and reaches to the hippocampus. SD in the hippocampus disrupts hippocampal synaptic transmission and plasticity. The impaired LTP induction likely

involves a decrease of postsynaptic AMPA receptor responses and an increase of GluN2A/B subunit of NMDA receptors. In addition, capsaicin could restore the CSD-induced LTP impairment.

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BIOGRAPHY

Mister Montree Maneepark was born on December 15th, 1982, in Bangkok, Thailand. He has received the Development and Promotion of Science and Technology Talents Project (DPST) Scholarship from the Institute for the Promotion of Teaching Science and Technology (IPST) since 2000. He graduated Bachelor of Science (B.Sc., 1st class honor) in 2004 and Master of Science (M.Sc.) in 2007 from Chulalongkorn University. He has publications and awards as listed below.

Publications

1. **Maneepak, M.**, le Grand S.M., and Srikiatkhachorn, A. 2009. Serotonin depletion increases nociception-evoked trigeminal NMDA receptor phosphorylation. Headache 49(3): 375-82.

2. Saiwichai, T., **Maneepak, M.**, Songprakhon, P., Harnyuttanakorn, P., and Nithiuthai, S. 2009. Species-specific nested PCR for detecting *Plasmodium gallinaceum* in fresh chicken blood. The Journal of Tropical Medicine and Parasitology 32(2).

Awards

1. "Outstanding oral presentation award" at 8th National Graduate Research Conference, given by the Deans of the Graduate Schools of the Public Universities of Thailand (DGPU) and the Faulty of Graduate Studies, Mahidol University

2. "Outstanding research award" given by the Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University

3. "Outstanding research award" at Chula Medical Expo 2007, given by the Faculty of Medicine, Chulalongkorn University