SUMATRIPTAN-INDUCED SENSITIZATION OF THE TRIGEMINAL SYSTEM TO CORTICAL SPREADING DEPRESSION (CSD) IS BLOCKED BY TOPIRAMATE

By

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DEDICATION

I dedicate this dissertation to my wife Weixi Kong and my parents Chunxin Gu and Lianzhen Liu.
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ABSTRACT

The studies in this thesis research were conducted to investigate if sensitivity to induced cortical spread depression (CSD) or the consequence of a CSD event is affected by sumatriptan induced latent sensitization. Previous studies in our lab showed persistent exposure of sumatripan to rats produced a latent state of sensitization. Using persistent sumatripan exposed rats as a model for medication overuse headache, behavior, electrical stimulation threshold to provoke a CSD event and the immunoreactivity of c-Fos in the trigeminal nucleus caudalis (TNC) were characterized. Current results showed no statistical difference of electrically induced CSD thresholds in anesthetized rats measured at day 20 in sumatripan exposed rats compared with saline treated rats. Topiramate (80 mg/kg, i.p.) used clinically for prophylaxis of migraine headache significantly increased CSD threshold in both saline and sumatriptan infused rats. CSD events appear to be associated with trigeminal vascular system activation in TNC because c-Fos expression significantly enhanced in rats with electrically stimulated CSD events. As compared to saline treated rats, sumatriptan-exposed rats demonstrated a significantly higher number of c-Fos positive cells following the electrically stimulated CSD event. Under environmental stress (bright light), sumatripan exposed rats
demonstrated decreased response thresholds to periorbital and hindpaw tactile stimuli (i.e., allodynia) and enhanced c-Fos expression in TNC. A single dose of topiramate (80 mg/kg, i.p.) reversed environmental stress induced allodynia and c-Fos over-activity. Taken together, these results suggest that latent sensitization induced by persistent sumatripan exposure seems not correlated to the threshold of electrically stimulated CSD in current model. However, CSD enhanced the responses of trigeminal system in rats with sumatriptan-induced latent sensitization. The protective effects of topiramate shown in this model may be related to blocking the initiation of CSD events resulting from environmental stimulation as well as inhibiting the consequences of CSD events in primary afferents. These findings correlate with clinical observations of protective effects of topiramate for migraine prophylaxis.
1 INTRODUCTION

1.1 Overview of migraine headache

1.1.1 Symptoms and prevalence

Migraine headache is a complex paroxysmal neurological disorder characterized as an episodic, unilateral throbbing cephalic pain (De Felice et al., 2010b; Mehrotra et al., 2008; Rasmussen et al., 1991).

According to the International Headache Society, the criteria for migraine headache is repeated episodic headache (4 – 72 hours) with the following features of any two of symptoms such as unilateral, throbbing, nausea, vomiting, photophobia and phonophobia (Olesen et al., 2004). These symptoms will be worse with physical movement (Ferrari et al., 1993; Olesen et al., 1994).

As a worldwide disease, the prevalence of migraine does not discriminate between economic and race background. However, it does appear to have strong association with age, sex and genetics. Generally, females are more susceptible to migraine attack than males (15~18% vs. 6%) (Stewart et al., 1992). Also, people who have one or more relatives suffering from migraine are more likely to experience this disease during their lifetime than those without family history. Based on several sources, migraine headache most commonly starts between 15 and 24 years of age and occurs
most frequently in those 35 to 45 years of age (Bartleson et al., 2010). Approximately 4 - 5% of children aged under 12 suffer from migraine but no apparent differences occur between boys and girls (Mortimer et al., 1992). A rapid growth in incidence of migraine is seen amongst girls occurs after puberty, which continues throughout most of the adult life. After menopause, attacks in women tend to decline dramatically, so that after the age over 50, approximately equal numbers of males and females will experience migraine headache (Lipton et al., 1993; Stovner et al., 2006).

In addition to human suffering, migraine headache causes a substantial economic and societal burden. Since it generally affects the people in the age range of 15 - 50, these people are most industrious in term of societal influence and contribution. Also, migraine attack will disrupt the ability to work, care for families, or meet social obligations. Days lost from work represent a societal and indirect economic loss from migraine. Translated into dollars, estimations of the cost of lost productivity range from $ 1.2 billion to $ 17.2 billion per year (Andlin-Sobocki et al., 2005).

### 1.1.2 Classification of migraine and aura

Migraine headache could be classified into two main subtypes, migraine with aura and migraine without aura. Aura is characterized as a transient episode of focal neurologic phenomena that precedes or
accompanies the migraine attack. It is caused by an imbalance between excitatory and inhibitory neuronal activity at different levels in the central nervous system and about 15% - 30% of migraine patients may have aura at some time (D’Andrea et al., 2011). Aura appears gradually over 5 to 20 min and generally last less than 1 h (Kallela et al., 2012). The headache phase of the migraine attack usually begins within 60 min of the end of the aura phase, but sometimes delays up to several hours, or it may miss completely. (Kallela et al., 2012; Panayiotopoulos, 2012; Tfelt-Hansen, 2010).

Symptoms of migraine aura can be sensory or motor in nature. Visual aura is the most common aura that can occur without any headache. Visual aura is a disturbance of vision consisting of unformed flashes of white or black, or formations of dazzling zigzag lines often arranged like the battlement of a castle. The other form of aura is somatosensory aura. It may perform like a tingling feeling in the hand and arm. This paresthesia may migrate from the arm and then extend to the face, lips and tongue. Other symptoms of the aura phase include auditory, gustatory or olfactory hallucinations, temporary aphasia, vertigo, and hypersensitivity to touch (Panayiotopoulos, 2012).

1.1.3 **Triggers and four phases of migraine**

Many triggers are known to induce migraine headache such as light, stress, emotion, altered sleep pattern, menses, and etc. A trigger may occur up
to 24 h prior to the onset of symptoms (Bartleson et al., 2010). Once a migraine headache is induced, four phases of this disease occur, although not all the phases are necessarily experienced. The first phase is prodrome that occurs days or hours before migraine attack in 40 - 60% of migraine patients. In this phase, patients will experience symptoms, such as depression or euphoria, light sensitivity, yawning and etc. The second phase is aura (the details see above). The third phase is a pain phase, usually starts within 60 min of aura and occurs on one side of the brain. This throbbing pain might be associated with nausea, vomiting, photophobia, phonophobia, and cutaneous hypersensitivity throughout the body. Those symptoms usually last from 4 to 72 h. The last phase is postdrome with symptoms like fatigue, changes in mood or behavior. About 25% of migraine patients will experience this last phase (Bartleson et al., 2010; Kelman, 2006). It is necessary to point out that the migraine phases and symptoms experienced can be different from one migraine attack to another even in a same person (2004).

1.2 Possible mechanisms of migraine

Migraine is a form of sensory disturbance in the central nerve system (CNS). Two major hypotheses, vascular hypothesis and neurogenic hypothesis, have been proposed for the mechanism of migraine headache.
Vascular hypothesis explained the pain of migraine is caused by dilation of meningeal vessels. It is first proposed by Willis in 1664 and best articulated by Wolff in 1948 (Goadsby, 2009). Neurogenic hypothesis proposed that vasodilation is only an epiphenomenon for migraine attack but activation of certain receptors in trigeminal vascular system is the key event during the occurrence of migraine pain (Schoonman et al., 2008). In this hypothesis, cortical spreading depression (CSD) is believed to activate trigeminal vascular system to send pain signals to the trigeminal nucleus in the brain stem. The trigeminal nucleus conveys the signals to the thalamus, which relays them to the sensory cortex that involved in the sensation of pain (Ayata, 2009; Dalkara et al., 2006).

Unfortunately, the available data are not fully support neither of these hypotheses alone (Bergerot et al., 2006; Goadsby, 2007). It is important to note that migraine is a complex disorder. Any unitary theory seems a long way off to clearly elucidate the formation of this disease. The bright side for migraine headache study is that more and more researchers agree that dysregulation of trigeminal nerve system is a key component for migraine pain.

The trigeminal, or the fifth cranial nerve (V), is a nerve responsible for sensation in the face and certain motor functions such as biting, chewing, and swallowing. The trigeminal nerve has three major branches: the ophthalmic
nerve (V1), the maxillary nerve (V2), and the mandibular nerve (V3). The ophthalmic and maxillary nerves are purely sensory. The mandibular nerve has both sensory and motor functions. All together, these three main branches provide somatosensory innervation to distinct regions of the cranium. The dura is the outermost of the three layers of the meninges surrounding the brain and spinal cord. It consists of dense vascular system and is innervated by a large quantity of sensory neurons. Part of the sensory neurons are also referred as nociceptive fibers which can sense the dilation and constriction of vessels and can be activated by pro-nociceptive peptides such as CGRP (calcitonin gene-related peptide), substance P and histamine (Goadsby et al., 1990; Ma et al., 2001; Potrebic et al., 2003; Schwenger et al., 2007). The major function of these sensory neurons is to collect sensory information and convey information to upper level of neurons. Sensory pathways from periphery to cortex are illustrated below:
Sensory information collected from primary afferents travel to the trigeminal nuclei located in the brainstem. The trigeminal nucleus caudalis (TNC) is believed to be the most relevant trigeminal nucleus for transmission of headache related information (Buzzi, 2001). Primary afferent terminate in the TNC where they synapse with the second-order neurons that project to the thalamus. The thalamus conveys the information to certain somatosensory cortex (i.e., insula and cingulate cortex) via the third-order neurons, where the brain will analyze this information and make the corresponding response.

Among all the proposed mechanisms of migraine, Cortical Spreading Depression (CSD) has received attention recently, although it has been described decades ago (Kunkler et al., 2003; Lauritzen et al., 1982; Milner, 1958). The phenomenon of CSD was first reported by Dr. Leão in rabbit (Leao,
It is characterized as a transient wave of neuronal and glial depolarization, followed by long-lasting suppression of neuronal activity. CSD will propagate in all directions at a rate of 3 - 6 mm/min and last approximately 1 min once it is triggered. The critical event in the generation and propagation of CSD is a significant decrease in neuronal membrane resistance associated with a massive increase in extracellular K\(^+\) and neurotransmitters, as well as an increase in intracellular Na\(^+\) and Ca\(^{2+}\). These ionic shifts produce the characteristic slow DC potential shift that can be recorded extracellularly. Several different stimuli can trigger CSD including direct cortical trauma, exposure to high concentration of K\(^+\), and direct electrical stimulation (Ayata et al., 2006; Bergerot et al., 2006; Sanchez-Del-Rio et al., 2006).

There is considerable evidence supporting CSD as a potential mechanism in migraine headache. First of all, recent studies have established a link between migraine aura and CSD using magnetoencephalography, or highfield strength, high-resolution magnetic resonance imaging (Cao et al., 1999; Hadjikhani et al., 2001), based on the fact that visual or somatosensory symptoms of migraine aura propagate at a rate that corresponds well to the propagation speed of CSD. The other evidence is that CSD is associated with a brief hyperaemia that lasts a few minutes, followed by a hypoperfusion phase lasting up to 1 h. A similar long-lasting hypoperfusion in magnitude and
duration has been detected during migraine attacks in a few studies after the
hyperaemia phase finished (Lauritzen et al., 1983a; Lauritzen et al., 1983b).
Further evidence comes from the genetic studies performed in migraine
patients with aura. Results showed that the patients who have mutations on
familial hemiplegic migraine (FHM) genes such as CACNA1A (Cav2.1),
ATP1A2 (Na/K ATPase), and/or SCN1A (Nav1.1) demonstrated decreased
CSD threshold (De Fusco et al., 2003; Dichgans et al., 2005; Tottene et al.,
2002; van den Maagdenberg et al., 2004). These patients with mutation
became more susceptible to CSD induced by either excessive synaptic
 glutamate release or decreased removal of glutamate and potassium from the
synaptic cleft, or persistent sodium influx (Moskowitz et al., 2004). Moreover, a
recent study showed that chronic administration of prophylaxis medications
such as topiramate, valproate, propranolol, amitriptyline, or methysergide
significantly reduced the number of potassium chloride evoked CSD and
elevated the electrical stimulation threshold to evoke CSD in rats (Ayata et al.,
2006). Finally, as mentioned before, migraine is more prevalent in women than
in men during particular age (i.e. menarche to menopause). Recent studies
show that CSD threshold is significantly higher in female mice over male. More
interestingly, this sex difference on CSD threshold was abolished by
gonadectomy and ageing (Brennan et al., 2007; Eikermann-Haerter et al.,
Similar with other proposed mechanisms, CSD events have shown to activate the trigeminovascular system (Bolay et al., 2002; Moskowitz, 1984; Moskowitz et al., 1993b). It has resulted in elevated c-Fos expression in TNC (Moskowitz et al., 1993b), dilation of meningeal arteries (Bolay et al., 2002), plasma protein extravasation and inflammation (Buzzi et al., 1995), stimulation the release of calcitonin gene-related peptide and nitric oxide, and activation of perivascular nerves (Read et al., 1997; Reuter et al., 1998; Wahl et al., 1994).

However, it is still under debate whether CSD should be considered as a mechanism of migraine headache. Aura occurs only in 15 - 30% of migraine patients, and it is unclear whether CSD events occur in the migraine patients without aura. Although a recent study showed the silent CSD exists in migraine patients without aura, it still remains to determine whether CSD relates to migraine (Geraud et al., 2005; Kruit et al., 2005; Kruit et al., 2004). In addition, although the CSD theory of migraine can explain the mechanisms of aura and headache, it does not account for the diverse premonitory symptoms (e.g. fatigue, phonophobia, yawning, nausea, mood changes) that often occur prior to a migraine attack by hours. Moreover, since chronic administration of prophylaxis drugs such as topiramate, valproate, propranolol, amitriptyline, or methysergide significantly reduced the number of potassium chloride evoked
CSD and elevated the electrical stimulation threshold to evoke CSD in rats (Ayata et al., 2006), it is difficult to explain why the prophylaxis drugs are only effective in a small part of the patients if CSD is the underlying mechanism of migraine.

Taken together, it is plausible to consider CSD as a possible mechanism of migraine headache based on current clinical and experimental evidences. However, this hypothesis needs further investigation.

1.3 Treatment of migraine

Current treatments for migraine headache are not as effective as expected due to the lack of understanding on the mechanisms of this disease. The available treatments can only temporarily relieve the symptoms but do not cure. To date, the pharmacological treatments for migraine headache can be divided into two major categories: abortive and prophylactic therapy.

The goal of abortive therapy is to stop the migraine headache once it starts. Abortive agents include NSAIDs (non-steroidal anti-inflammatory drugs), opiates, ergot, triptans and CGRP (calcitonin gene-related peptide) antagonists. These medications usually are taken during aura phase, headache onset or in between as the drugs are more effective if taken earlier in an attack. Otherwise their efficacy will decrease towards to relieve
symptoms (Bartleson et al., 2010). NSAIDs such as ibuprofen, acetaminophen, aspirin and naproxen are used to treat migraine headache due to their anti-inflammatory effects. However, in most cases the NSAIDs are not effective at relieving migraine pain and long term use will cause severe gastrointestinal (GI) side effects, which limit the application of NSAIDs in migraine treatment (Brandes et al., 2007; Derry et al., 2010; Kirthi et al., 2010; Rabbie et al., 2010). Opioids decrease the transmission of painful information due to their functions of suppressing neuronal excitability and neuropeptide release (Tepper, 2012; Tepper et al., 2012). Morphine, as one of the representative drug of opioids, activates the G-protein coupled opioid receptors located on sensory fibers throughout the trigeminal vascular system (Kelley et al., 2012b; Taheraghdam et al., 2011). However, opioids are not the common treatment for migraine because FDA does not approve for this indication. They are only used for severe migraine patients in the emergency room.

Triptans are the most commonly prescribed abortive therapies for migraine headache. Also, they are the only drug family used specifically for migraine. However, the mechanism(s) of triptans to relieve migraine pain is still unclear (Johnston et al., 2010). Drugs in this category such as sumatriptan are 5-HT agonists (serotonergic agonists). Sumatriptan is selective for the 5-HT1B and 5-HT1D receptor and decreases dural vasodilatation and neuronal
excitability (Classey et al., 2010; De Vries et al., 1999; Goadsby, 2000; Goadsby et al., 2002; Moskowitz et al., 1993a). Due to the fact that 5-HT receptors exist in coronary vasculature, triptans also cause vasoconstriction in this system and lead to cardiac perfusion problems in patients with cardiovascular disease (Johnston et al., 2010). In addition to triptans, the therapeutic activity of ergot derivatives such as dihydroergotamine are also shown to be mediated through activation of 5-HT receptors (Kelley et al., 2012a).

Recently, CGRP (calcitonin gene related peptide) has been found to play a role in the pathogenesis of the pain associated with migraine. This finding is based on the evidence that CGRP levels are elevated in the blood of migraine patients. Also, clinical studies showed CGRP administration will trigger migraine attack in migraine patients (Lassen et al., 2002). Triptans as one of the most effective treatments for migraine have demonstrated the abilities to reduce CGRP release from trigeminal nerve endings and suppress the vasodilation function of CGRP in meningeal blood vessels (Tepper et al., 2008). CGRP receptor antagonists, specifically olcegepant and telcagepant, are being investigated both in vitro and in vivo for the treatment of migraine. Preclinical and clinical results showed both of them were effective in the acute treatment of migraine headache (Durham et al., 2010).
The other major category for migraine treatment is prophylactic therapy. It is recommended to reduce the frequency and intensity of migraine headache when patients experience more than two attacks per month (Brandes, 2005; Modi et al., 2006). The prophylactic medications are usually given daily for months or years, however, treatment can be episodic, subacute, or chronic. The classes of drugs belonging to this category include antiepileptics, beta-blockers, calcium channel blockers and tricyclic antidepressants. Most of migraine preventive medications are designed to treat other medical disorders (e.g., propranolol for hypertension, topiramate and valproate for epilepsy, etc.). However, they are found serendipitously to be beneficial in migraine treatment. As a result, mechanism of prophylaxis is still not well understood (Mannix, 2004).

Antiepileptic medications like topiramate and valproate have been shown to decrease abnormal neuronal excitation in the brain (Brandes, 2005). In addition, beta-blockers such as propranolol are able to decrease and stabilize blood pressure and keep the blood vessels in a relaxed state thereby decrease the intensity and duration of the migraine attacks (Silberstein, 2005). If the patient is unresponsive to these first line preventive medications, calcium channel blockers can also be used. Drugs in this category like flunarizine are considered to prevent migraine by reducing reflex narrowing of the blood
vessels after vasodilatation during an attack (Silberstein, 2004). Tricyclic antidepressants such as amitriptyline are also prescribed for migraine headache when other treatments fail (Yaldo et al., 2008).

1.4 Statement of problem and research hypothesis

Although several treatment options exist, there are still over 50% of migraine patients suffering from migraine headache. Even worse, the frequent use of abortive migraine medications over an extended period of time may result in medication overuse headache (MOH), in which the headaches become more severe and more frequent. MOH may occur with opioids, ergot alkaloids, serotonin 5-HT(1B/1D) receptor agonists (‘triptans’) and medications containing barbiturates, codeine, caffeine, tranquillizers and mixed analgesics (2004; Dowson et al., 2005). The International Headache Society defines MOH as more than 15 headaches per month during the regular overuse (> 15 day per month) of acute analgesics or symptomatic drugs for more than three months (Ghiotto et al., 2009; Olesen et al., 2006; Silberstein et al., 2005). Since frequent use of triptans has shown to cause MOH in migraine patients (more than 10 doses of triptans per month for a period of 3 months) (Diener et al., 2004; Ghiotto et al., 2009; Olesen et al., 2006; Silberstein et al., 2005), attention was focused on triptan (sumatriptan) -induced MOH in this research
based on the previous studies in our lab.

Recently, latent sensitization as one of the potential mechanisms of MOH attracts more and more attention (De Felice et al., 2010a; De Felice et al., 2010b). One clinical study showed MOH patient demonstrated facilitation of trigeminal and somatic parameters in the measurement of pain related cortical potentials (Ayzenberg et al., 2006). Similar results were obtained from the studies performed in our lab using rats with sustained or repetitive administration of triptans (naratriptan or sumatriptan). Persistent sumatriptan exposure elicited a time-dependent and reversible cutaneous tactile allodynia and induced a state of latent sensitization that is sensitive to presumed migraine triggers such as environmental stress (bright light stress) and NO donor challenge (De Felice et al., 2010b). Cutaneous allodynia is accepted as a marker of central sensitization (Burstein et al., 2000). The results suggested that sensitization of primary afferent neurons may lead to further sensitization of higher level neurons in the trigeminal nucleus caudalis and thalamus which eventually result in cephalic and extracephalic cutaneous allodynia (Burstein et al., 2004; De Felice et al., 2010b; Landy et al., 2004). Based on the previous studies in our lab, sumatriptan over-exposed rats will be used as a “migraine model” to investigate the correlation of persistent sumatriptan exposure induced latent sensitization and CSD.
The hypothesis of this research is that persistent sumatriptan exposure induced a state of latent sensitization that is associated with increased responsiveness to electrically stimulated CSD or environmental stress in rats. To support this hypothesis, three specific aims were tested:

Specific aim 1: Develop a method to determine electrical stimulation threshold to produce a CSD in anesthetized rats.

Specific aim 2: Determine if persistent sumatriptan exposure reduces the stimulation threshold to produce a CSD and the effect of topiramate on CSD threshold.

Specific aim 3: Determine the effects of topiramate in sumatriptan induced latent sensitization under bright light, i.e., environmental stress.
2 METHODS AND MATERIALS

2.1 Chemicals

Sumatriptan was purchased from GlaxoSmithKline (Philadelphia, PA, USA). Topiramate tablets (100 mg/tablet, Johnson & Johnson) were obtained from pharmacy. Isoflurane was purchased from Piramal Healthcare. Other used chemicals including urethane, paraformaldehyde and general reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise, and used without further purification.

2.2 Animals

Male Sprague-Dawley (SD) rats, 175 - 200 g at the time of arrival, were obtained from Harlan laboratory Inc. (Indianapolis, IN). They were housed in the University of Arizona Animal Care Facility (UAC) which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Upon receipt, the animals were taken to a designated animal room where they were acclimated for 4 - 7 days in polyethylene cages (three animals per cage) before being used in the experiments. The room temperature was maintained between 20 - 25 °C and the relative humidity between 40 - 60%. A light/dark cycle was maintained at 12 hour intervals.
Biannual testing for coliform and nitrates was performed by the UAC Diagnostics Laboratory Medical Technologist. During the acclimation period, rats were fed standard commercial diets (Harlan Teklad 4% rodent diet, Harlan, Indianapolis, IN) and were allowed food and water *ad libitum*. All experimental procedures were performed in accordance with the policies and recommendations of the International Association for the Study of Pain, the National Institutes of Health guidelines for the handling and use of laboratory animals, and the Animal Care and Use Committees of the University of Arizona.

2.3 Behavioral testing protocols

2.3.1 Periorbital sensory testing of non-noxious tactile stimuli in rats

Animals were acclimated to suspended plexiglass chambers (30 cm L x 15 cm W x 20 cm H) with a wire mesh bottom (1 cm²) for 45 min. The animals were allowed to freely move in their chambers during the entire testing protocol. The response thresholds to tactile stimuli were determined in response to probing the periorbital region with calibrated von Frey filaments (model 58011, Stoelting). Each von Frey filament was applied for 3 to 6 sec, perpendicular to the midline of the forehead, within a 3 mm diameter area at the level of the eyes, until buckling slightly. A positive response was indicated by a sharp withdrawal of the head, which sometimes included an attempt to grasp and/or
bite the filament. The withdrawal thresholds were determined by Dixon’s up-down method (Chaplan et al., 1994; Dixon, 1980). Maximum filament strength was 8 g.

2.3.2 Hindpaw sensory testing of non-noxious tactile stimuli in rats

Hindpaw measurements were determined in the same animals that received the periorbital testing. The hindpaw withdrawal thresholds to tactile stimuli were also determined in response to probing with calibrated Von Frey filaments. Each Von Frey filament was applied perpendicularly to the plantar surface of hindpaw until it buckled slightly, and was held for 3 to 6 sec. A positive response was indicated by a sharp withdrawal of the hindpaw. The withdrawal thresholds were determined by Dixon’s up-down method (Chaplan et al., 1994; Dixon, 1980). Maximum filament strengths were 15 g for hindpaw.

2.4 Drug infusion

Alzet osmotic mini-pumps (Alzet, Cupertino CA, USA; model 2001) with a nominal flow rate of 1 µL/h for 7 days were used for subcutaneous drug infusion. The mini-pumps were implanted subcutaneously in rats under anaesthesia with isoflurane (2% in air at 2 L/min). The day of the pump implant was considered as day 0. Drug administered by infusion was sumatriptan (0.6 mg/kg/day; GlaxoSmithKline, Philadelphia, PA, USA). At day 6, minipumps
were removed under isoflurane anesthesia. Withdrawal thresholds were tested at day 0, day 6 and day 20.

2.5 Implantation of electrodes

On day 20, rats were deeply anesthetized with urethane (1.2 g/kg, i.p.) and fixed to a stereotaxic frame (Stoelting) to prepare the rats for electrical stimulation and recording. Recording electrodes were made from 0.25 mm diameter Ag wire (A-M Systems, Inc., Everett, WA). The wire was flamed to produce spherical tips (1 mm diameter) and then coated with AgCl. Electrodes were placed in burr holes through the skull made with electrical drills, and one screw (#MPX-080-3F-1M, Small Parts Inc., Miami Lakes, FL) was placed over the uninjured hemisphere (left) that served as a head-mount anchor. Recording electrodes (electrode 1 and 2) were placed over frontal and parietal cortices (2.0 mm lateral, 1.5 mm anterior and 2.5 mm posterior to bregma, respectively) (Figure 2.1). A reference electrode was located posterior to lambda (11.5mm from bregma). The wires at the free ends of the electrodes were soldered to a multi-pin connector (Continental Connector, Hatfield, PA) and the assembly was fixed to the skull with dental cement (Figure 2.3). An additional burr hole (3.0 mm diameter) was made with an electrical drill to expose the dura at 6.5 mm posterior and 3.0 mm lateral (right) to bregma. The
dura was carefully cut with a number 11 scalpel. A pair of electrodes, with tips 1 mm apart, (A-M systems, tungsten, 1 mm tip exposure, 20 degree, WA,98382) (Figure 2.2) was inserted through the broken dura and placed 1.2 mm into cortical tissue.

2.6 Electrophysiological recording of CSD event

2.6.1 Electrophysiological recording

After surgery, rats were placed in suspended Faraday cages (40 cm L x 49 cm W x 37 cm H) with wire mesh bottom (0.5 cm²) during the recording period. A feedback-controlled heating pad (Harvard Apparatus, 110 v, 60 Hz) was used to maintain body temperature of rats consistent at 37 °C during recording (Figure 2.4). The multi-pin connector was attached to an electro-cannular swivel (#CAY-675-6 commutator, Airflyte, Bayonne, NJ) fixed to the chamber’s ceiling.

The stimulating electrodes were connected to an electrical stimulator (A-M systems, isolated pulse stimulator, Model 2100). The duration and intensity of the current was monitored and verified by an oscilloscope (Figure 2.5). Baseline ECoG (Electrocorticogram) and DC (Direct Current) recordings were obtained for 0.5 h before any electrical stimulation was initiated. Only rats with stable electrical recordings were included in the experiment. Signals were recorded through shielded cables, input to separate channels for amplification.
of DC and AC currents with a Grass (West Warwick, RI) Model 15 amplifier system (15A12 DC and 15A54AC amplifiers), digitized at 100 Hz, and collected with EEG recording analysis software Gamma v.4.9 (Astro-Med, Inc. West Warwick, RI).
Figure 2.1 Schematic of skull indicating positions of electrodes and ECoG/DC traced from anesthetized (Urethane 1.2 g/kg) rats after receiving a electrical stimulation (horizontal arrow). The electrophysiological recording clearly demonstrated a wave of CSD moving from electrode 2 (DC 2) in the parietal region to electrode 1 (DC 1) located at the frontal cortex.
Figure 2.2 A stimulating electrode which is formed as a pair of tungsten electrodes with tips 1 mm apart, (A-M systems, 1 mm tip exposure, 20 degree, WA, 98382) used in the experiment to deliver current into cortex. It was inserted through the broken dura and placed 1.2mm into the cortex.
Figure 2.3 A representative picture of the post-surgery rat. The free ends of the electrode wires were soldered to a multi-pin connector and the assembly was fixed to the skull with dental cement. Via the multi-pin connector implanted on the head, rats were attached to an electro-cannular swivel (the green part). The stimulating electrode (the white part) was connected with an electrical stimulator (A-M systems, isolated pulse stimulator, Model 2100) which delivers current into cortex through the stimulating electrode.
Recording were performed in an isolated, quiet room. The rat was placed on the pad of a homeothermic monitor (Harvard Apparatus, 110 v, 60 Hz) to keep the body temperature at 37 °C during the recording.
**Figure 2.5** The oscilloscope was used to verify the duration and intensity of current delivered into cortex. Here it indicates a current pulse of 300 millisecond (msec) duration at an intensity of 2 milliampere (mA).
### 2.6.2 CSD parameters and criteria to define a CSD event

As shown in Figure 2.6 A, DC shift is defined as the voltage difference measured at parietal lobe (DC2) or frontal lobe (DC1) during a CSD event. CSD duration is the time difference of a CSD passing through electrode 2 to electrode 1. To measure CSD duration, the start points are the time (unit: seconds) when the DC currents start to decrease (shown as two vertical arrows in Figure 2.6 B). Electrocorticogram (ECoG) amplitude was recorded with 1 min time frame before and during the occurrence of a CSD (Figure 2.6 C).

Two criteria are used to define the electrical recording is a CSD event: DC shift and reduction of ECoG amplitude. First, CSD is recognized by a negative deflection of the monitored DC current occurring first in the parietal lobe (DC2), and then the frontal lobe (DC1) following an effective electrical stimulation (Figure 2.6 B). Second, the amplitude of the ECoG trace will decrease during a CSD event compared with the amplitude measured before the CSD event (Figure 2.6 B).
Figure 2.6 CSD parameters recorded during a CSD event. (A) DC shift (B) CSD duration (C) ECoG amplitude.
2.6.3 CSD threshold measurement

The stimulation threshold to produce a CSD event (CSD threshold) was determined by electrical stimulation of the cortex applied through the pair of stimulating electrodes with 1 mm apart, placed 1.2 mm into cortical tissue. Square wave pulses of increasing intensity (10 to 4,000 µCoulombs) were applied at 4 min intervals by adjusting the current and duration of stimulus (Table 2.1) until a CSD was generated. The CSD threshold was determined as total charge (µCoulombs), and calculated using the following equation:

\[
\text{CSD threshold (µC)} = \text{Current (mA)} \times \text{Duration (mS)}
\]

<table>
<thead>
<tr>
<th>Stimulation protocol</th>
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<tbody>
<tr>
<td><strong>Current (mA)</strong></td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>Duration (mS)</td>
</tr>
<tr>
<td>Charge (µC)</td>
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</table>

**Table 2.1** Electrical stimulation protocol to produce a CSD

2.6.4 CSD propagation speed calculation

CSD propagation speed was calculated based on the CSD duration and the distance between two recorder electrodes (electrode 1 and electrode
2). As the distance between these two electrodes is 4 mm, the propagation speed of CSD (mm/s) can be calculated by the following equation:

$$\text{CSD propagation speed (mm/s)} = \frac{\text{Distance (4 mm)}}{\text{CSD duration (s)}}$$

### 2.7 Immunolabeling

Rats were deeply anesthetized with 100 mg/kg of an 80 : 12 mixture of ketamine and xylazine and perfused transcardially with 250 ml of phosphatebuffered saline (PBS; 0.1 M; pH 7.4), followed by 4% paraformaldehyde in 0.1 M PBS for 20 min. The brainstem was removed, postfixed in 4% paraformaldehyde overnight, and cryoprotected in 30% sucrose for 48 h at 4 °C. Transverse sections (30 mm thick) were cut from 2 - 4 mm caudal to obex and collected for immunohistochemistry (IHC) labeling. Sections were first incubated in blocking buffer (0.1 M PBS, 0.05% triton X 100, 1% BSA and 5% goat serum) for 90 min following 10 min permeabilization (0.1 M PBS, 0.2% triton X 100). Then, sections were immunoblotted at room temperature for c-Fos primary antibody overnight (rabbit polyclonal anti c-Fos sc-52, dilution 1 : 20000, Santa Cruz Biotechnology) and biotinylated goat anti-rabbit IgG for 90 min (dilution 1 : 600, Vector Laboratories, Burlingame, CA). Next, tissue sections were quenched by endogenous peroxidases with 0.1 M PBS, 10% methanol and 0.3% H₂O₂ for 30 min and subjected to ABC
complex (Vector Laboratories, Burlingame, CA) incubation for 60 min. At the end, sections were soaked in TSA (Tyramide Signal Amplification, dilution 1 : 50, Perkinelmer, Inc., Waltham, MA) solution, air dried for 30 min and then cover-slipped with Vectashield (Vector Laboratories, Burlingame, CA) for future study. A three-time of buffer wash (0.1 M PBS, 0.05% Triton X 100) was required in the interval when the incubation solution changed in the IHC study.

2.8 Determination of c-Fos labeled cells

The sections of trigeminal nucleus caudalis (TNC) were examined using an Olympus microscope (BX51) equipped with a digital Hamamatsu C4742-95 color camera along with Wasabi software (version 1.5) for fluorescence imaging. A digital Infinity 3 color camera with Infinity Capture Application (version 3.7.5.) was used for light imaging. Following a systematic sampling through the extension of the trigeminal nucleus caudalis, most c-Fos immunoreactivity was identified in laminae I and II, 2 - 4 mm below the obex. Thus cell counts were obtained from the same area for all testing groups. Both sides of each slice were counted (Fioravanti et al., 2011).

2.9 Bright light stress

In this experiment, 30 adult male Sprague Dawley rats (175 - 250 g) were selected based on the behavior results obtained from periorbital region
and hindpaw withdrawal thresholds tested using Von Frey (VF) filaments at day 0, day 6 and day 20 (Section 2.2, 2.3 and 2.4). On day 20, these rats were divided into 4 groups: saline-infused with saline injection (6 rats); saline-infused with topiramate injection (6 rats); sumatriptan-infused with saline injection (9 rats) and sumatriptan-infused with topiramate injection (9 rats). To induce an environmental stress, rats were placed in an open box (20 - 30 cm) and exposed to bright light for 1 h. The light was positioned to avoid temperature changes in the box. Periorbital and hindpaw allodynia are measured at 1 h intervals for 6 h, starting 1 h after the end of the light stress stimulus exposure. This stress was repeated 24 h after the first exposure. Saline or topiramate (80 mg/kg, i.p.) was injected 30 min before the environmental stress. The flow of this experiment is shown in Table 2.2.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 6</th>
<th>Day 20</th>
<th>Day 21</th>
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<tr>
<td>Baseline VF thresholds</td>
<td>VF thresholds</td>
<td>Topiramate (30 min before light)</td>
<td>Bright light (1h); VF thresholds</td>
</tr>
<tr>
<td>Implant minipumps</td>
<td>Remove minipumps</td>
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**Table 2.2** Experimental flow of bright light exposure
2.10 Statistical analysis

All data were expressed as mean ± SEM. Comparisons among multiple means or treatment groups were determined by one-way ANOVA followed by the post hoc Student-Newman-Keuls test. In case of comparison between two groups, t-test is performed. A p-value < 0.05 was considered significant and is indicated by an asterisk (*). A p-value < 0.001 was indicated by two asterisk (**).
3 RESULTS

3.1 Persistent sumatriptan exposure produces generalized tactile allodynia

To confirm our migraine pain model that using persistent sumatriptan exposure produces tactile allodynia, SD rats were infused with sumatriptan (0.6 mg/kg/day) or saline by osmotic minipump for 6 days. At day 6, the minipumps were removed. The hindpaw withdrawal thresholds were measured at day 0 prior to and on days 6 and 20 following minipump implantation. Results showed persistent sumatriptan exposure significantly reduced paw withdrawal thresholds at day 6 from 15.0 ± 0.0 g to 8.5 ± 1.5 g compared to saline group (Figure 3.1). Removal of the minipump resulted in a return of withdrawal thresholds to pre-implantation baseline levels by day 20.
Figure 3.1 Sustained infusion of sumatriptan (0.6 mg/kg/day) decreased withdrawal thresholds to light tactile stimuli applied to the hindpaw of rats. Sumatriptan or saline was continuously administered through an osmotic minipump for 6 days, after which the minipumps were removed. Data are shown as mean ± SEM. N=10, * p < 0.05 versus saline infused rats.
3.2 Measurement of electrical stimulated Cortical Spreading Depression (CSD)

At day 20 after minipump implantation, CSD threshold was measured in both saline and sumatriptan infused rats by the methods described in section 2.5 and 2.6. According to the two criteria, two blocks of ECoG recordings (1 min duration) were measured before and during CSD events and used for data analysis. The results showed ECoG amplitude was significantly reduced during CSD occurrence as compared to that recorded before CSD (Figure 3.2). This reduction was measured in both parietal lobe (DC2) and frontal lobe (DC1) in all treatment groups.

DC shifts measured in parietal lobes (DC2) and frontal lobes (DC1) are shown in Figure 3.3. Statistical analysis indicated no significant difference of DC-shift among all groups. Similarly, no significant difference of the other two CSD parameters, CSD propagation speed and CSD duration, were detected among all groups (Figure 3.4 and 3.5).
Figure 3.2 Reduction of ECoG amplitude during the CSD event measured in frontal and parietal lobes of saline or sumatripan infused rats which pre-treated with or without topiramate. Data are expressed at mean ± SEM. N= 7 - 9, * p < 0.05 versus ECoG amplitude recorded before CSD occurrence.
Figure 3.3 DC-shift measured in (A) frontal and (B) parietal lobes of saline or sumatriptan infused rats with or without topiramate injection. Data are expressed at mean ± SEM. N=8 - 10.
Figure 3.4 CSD propagation speed measured in saline and sumatripan treated rats with or without topiramate pretreatment at day 20. Data are expressed as mean ± SEM. N = 8 - 10.
Figure 3.5 CSD duration measured in saline and sumatripan treated rats with or without topiramate pretreatment at day 20. Data are expressed as mean ± SEM. N = 8 - 10.
3.3 Evaluation of CSD threshold

Since persistent sumatripan exposure caused sensitization to tactile stimuli in behavior testing (shown in Figure 3.1), it would be interesting to know if sumatripan-exposed rats demonstrate increased responsiveness to electrical stimulated CSD events. Therefore, electrical stimulation threshold of CSD was evaluated in our migraine model at day 20 following sumatripan minipump implantation (14 days after removal of minipumps). Also, the protective effects of topiramate, a prophylaxis of migraine headache used in clinic, were investigated at the same time. Rats were divided into 4 groups at day 20 following the minipumps implantation: saline-infused rats with saline injection (Saline-S); saline-infused rats with topiramate injection (Saline-T); sumatriptan infused rats with saline injection (Suma-S) and sumatriptan-infused rats with topiramate injection (Suma-T). Saline or topiramate (80 mg/kg, i.p.) was injected 30 min before CSD testing. The results showed that there is no statistically significant difference of CSD thresholds between Saline-S rats and Suma-S rats (Figure 3.6). However, topiramate injection significantly increased CSD thresholds in both saline and sumatriptan-infused rats (p < 0.05) in contrast to the rats with saline injection. CSD threshold increased from 1687.5 ± 535.8 to 3100.0 ± 621.7 μC after topiramate injection (80 mg/kg; i.p.) in saline-infused rats. In sumatriptan-infused rats, CSD threshold increased from
1288.9 ± 334.5 to 3000.0 ± 641.7 μC after topiramate injection.
Figure 3.6 Evaluation of electrical stimulation threshold of CSD in saline and sumatripan infused rats with or without topiramate pretreatment. A single dose injection of topiramate (80 mg/kg, i.p.) increased CSD thresholds measured in both saline and sumatripan treated rats at day 20. Data are shown as mean ± SEM. N= 8 - 10, * p < 0.05 versus saline injection.
3.4 CSD is associated with the enhanced c-Fos expression in the trigeminal nucleus caudalis

To determine if electrical stimulated CSD event is associated with the neuron activation in the trigeminal nucleus caudalis (TNC) of rats, the expression of c-Fos as a biomarker of neuron activation was evaluated via immunohistochemistry. Brainstems were harvested for immunofluorescence staining from all treatment groups: saline or sumatriptan-infused rats without any stimulation; saline or sumatriptan-infused rats with electrical stimulated CSD events; saline or sumatriptan-infused rats with electrical stimulated CSD events and topiramate injection (80 mg/kg, i.p.) 30 min prior to CSD testing. Due to our surgery is performed on the right side of the brain, c-Fos expression on both sides of TNC slice was analyzed to discriminate the position difference. Results indicated no significant difference on c-Fos expression between ipsilateral and contralateral side of TNC slices (Figure 3.7). Then, c-Fos expression on both sides of the slices were combined and shown in Figure 12. Results showed that c-Fos expression significantly enhanced in both saline and sumatriptan-infused rats with electrical stimulated CSD events. Compared with rats without any stimulation, the number of c-Fos positive cells increased from $1.0 \pm 0.6$ to $7.5 \pm 2.3$ in saline-infused rats and from $1.1 \pm 0.6$ to $14.3 \pm 3.1$ in sumatriptan-infused rats following electrical stimulation ($p < 0.001$).
Interestingly, sumatriptan infused rats demonstrated significant higher number of c-Fos positive cells ($14.3 \pm 3.1$) than that in saline-infused rats ($7.5 \pm 2.3$) following the same electrical stimulated CSD event. Injection of topriamate 30 minutes before electrical stimulation did not significantly reduce the number of c-Fos positive cells in the TNC of the rats (Figure 3.8).
Figure 3.7 Evaluation of c-Fos expression on both the ipsilateral and contralateral side of TNC slices. (A) Contralateral side (left TNC) (B) Ipsilateral side (right TNC). Data is expressed as mean ± SEM. N=2 - 4. No significant difference on c-Fos expression was measured between ipsilateral and contralateral side of TNC slices.
**Figure 3.8** Electrical stimulated CSD is correlated to the enhanced c-Fos expression in both saline and sumatripan infused rats. Data are shown as mean ± SEM, N=2 - 4. ** p < 0.001 versus rats without CSD, ## p < 0.001 versus saline infused rats with CSD.
3.5 Topiramate reverses environmental stress induced allodynia in persistent sumatriptan exposed rats

Our previous work has shown persistent sumatriptan exposure increased sensitivity to the traditional migraine triggers such as environmental stress (De Felice et al., 2010a; De Felice et al., 2010b). To test if topiramate could block this sensitization in current migraine pain model, the rats were stressed by bright light for 1 h at day 20 (14 days after termination of sumatriptan exposure). At the same day, rats were administrated with saline or topiramate (80 mg/kg, i.p.) 30 min before bright light exposure. Results showed the response threshold of sumatriptan exposed rats to periorbital tactile stimuli significantly reduced from the baseline level (8.0 ± 0.0 g) to 6.6 ± 0.1 g. Similarly, the hindpaw withdrawal threshold reduced from the baseline value (14.7 ± 0.3 g) to 6.7 ± 0.5g (Figure 3.9 A and 3.9 B). At the following day (i.e. day 21, 15 days after termination of sumatriptan infusion), these rats were exposed to bright light again for 1 h. Results showed the threshold of periorbital withdrawal and hindpaw withdrawal in sumatriptan exposed rats significantly reduced to 2.9 ± 0.3 g and 6.2 ± 1.0 g respectively (Figure 3.9 C and 3.9 D). More importantly, a single bolus injection of topiramate (80 mg/kg, i.p.) given 30 min before the exposure to bright light almost completely blocked environmental stress-induced cutaneous allodynia in periorbital region and the
hindpaw (Figure 3.10) on any days tested.
Figure 3.9 Persistent sumatriptan exposure induced latent sensitization in rats. On Days 20 and 21 after pump implant, rats were injected topiramate (80 mg/kg, i.p.) or saline 30 min prior to bright light exposure. Sumatriptan exposed rats demonstrated hind paw (A: Day 20, C: Day 21) and periorbital (B: Day 20, D: Day 21) tactile alldynia. Data are expressed as mean ± SEM. N=4 - 5, * p < 0.05, versus saline infused rats.
Figure 3.10 Environmental stress-induced allodynia is blocked by a single dose of topiramate (80 mg/kg, i.p.). Sumatripan infused rats were challenged on Days 20 and 21 with a single dose injection of topiramate (80 mg/kg, i.p.) 30 min prior to bright light exposure. Topiramate (80 mg/kg, i.p.) blocked the expression of hindpaw (A: Day 20, C: Day 21) and periorbital (B: Day 20, D: Day 21) tactile allodynia in these rats. Data are shown as mean ± SEM. N=4 - 5.
3.6 Topiramate attenuates the enhanced expression of c-Fos in TNC of saline and sumatriptan infused rats

Two hour after the second bright light exposure on day 21 (15 days after termination of sumatriptan and saline infusion), rats from all groups: saline-infused with saline injection; saline-infused with topiramate injection; sumatriptan-infused with saline injection and sumatriptan-infused with topiramate injection were anesthetized and the brainstems were removed for immunofluorescence staining. Results showed c-Fos immunoreactivity in the superficial laminae of the TNC from sumatriptan infused rats with saline injection significantly increased (23.2 ± 3.2) compared with saline-infused rats with saline injection (15.3 ± 1.5) (p < 0.05). Importantly, topiramate significantly reduced c-Fos expression in both saline (10.7 ± 1.9) and sumatriptan (14.4 ± 1.6) infused rats. About 31% and 38% of c-Fos positive cells were decreased by topiramate pretreatment in saline and sumatriptan infused rats respectively (Figure 3.11).
Figure 3.11 Pretreatment of topiramate (80 mg/kg, i.p.) attenuates c-Fos expression in both saline and sumatriptan exposed rats in TNC following bright light exposure. Data are expressed as mean ± SEM. N= 2 - 4, * p< 0.05 versus saline infused rat with saline injection, # p< 0.05 versus sumatripan infused rats with saline injection.
4 DISCUSSION AND CONCLUSIONS

Migraine headache is a common and complex neurological disorder. Although it is clear that head pain is a key manifestation of this disorder for most patients, what drives the activation of neuronal pain pathways in migraine patients is still not well understood. The main barriers for migraine pain study are that such pain occurs without tissue damage and patients with this disease perform normally between periods of migraine attack (De Felice et al., 2010b).

A relatively reliable model is pivotal for migraine research. So far, several migraine models have been developed both in vivo and in vitro, including vascular (vasodilation and vasoconstriction) model, neurovascular model (plasma protein extravasation, activation of TNC, CSD, effects of NO donors) and mutant mouse model (i.e., mutant Cav2.1 channels) (Bergerot et al., 2006). In current research, sumatriptan over-exposed rats were used as a “migraine model” which was originally developed in our lab. This model is based on the clinical phenomenon that migraine patient with frequent use of triptans will lead to medication overuse headache (MOH) during which frequency of migraine attack increases (De Felice et al., 2010b; Diener et al., 2004). Studies in our lab showed that persistent neural adaptation in trigeminal ganglion cells was observed after repeated or sustained triptan administration.
It was characterized as increased labeling for calcitonin gene related peptide (CGRP) and neuronal nitric oxide synthase (nNOS) in identified rat trigeminal dural afferents. In addition, a behavioral reaction of increased sensitivity to presumed migraine triggers such as environmental stress and nitric oxide (NO) donor was maintained for weeks following discontinuation of triptan administration. This phenomenon is termed as “triptan-induced latent sensitization” (De Felice et al., 2010a; De Felice et al., 2010b).

Results suggested that persistent sumatripan exposure induced latent sensitization in rats mimic several common characteristics of clinical migraine. First of all, in this model, no tissue injury was observed and normal sensory threshold was maintained between periods of pain. These are consistent with human migraine headache. Secondly, challenge of traditional migraine triggers such as environmental stress (bright light stress) and NO donor reduced sensory threshold and caused generalized tactile allodynia (defined as a hypersensitivity of the skin to touch or mechanical stimuli that is considered non-noxious under normal circumstances). Finally, after NO donor challenge, CGRP level in blood was elevated. CGRP receptor, but not NK-1 (neurokinin-1) antagonists can reverse allodynia caused by NO donor challenge (De Felice et al., 2010b). Indeed, the results of current research confirmed our previous observations (De Felice et al., 2010b). Current results showed that persistent
sumatriptan (0.6 mg/kg/day) infusion significantly reduced hindpaw withdrawal threshold at day 6 (Figure 3.1) suggesting these rats exhibited tactile allodynia. Removal of the sumatriptan resulted in a return of withdrawal threshold to pre-implantation baseline levels by day 20 (Figure 3.1).

Next, a stable electrical stimulation technique to evoke CSD events in rats was established. Efforts were focused on finding a reliable electrode to deliver current into the cortex of rats. This is a time consuming process in which at least twelve electrodes in different material, size and form have been tested. Eventually, a tungsten electrode was found to be the most reliable one in terms of delivering the appropriate current stably in this study. Therefore, this tungsten electrode was used in the following experiments.

Two criteria were used to define a CSD event. The first one is the occurrence of DC shift that is caused by the ionic movement, i.e., Ca$^{2+}$, Na$^+$, K$^+$. During a CSD event, DC shift occurs first at parietal lobe (relatively close to electrical stimulation site) then frontal lobe. The other criterion is the reduction of ECoG amplitude during CSD development (Figure 3.2), which is exactly like the name of CSD (spreading depression). These two criteria were used throughout all experiments to confirm the occurrence of CSD event. Several parameters of CSD such as DC shift, ECoG amplitude, propagation speed and CSD duration were measured in this study to define if an electrical recording is
the same CSD event. Indeed, no significant difference on DC shift, ECoG amplitude and DC duration were measured among all groups. Therefore, the same CSD events were visualized, recorded and compared among all groups.

Because slowing of propagation speed has been associated with CSD propagation failure (Akerman et al., 2005), the speed of propagation of CSD were measured. Results showed no significant differences on CSD propagation speed among all tested groups. Topiramate only slightly decreased the propagation speed in sumatriptan and saline group (Figure 3.4) This result is not consistent with the study reported by Ayata et al. that CSD propagation speed was significantly reduced by topiramate (Ayata et al., 2006). The possible reason for this discrepancy is the different duration of topiramate treatment used in current study. In our study, one single dose of topiramate (80 mg/kg) was administrated Intraperitoneally instead of chronic administration (60 - 80 mg/kg, i. p.) for 4 - 17 weeks in their study.

A slight decrease of CSD threshold in sumatriptan-exposed rats was measured compared with saline treated rats (Figure 3.6). However, statistical analysis did not show a significant difference between these two groups. These results are unexpected. Our original expectation was that sumatriptan exposure might decrease the stimulation threshold to produce a CSD. This outcome suggests that increased migraine frequency after triptan exposure is
not likely due to an increase in susceptibility to generate CSD events. However, a CSD may still trigger enhanced responses in the condition of latent sensitization. Interestingly, a single dose of topiramate (80 mg/kg, i.p.) elevated CSD threshold in both sumatriptan and saline treated rats (Figure 3.6). As a prophylaxis of migraine headache, chronic daily i.p. administration of topiramate dose-dependently suppressed CSD frequency and increased the electrically stimulated threshold of CSD in rats (Ayata et al., 2006). The other study showed intravenous injection of topiramate (30 mg/kg) inhibited occurrence of CSD evoked by a cortical needle plunge in rats (Akerman et al., 2005). Our results are partly consistent with these reported studies. Our interpretation for the results is that the elevated CSD threshold by topiramate is probably due to its anti-epileptic mechanisms that can decrease abnormal neuronal excitation in the brain (Brandes, 2005; Rogawski, 2012).

C-Fos immunoreactivity in TNC as a biomarker of the activation of trigeminal vascular system has been widely used in migraine research (Goadsby et al., 1997; Hoskin et al., 1999; Sugimoto et al., 1998). To determine if electrical stimulation induced CSD is associated with the activation of TNC, the expression of c-Fos in TNC was evaluated by immunohistochemistry. Results showed c-Fos expression was significantly elevated in both sumatriptan and saline treated rats following the occurrence of electrical
stimulated CSD, which indicated that CSD event can activate trigeminal vascular system (Figure 3.8). This result is consistent with previous findings showed that CSD provokes c-Fos expression within TNC (Bolay et al., 2002; Moskowitz, 1984; Moskowitz et al., 1993b). More importantly, after CSD events, the level of c-Fos expression in persistent sumatriptan exposed rats was significantly higher than that measured in saline rats (Figure 3.8), regardless of the c-Fos positive cells from the combination or separation of ipsilateral and contralateral side of the TNC slices (Figure 3.7, 3.8). As observed in sumatriptan-exposed rats, the enhanced expression of c-Fos is most likely caused by sumatriptan induced latent sensitization. The previous data from our lab have showed the primary afferents were sensitized after persistent sumatriptan exposure. It was characterized as the increased labeling of CGRP and nNOS in identified trigeminal dura afferents (De Felice et al., 2010a; De Felice et al., 2010b). The sensitization of the 1st order neurons may lead to sensitize the 2nd order neurons or even the 3rd order neurons (Burstein et al., 2004; De Felice et al., 2010b; Landy et al., 2004). Here, further study is necessary to investigate which level of neurons was sensitized to cause the enhanced expression of c-Fos in sumatriptan exposed rats.

Unpublished data from our laboratories showed CSD event was induced by bright light stress in sumatriptan exposed rats. Also because CSD
threshold was elevated by topiramate (Figure 3.6), next experiment was designed to investigate whether preventive administration of topiramate could block the allodynia in sumatriptan exposed rats following bright light stress. The results showed that a single dose of topiramate (80 mg/kg, i.p.) decreased trigeminal vascular sensitivity in sumatriptan exposure model as evidenced by the reverse of bright light stress induced allodynia and attenuation of c-Fos immunoactivity in TNC (Figure 3.9 and 3.10). This protective effects are similar as observed in migraine patients who treated by topiramate (Brandes, 2005; Krymchantowski et al., 2004; Silberstein, 2004). These results, coupled with the results obtained in CSD threshold experiment, indicate topiramate prevents the occurrence of sumatriptan induced latent sensitization possibly via increasing the electrical stimulation threshold of CSD.

In summary, this study found that persistent sumatriptan exposure does not increase responsiveness to electrically stimulated CSD in cortex, since the stimulation thresholds were not decreased in sumatriptan exposed rats. However, the trigeminal system is still in a state of latent sensitization, as indicated by the production of tactile allodynia after exposure to bright light stress. This observation is supported by the enhanced c-Fos expression found in the TNC of sumatriptan exposed animals after bright light stress. Therefore, a normal CSD event could still trigger enhanced responses in the trigeminal
pathway, as shown by the increased c-Fos expression observed in sumatriptan-exposed animals following CSD event. Topiramate may exert its prophylactic effect by inhibiting the generation of CSD events, as indicated by the increased stimulation thresholds to elicit a CSD. Moreover, we found that topiramate produced a slight attenuation of the increased c-Fos expression in TNC after CSD, and blocked the sumatriptan-induced increased c-Fos expression after bright-light stress as well as the stress-induced tactile allodynia. These observations suggest that the protective effects of topiramate against medication overuse headache (MOH) are mediated by three possible mechanisms: blocking the initiation of CSD events; inhibiting latent sensitization or both. The relevance of these protective mechanisms of topiramate to pathogenesis of medication overuse headache is remained to be established.
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