CHAPTER II
LITERATURE REVIEWS

MIGRAINE

Migraine is a recurring neurological disorder characterized by a throbbing unilateral head pain that is readily aggravated by routine physical activities. Clinical definition of migraine includes a host of neurological symptoms other than pain: nausea, photophobia, phonophobia, osmophobia, fatigue, and numerous disturbances in automatic, mental, sensory, and motor function. The headache is sometimes preceded by a period of transient neurological symptoms know as "aura". The aura can be in the form of visual (scotoma, or scintillation), cognitive (dysphasia) or motor (hemiparesis) dysfunction

The above clinical syndromes reflect are relative to migraine pathogenesis. Transient deficits are observed during aura period that some certain area in the cerebral cortex which are usually completely recovered. The gradual feature of the aura indicates the phenomenon sweeping over the cortical surface. The pulsating character of headache reveals the importance of cranial vasculatures as the site of pain generation. During the attack of migraine, the sensory systems are highly susceptible to their respective stimuli by increase of sensory perception. Therefore, it seems that the function of the central control system may be compromised during the attack, resulting in the change of the sensory perception. Recently, the change of trigeminal nociceptive system hyperexcitability was considered to be the explanation of above clinical syndromes.

Migraine: definition and classification

Migraine is a primary neurobiology disorder, resulting from dysfunction of the trigeminovascular system. The disorder manifests a recurring attack, usually lasting 4.72 hours, which can interfere with normal function especially unilateral throbbing headpain from moderate to severe intensity [4]. They also involve nausea, sometimes vomiting, and light, sound, and sensitivity to other sensory stimuli. Migraine may occur with or without an aura generally lasts between 5 and 60 minutes. Aura occurs in about 15-20% of patients with migraine. The most common type of aura disturbs the visual field but also can be in somatosensory perception. The

typical presentation of the visual aura is the scintillating scotoma. In most patients who experience aura, the aura develops before the headpain being. An aura is present before every migraine attack in some individuals, but in the other patients, aura accompanies only a small proportion of attacks. The intensity of aura varies among attacks and many remain constant from attack in a particular person or may vary in successive attacks in the same person. Furthermore, premonitory symptoms, called prodome, may precede migraine attack; these symptoms occur 24-72 hours before the onset of other symptoms. During this period, patients may experience feeling of well-being, talkativeness, surges, hunger, anorexia, excessive yawning, depression, irritability, restlessness, and tension.

Migraine headaches are classified according to their clinical features, as well as according to current concepts of pathophysiology. Patients who have migraine without aura have general cerebral blood flow and do not report focal neurologic symptoms. In those who have migraine with aura, changes in regional cerebral blood flow can be demonstrated and neurologic symptoms originating in the brain or brain stem [5]

Migraine without aura

Migraine without aura is an idiopathic headache disorder that manifests in the form of attacks that last 4-72 hours. Typically, the headache is unilateral, has a pulsating quality, and moderate to severe intensity which is aggravated by routine physical activity and are associated with nausea or vomiting, photophobia, and phonophobia.

Migraine with aura

Migraine with aura is also an idiopathic recurring disorder that manifests in some patients as migraine without aura but is also accompanied by transient neurological symptoms. Aura symptoms usually develop gradually, over 5-20 minutes, and last less than 60 minutes. Headache, nausea, or photophobia usually follows neurologic aura symptoms directly or after a free interval of less than 1 hour. The headache usually lasts 4-72 hours, but will be completely absent (migraine aura without headache)

Trigeminal system and pathophysiology of migraine

The trigeminal nerve provides the principal afferent pathway for the transmission of headpain in human. The cranial tissues are innervated mainly by branches of 3 divisions (opthalamic, maxillary, mandibula) of the trigeminal nerve (Fig 2.1) [6]. The first division innervates the integument and underlying muscles of the forehead, nose, and anterior scalp, eyebrow, eyelid, and content of the orbit via its three main branches, the frontal, lacrimal, and nasal nerve. Within the cranium, the dura matter of anterior and middle fossae are supplied by both opthalamic and maxillaly brances of the trigeminal nerve. The information regarding cranial nociception in conveyed via A-delta and C-fiber afferent nerve fibers which terminate in the cranial tissue as free nerve endings. Noxious stimuli can activate these free nerves endings, resulting in the excitation of A-delta or C-fiber afferents.

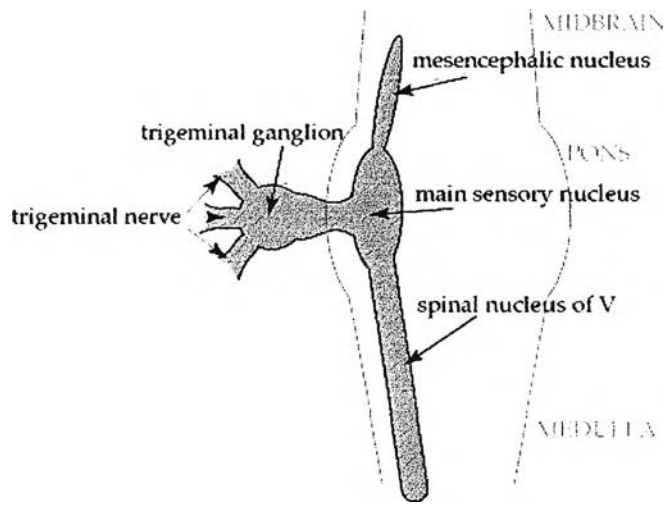

Figure 2.1 Structure of trigeminal nociceptive system (Snell, 1992)

The descending axons travel to the peripheral neuron through trigeminal ganglion (TG) and finally reenter the central nervous system at trigeminal nucleus caudalis (TNC). TNC receives nociceptive fibers from the ophthalmic division of the trigeminal nerve providing functional and anatomical organization of nociceptive neurons as same as the dorsal horn of spinal cord. Basically, nociceptive fibers form spinal tract of V ascending into spinal nucleus of V. The spinal nucleus of V can be divided into three regions along its length as described below; the nearest area of the mouth is called subnucleus oralis, the middle area is called subnucleus interpolaris,

and the nearest area of the tail is called subnucleus caudalis. The nociceptive fibers actually synapse to neurons in subnucleus caudalis which ascend across to the opposite side and converge with spinothalamic tract through to thalamus (Fig2.2).

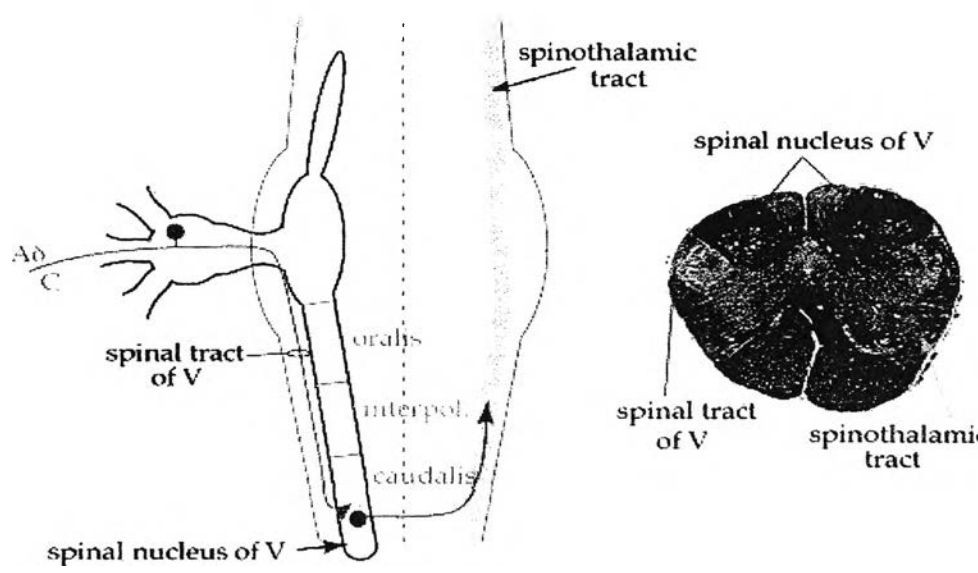

Fig 2.2 Trigeminal pathway (Snell, 1992)

Because neurons locating in the superficial dorsal horn of marginal layer (lamina I) respond exclusively to noxious stimuli, they are called "nociceptive – specific neurons" [7], interestingly they project to higher brain center. Wide-dynamic range neurons in lamina I respond to graded fashion of both unnoxious and noxious stimulations. The substantial gelatinosa (laminaII) is made up almost exclusively of interneuron (both excitatory and inhibitory). Some neurons in lamina II respond only to nociceptive inputs while others respond to unnoxious stimuli. Lamina III and IV contains the neurons that receive monosynaptic input from Aβ fibers. Lamina V contains the primarily wide dynamic-range neurons that project to brain stem and thalamus. These neurons receive monosynaptic input from Aβ and Aδ fibers. They also receive input from C fibers, either directly on their dendrites, which extend dorsally into the superficial dorsal horn, or indirectly via excitatory interneurons that receive input directly from C fibers. Furthermore, the role of the trigeminal system in cranial nociception and vasomotor control was further confirmed by the discovery of circuit between trigeminal system and parasympathetic system (Figure 2.3). This

circuit provides the activation of trigeminal system to superior salivatory nucleus in brainstem. Trigeminal neuron projects the impulse to the sphenopalatine ganglion which projects axons terminate in the cranial vessels, causing vasodilatation. This responding is called "trigeminovascular reflex" and is believed to play an important role in migraine pathogenesis

Furthermore, albeit indirect, the involvement of trigeminovascular system during migraine development comes from comparative studies between clinical features of migraine pain and observations of sensitization in rodent models. It has been proposed that sensitization of peripheral sensory nerve endings was followed by sensitization of central trigeminal neurons, render the nociceptive fibers hypersensitivity to arterial pulse and head movements, which account for the throbbing nature of migraine pain and it's worsening during coughing, bending over and rapid head movement [8]. The sensitization of central neurons may also account for the extracranial tenderness and cutaneous allodynia seen in these patients. In fact, development of cutaneous allodynia in the ipsilateral side of headache, followed by the contralateral side over the course of a migraine attack has been documented [9]. Basic and clinical pharmacological observations also share several similarities, suggesting involvement of the trigeminovascular system during migraine. The clinically proven potent anti-migraine drugs dihydroergotamine and sumatriptan are effective in blocking neuropeptide release and neurogenic inflammation induced by electrical stimulation of the trigeminal ganglion in rodents [10]. Inhibition of peptide release within trigeminal nucleus caudalis has also been inferred [11]. Stimulation of the trigeminal ganglion causes calcitonin gene-related peptide (CGRP) release in the plasma obtained from cerebral venous blood in experimental animals and humans. Triptans inhibit CGRP release induced by trigeminal ganglion activation in experimental animals as well as after successful treatment of migraine attacks [10, 12]. In parallel with preclinical data, a potent CGRP blocker, BIBN4096BS, was reportedly effective in aborting acute migraine attacks [13], conforming to the idea that animal models of neurogenic inflammation may serve as screening tests for discovering potential anti-migraine compounds despite some limitations and expected species differences.

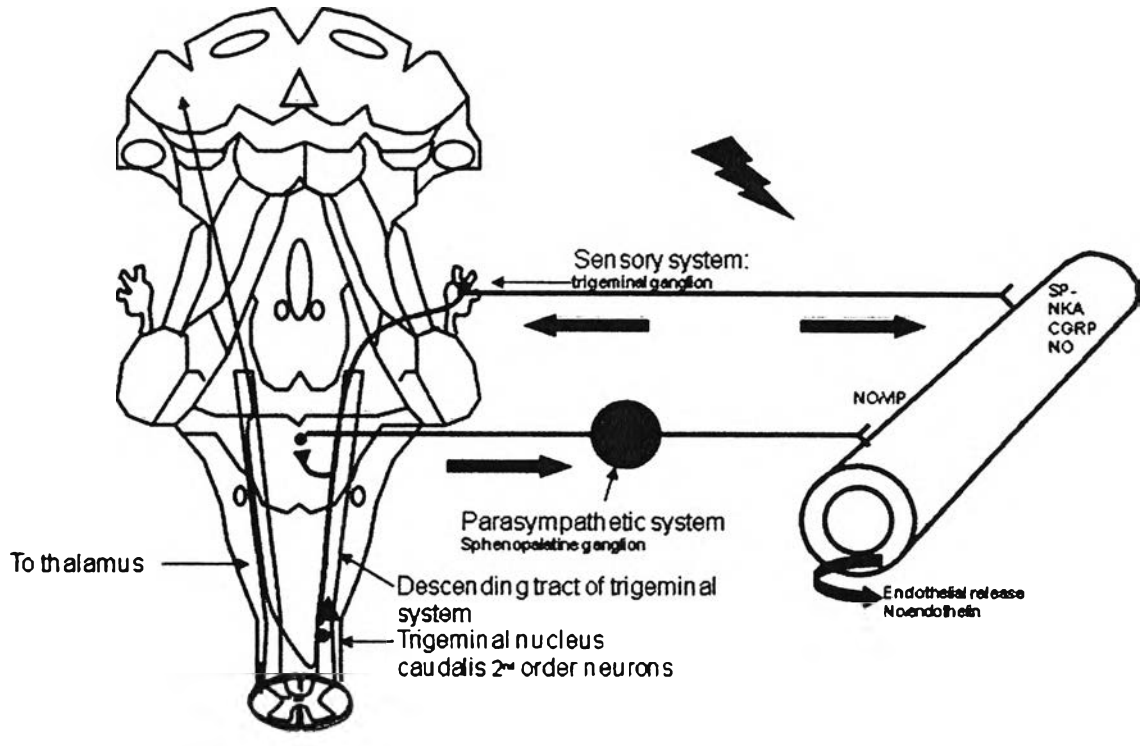

Figure 2.3 Trigeminovascular system describes the relationship between trigeminal nerve and the cranial vasculature

Activation of trigeminal nociceptive fibers result in the release of several chemical messenger especially powerful neuropeptide vasodilator substances, such as nitric oxide (NO), substance P (SP), calcitonin gene-related peptide (CGRP), neurokinin A (NKA), vasoactive intestinal polypeptide (VIP), etc. [14]. Importantly, from the viewpoint of the cranial nociception above neuropeptides are located in neurons in the trigeminal system [15].

Trigeminal nociceptive System

The dura and cranial blood vessels, which contributing to pain sensitive, are also innervated by trigeminal affrents, particularly from the ophthalmic and mandibular divisions [16]. Surrounding the large, supratentorial cerebral vessels, pial vessels, large venous sinuses, and dura mater is a plexus of nociceptive fibers, largely myelinated (Aδ) fibers and unmyelinated (C) fibers, that arise from the trigeminal ganglion, while the posterior fossa innervations arises from the upper cervical dorsal root [17] (Figure 2.4). The cell bodies of the afferent fibers lie in the trigeminal (Semilunar or Gasserian) ganglion accumulated in the middle cranial fossa at the base

of the skull [18]. Trigeminal ganglion cells are pseudounipolar and can also be classified on the basis of ultrastructural and immonocytochemical differences [19], into large, type A cell and smaller, type B cells, with subclasses of each trigeminal ganglion cells also contain amino acids and neuropeptides similar to those in spinal ganglia [19,20,21]. Some of the peptides that have been identified in dorsal root ganglion cells by immunohistochemical staining include the following: SP, VIP, CGRP, NKA, somatostatin (SOM), cholecytokinin (CCK), bombesin, galanin, vasopressin, oxytocin, dynorphin (DYN), enkephalin (ENK), α-neoendrophin, and corticotrophin releasing factor. At present, it is unclear if the presence of a particular set of peptides can predict the function type of sensory receptor. It seems likely that the peptides which are the neuromodulators can be act in concert with fast-acting neurotransmitters, either enhancing or diminishing their actions [22].

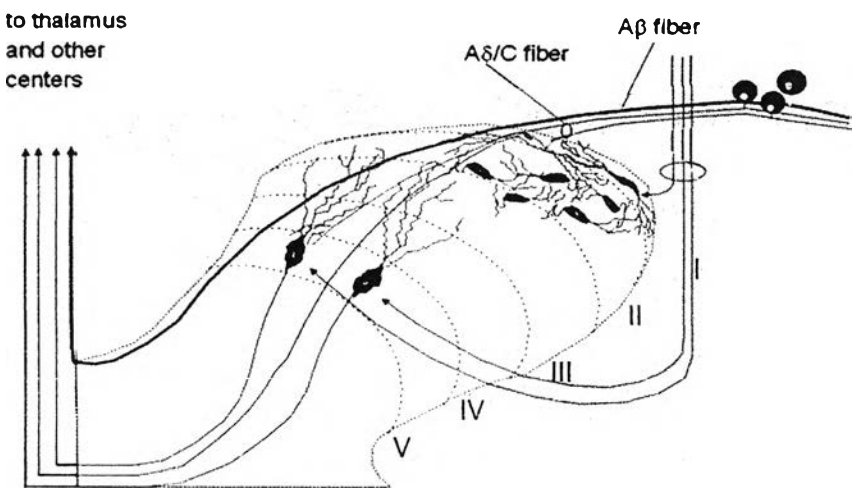

Figure 2.4 Nociceptive afferent fibers terminate on projection neurons in the dorsal horn of the spinal cord. Projection neurons in laminar I receive direct input from myelinated (Aδ) nociceptive afferent fibers via stalk cell interneurons in laminar II. The neurons in laminar V are predominately of the wide dynamic-range type which receive low threshold input from the large-diameter myelinated fibers (Aβ) of mechanoreceptors as well as both direct and indirect input from nociceptive afferent fibers (Aδ and C). In this figure the laminar V neurons send dendrites up through laminar IV, where it is contacted by the terminal of Aβ primary afferent. Dendrites in laminar III arising from a cell in laminar V are contacted by the axon terminal of interneurons in laminar II.

Trigeminal nociceptive system can be activated by applying different stimuli (mechanical, electrical or chemical) in intra- or extracranial structure innervated by the trigeminal nerve. Of particular relevance to the study of migraine has been the observation that induction of spreading depression [23], an electrophysiological wave of depolarization hypothesized to underlie migraine aura, which induced c-Fos expression within cells in TNC. Cells in lamina I and II receive direct synaptic input from primary afferent fibers to second and higher order neurons which transmit nociceptive information to rostral centers [24]

Pathophysiology of migraine aura: The cortical spreading depression theory

The migraine aura is defined as any neurological disturbance, appears shortly before or during the development of migraine headache. Seemingly similar migraine aura may have different features, suggesting involvement of different brain regions. The headache is most often throbbing and unilateral, on a side of the head relevant for the focal symptoms [25]. To explain this sequence of event, Harold G. Wolff developed the vascular theory of migraine. He hypothesized that migraine attacks are initiated by vasoconstriction in the cranial vasculature that leading to oligemia and a reduction in cerebral blood flow that could be severe enough to generate an aura. Compensatory vasodilatations occurring in intracranial or extracranial blood vessels after vasoconstriction is assumed to result in perivascular edema and inflammation that, in turn, triggered headache pain. Whereas most of the brain is insensitive to pain, meningeal blood vessels are highly innervated by pain fibers. Blood vessel dilation is presumed to activate the trigeminal sensory nerves that surround the meningeal blood vessels, causing pain. Wolff's theory was accepted for nearly 50 years. However, Jes Olesen's careful measurements of regional cerebral blood flow during migraine attacks using xenon 133 single-photon emission computed tomography demonstrated that while there were reductions in blood flow at the time of aural symptoms, blood flow could be decreased during the headache. Blood flow might then become abnormally high without a change in the headache. The lack of correlation between the changes in blood flow and migraine symptoms raised doubts about the vascular etiology of migraines and opened the door for the neural theory.

Cortical spreading depression (CSD)

As mentioned above, the trigeminogeminal nerves innervate the meninges and very likely participate in the genesis of migraine pain. However, the mechanisms that trigger migraine attacks are not well understood. Although a link between CSD and migraine pathogenesis was hypothesized more than 40 years ago [26, 27, and 28], unequivocal evidence has been difficult to obtain until recent developments in magnetic resonance imaging, optical imaging, magnetoencephalography. Moreover, migraine genetics provide more compelling evidence for the potential role of CSD in inducing migraine pain. On the other hand, recent experimental data also clearly demonstrated that, in the rat, CSD might be activated the trigeminovascular afferents, as suggested by earlier studies [29], and induced a long-lasting blood-flow enhancement within the middle meningeal artery and plasma protein leakage in the dura mater [30].

CSD is increasingly accepted as the likeliest basis for migraine aura and the trigger for headache pain. Cortical spreading depression is characterized by rapid and nearly complete depolarization of a sizable population of cortical neurons with massive efflux of potassium ions from intracellular to extracellular compartments [31]. The process represents a regenerative all-or-none process that propagates slowly as a wave in brain tissue. CSD was discovered in the 1940s by a Brazilian doctoral student A.P. Leao while working in the Department of Physiology at Harvard Medical School. Leao had set out to study the response of cortical tissue to electrical stimulation in an attempt to understand the basis of cortical electroencephalography in a model of experimental epilepsy. His experiments were performed in the exposed cortex of rabbits and a few pigeons and cats. Following a brief period (1-5 seconds) of repetitive electrical stimulation of the cortex or a few light touches with a small glass rod, he noticed a "marked and enduring depression" of the spontaneous electrical activity in the electroencephalogram signal that spread out slowly in all directions from the region stimulated (Figure 2.5)

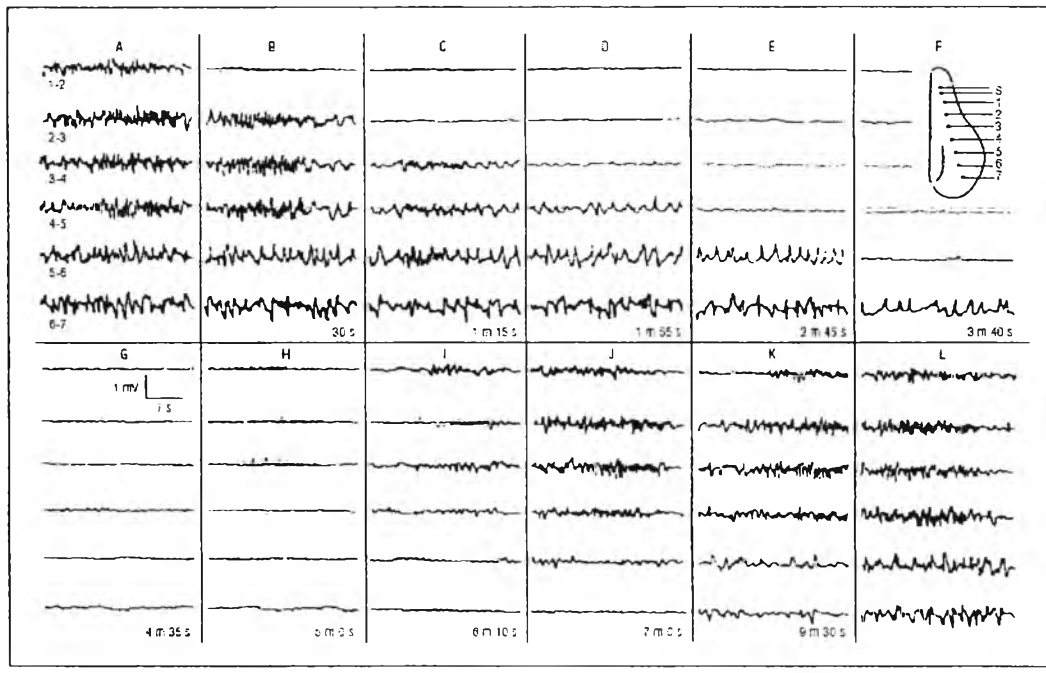

Figure 2.5 Demonstration of CSD in the rabbit neocortex (Leao, 1944)

The responsibility of CSD in migraine aura is always based on a rate of aural progression and spreading depression. Migraine aura is any transient neurological disturbance that appears shortly before or during the development of a migraine headache. Most commonly, the aura arises in the primary visual cortex and typically involves spreading scintillating scotoma (Figure 2.6) with a characteristic distribution of fortification figures. The disturbance usually starts at the center of the visual field and propagates to peripheral zones within 10 to 15 minutes. Function returns to normal within another 10 to 15 minutes [32]. The rate of development of the visual symptoms suggests that there is a hyperexcitation in the visual cortex that moves at a speed of approximately 3mm/min [26]. Milner noted that the speed of propagation of the visual symptoms was the same as that of CSD, leading to the hypothesis that CSD is the physiological basis for the aura. Interestingly, in individuals experiencing somatosensory symptoms, the spread of symptoms along the sensory homunculus occurs at a similar rate. Numerous neuroimaging studies in humans have supported the concept that CSD-like phenomena in the neocortex occur simultaneously with migraine aura [33].

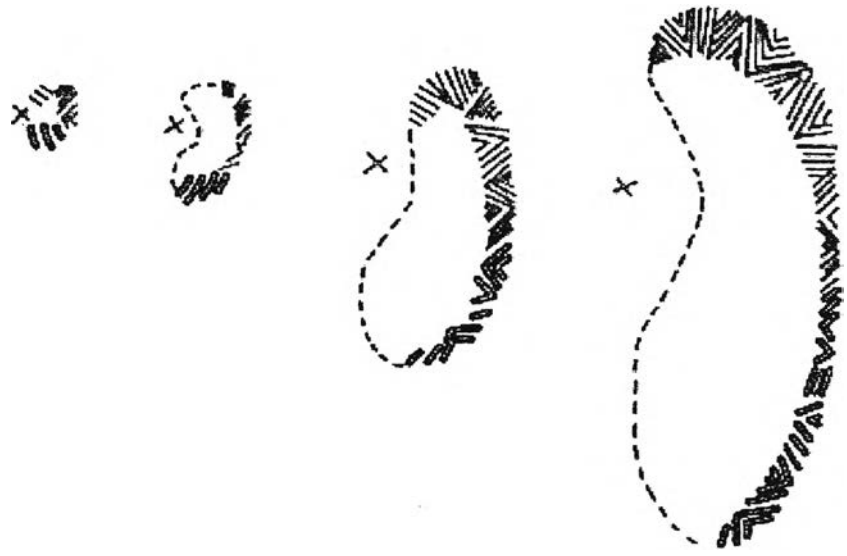

Figure 2.6 Successive maps of a scintillating-scotoma shows characteristic distribution of the fortification figures. In each case, the asterisk indicates the fixation point. The speed of propagation of the excitation-depression wave as ~ 3mm/min. (Lashley, 1941)

Basic features of CSD

In 1944, Leao published a seminal paper entitled "Spreading depression of activity in the cerebral cortex". The spreading depression has been subsequently known internationally as Leao's spreading depression. The EEG following mild noxious stimuli becomes completely extinguished for a minute and the depression propagates very slowly across a wide cortical region. CSD has been induced in most grey matter regions studied so far, e.g. in the cortex, the hippocampus and the cerebellum of variety of species [34]. It has been observed in human cortical tissue *in vitro* [35] and human hippocampus and striatum *in vivo* [36]. Thus, experiments in human cortical tissues can support the development of the CSD. However, a recording of CSD from the human neocortex *in vivo* is still missing.

CSD is a transient disturbance of mechanism maintaining ionic homeostasis which spread across the brain, moving from the back (occipital region) of the cerebral cortex toward the front at about 3-5 mm/minutes (Fig 2.7). These perturbations in ionic homeostasis are characterized by negative shift of extracellular potential, suggesting a cell depolarization (Fig 2.4). This electrical phenomenon can be induced

in animal with non-noxious stimuli, and is frequently referd to in the literature as the "spreading depression of Leao" (Lauritzen, 1994)

Successful elicitation of CSD in experiment depends on the trigger factor; e. g hypoglycemia and hypoxia lower the threshold [34]. Common methods of triggering CSD include local electrical or mechanical stimulation or injection of high concentration of KCl. Potassium plays a central role for CSD and it is reasonable to assume that any disturbance of K^+ homeostasis predispose the brain region to CSD [37]. Brain K^+ clearance system are heavily depended on the capacity of glial cells [38]. In humans the lowest glial-neuronal cell ratio is presented in the primary visual cortex [39]. Therefore one would expect human CSD to be initiated in occipital region. As it is well known, visual auras are indeed very frequent in migraine [25].

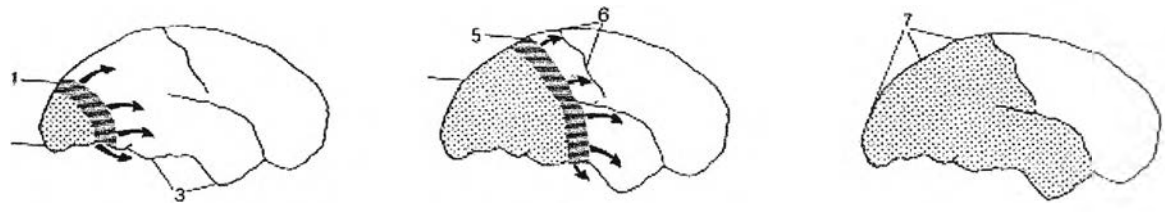

Figure 2.7 Hypothesis of the involvement of CSD development in migraine attack is summarized in the text. The figures represent lateral views of the human brain at different time intervals after the start of the attack, spaced by approximately 30 min. The dotted area represents the region of reduced cerebral blood flow (CBF), the striped area represents the region of neuronal depolarization during the first minute of CSD, and arrows represent the direction of CSD progression. 1. CSD is initially elicited at the occipital pole, spreading anteriorly at the lateral, mesial and ventral sides of the brain. At the CSD wave front, transient ionic and metabolic disequilibria trigger perturbed neuronal function, CBF changes and neurological symptoms. 2. Following CSD, cortical rCBF decreases by 20±30% for 2-6 h. 3. CBF in regions is not invaded by CSD remains normal until encountered by CSD. 4. The region of reduced CBF expands as the CSD moves anteriorly. 5. Somatosensory symptoms from the extremities appear when the CSD invades the primary sensory cortex at the postcentral gyrus. 6. CSD usually stops on reaching the central sulcus, but in many patients it does not even propagate this span. The ventral spread of CSD causes activation of pain-sensitive fibres and headache. 7. Full-scale attack. The CSD has stopped and is now detectable as a persistent reduction of cortical CBF. At this time the patient suffers from headache but has no focal deficits (Lauritzen, 1987b).

Neurons and glial cells depolarize during CSD, giving rise to an intense, but transient spike activity (second) when the reaction enters the tissue [40]. Neuronal silence immediately follows, lasting for a few minutes, but evoked potential usually take a longer time to recover in 15-30 min [34]. The sequence of brief excitation followed by a short-lasting depression is supposed to be the neurophysiological basis of the sensory symptom during migraine aura [41, 42, 43, and 44]. The depolarization is associated with dramatic changes in the distribution of ions between the intra- and extracellular compartment: K^+ and hydrogen ion leaves the cells, while Na^+, Ca^+ and Cl^- enter together with water, as the size of the extracellular space decrease to approximately half of the control values [38]

Most of ion concentrations return spontaneously to normal level after 30-60 s, whereas Ca^+ and H^+ concentrations usually take a few minutes to recover. There is, at the moment, no satisfactory explanation of the spreading depression mechanism of CSD, but the spread probably involves the diffusion of one or more chemical mediators, most likely K^+ and glutamate, into the extracellular compartment [45]. It has been suggested that a calcium wave in glial cells underlies CSD, but this still remains to be proven [46].

It is important to understand the transient nature of CSD. If the electrophysiological changes are sustained and propagation is absent, then the phenomenon is usually anoxia or hypoglycaemia rather than CSD. Repeated CSD increases the immunohistochemical staining of glial fibrillary acidic protein in the rat cortex that is associated with activation of this cell type [47] and prolonged period (24 h) of expression of the c-Fos proto-oncogene [48].

CSD phenomena occur in experiment animals in the penumbra zone, immediately adjacent to a cortical infarct, where nerve cells are viable but electrically silent [49, 50, and 51]. In many observations, the ionic disequilibria during CSD looks like transient ischemia, but there is usually no shortage of energy supply during CSD [52]. These dramatic changes of neuronal function and ion homeostasis are associated with profound change of the local circulation.

Several methods have been used to induce CSD in animal brain. These methods can be grouped into 2 categories including mechanical stimulation and KCl application

1) Mechanical stimulation

Blunt stabbing and pain pricking are common methods to elicit CSD. Several research groups such as Lambert et al., 1999 and Eberberger et al., 2001 choose the pinprick technique to induce CSD in the cortex of cats, rats, and rabbits [53, 54, 55, and 56]. This method is delivered by rapid insertion of a 26-30 gauge needles, through the dipole and dura (1-2 mm depth) into cortical tissue and immediately withdrawn. The location needs to be avoiding from cerebral vessels. However, mechanical stimulation has been disagreed with some research groups. They suggested that this technique may link to the noxious stimulation.

2) KCl application

As mentioned, potassium play a central role for CSD and it is reasonable to assume that any disturbance of K^+ homeostasis would predispose the brain region to CSD [35]. The KCl application is the most popular technique that has been chosen by many research groups, such as Read et al., 1997 [57], Smith et al., 2000 [58], Moskowitz et al., 1993 [59]. This technique can be further divided into 2 methods, including the microinjection of KCl on the cortical surface and the placement of solid KCl on the surface of cortex.

The microinjection of KCl technique is performed after craniotomy. A fused silica needle or PE-90 (a catheter tube) or glass micropipette is placed 1 mm below the dura surface. Then KCl is injection to the cortical area. The concentration of KCl vary 670 nM to 1 M. [59, 60, and 61]

The placement of solid KCl technique is also performed after craniotomy. A solid KCl is placed directly to the cortical surface area. The weights of KCl vary 3 to 30 mg. depending on the size of animal (for example; 3 mg in rat and 30 mg in cat) [57 and 58]. In addition, a remote intracortical injection and application of KCl-soaked paper on cortical surface are used in KCl application [62, 63]. For the sake of comparison, application of NaCl into the brain tissue is usually included as the control.

The transient receptor potential vanilloid subtype 1 (TRPV1): A molecular gateway to the pain pathway

The transient receptor potential vanilloid subtype 1 (TRPV1) is a non selective cation channel expressed in primary sensory neurons of the pain pathway. It can be activated by vanilloid compound (e.g. capsaicin), proton, or heat (over 43° C). After being activated, TRPV1 allows sodium, calcium, and possibly potassium ions to flow according to their concentration gradients, causing initial depolarization and neurotransmitter release.

Many evidences show that the expression of TRPV1 in dorsal root ganglion (DRG) cells is subjected to change. For example, Amaya et al., 2003 [64] demonstrated that inflammation can increase in TRPV1 positive neuron profile in rat DRG neurons using immunohistochemical methods. In their experiment, peripheral inflammation was generated by intraplantar injection of Freund's complete adjuvant (CFA) into the left hind paws. They found that the inflammation induced a 1.5 fold increase in percentage of TRPV1-like immunoreactivity positive profile. Area frequency histogram showed that TRPV1 expression was increases exclusively in small and medium-size neurons after inflammation.

Beside inflammation, injury in nervous system can also alter TRPV1 expression. In 2002, Fukuoka, Tokunaka and Tachibabana investigated the expression of TRPV1 in rat DRG neurons after spinal nerve ligation. They found that TRPV1 mRNA expression was increase in the small and medium-size DRG neurons. This change could be observed at the first day after injury and persisted for 28 days. An increase in TRPV1 expression was confirmed by immunoreactivity at the third day after surgery [65].

The above results indicate that both noxious stimulation and injury in nervous system can increase TRPV1 expression in DRG. Such change may relate to functional alteration of pain and may underlie the development of chronic pain syndrome.

Structure of TRPV1

The functional TRPV1 is a tetrameric membrane protein with four identical subunits assembled around a central aqueous pore. Each TRPV1 subunit protein shows a membrane domain composed of six transmembrane segments (S1-S6), with an amphipathic region between the fifth and sixth segment that forms the channel conductive pore. This region contains glutamic acids that are involved in the pH dependent gating of the receptor [66]. The protein also has a cytoplasmic N- and C-termini (Fig. 2.8). In the N-terminus, TRPV1 channels exhibit three ankyrin domains that mediate protein-protein interactions with cytosolic proteins, and show consensus sequences for protein kinases. The protein displays a cytosolic C-terminus domain containing phosphoinositide, and calmodulin binding (CAM) domains, as well as phosphorylation sites [67]. In addition, the C-end has a TRP-like motif that functions as an association domain of receptor subunits [68].

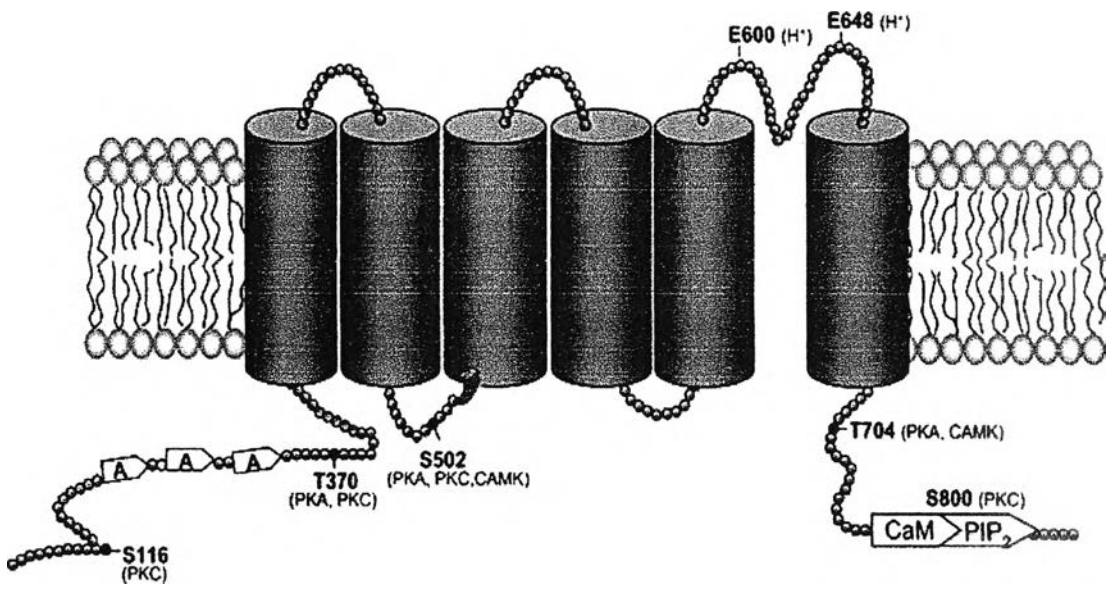

Fig. 2.8 Topological model of TRPV1 subunits. Each subunit consists of six putative transmembrane segments and intracellular N- and C- domains. Functional regulatory domains of the receptors and phosphorylation sites are indicated. (Messeguer, A., 2006) [79].

Distribution of TRPV1 in primary sensory neurons

As first shown by resiniferatoxin (RTX) autoradiography [71], TRPV1 are expressed along the entire length of vanilloid-sensitive sensory neurons, from the peripheral terminal to the axons to the somata. In corresponding areas the presence of TRPV1-like immunoreactivity has also been demonstrated [72, 73]. Northern blot analysis confirmed the presence of TRPV1 transcripts in dorsal root and trigeminal ganglia [74]. TRPV1-like immunoreactivity was detected in more than 50% of DRG neurons, with the expression being most prevalent in small to medium sized neurons [72]. TRPV1 also observed in both the central (e.g. dorsal horn of spinal cord) and peripheral (e.g. skin and cornea) processes of primary afferent neurons [72]. In DRG neurons, TRPV1-like immunoreactivity is associated with small clear vesicles in preterminal axons and plasma membrane of nerve terminal [72]. The latter finding agrees with an earlier observation of Szolcsanyi [75], according to which capsaicin depletes small clear vesicles from nerve ending.

The distribution of TRPV1-like immunoreactivity in the spinal dorsal horn deserves particular attention. The labeling is strongest in the Lissauer zone (laminaI). TRPV1 protein is also abundant in the inner, but not in the outer, layer of lamina II. It should be noted that several important proteins involved in pain transmission are also enriched in the inner layer of lamina II.

Distribution of TRPV1 in brain

TRPV1 is also presented in the neurons of the central nervous system. For instance, TRPV1 mRNA or protein is widely expressed in brain regions such as the olfactory nuclei, cerebral cortex, dentate gyrus, central amygdala, striatum, centromedian and paraventricular thalamic nuclei, hypothalamus, substantia nigra, reticular formation, locus coeruleus, inferior olive and cerebellar cortex [76]. The role of TRPV1 in the central nervous system is still elusive, although it may mediate endovanilloid signaling promoting the release of excitatory neurotransmitter such as L-glutamate, noradrenaline and dopamine [77, 78]

Taken together, all these findings illustrate that TRPV1 channels are widely expressed in neuronal cells of both endodermal and mesodermal origin, and involved in diverse physiological functions. These include thermosensory transduction, as well as chemical signaling presumably mediated by endovanilloid compounds. In addition, they hint that dysfunction of the channel may underlie the etiology of pathological

sensory transduction such as that occurring in inflammation and pain modulation. All these observations underscore the notion that TRPV1 is a widely expressed protein whose function may be critical for diverse physiological conditions, especially in pain pathway.

TRPV1 are predominantly expressed on Aδ and C fibers projecting to the dorsal horn of the spinal cord. In addition, they are expressed in trigeminal ganglion neurons projecting to the spinal nucleus of the trigeminal tract. Of the many stimuli that activate cation currents in primary sensory neurons, low extracellular pH and noxious stimuli (e.g. heat) have been proposed as physiological activators of TRPV1 [70].

TRPV1 as a receptor for noxious thermal stimuli

Many receptors and channels, show alterations in their structure or activity as a function of temperature, but TRPV1 has thermal response characteristics that make it especially interesting in the context of nociception. Most notably, TRPV1 is gated by heat, but only when ambient temperatures exceed over 43° C [73], that is a threshold of heat-evoked pain responses in humans and animals or heat-evoked electrophysiological responses in primary afferent nerve fibers or cultured sensory neurons [79, 80, 81]. Evidence for a direct relationship between TRPV1 expression and heat sensitivity is supported by several observations [73]. The sensitivity of TRPV1 to capsaicin and heat sensitivity is investigated by using TRPV1-transfected HEK293 cells. Both capsaicin- and heat-evoked currents are attenuated (in both frequency and magnitude) by application of capsazepine and ruthenium red, vanilloid receptor antagonists. In addition, both responses are characterized by outwardly rectifying current-voltage relations and relatively high permeability to calcium ions. Some distinctions exist, such as quantitative differences in cation permeability ratios or requirements for extracellular calcium in desensitization, suggesting that vanilloids and heat activate TRPV1 through overlapping but distinct mechanisms. In TRPV1-transfected HEK293 cells (as in sensory neurons), capsaicin or heat evokes single-channel currents in excised membrane patches, demonstrating that channel activation occurs via a membrane-delimited mechanism that does not require the action of diffusible cytoplasmic second messengers [73]. Site-specific mutations in TRPV1 can significantly alter capsaicin potency or thermal activation thresholds [82], providing

further evidence that TRPV1 transduces responses to these stimuli when expressed in heterologous cells.

These observations of heterologous nonneuronal systems have indicated that TRPV1 functions as a molecular transducer of noxious thermal stimuli *in vivo*. Consistent with this hypothesis, sensitivity to capsaicin and sensitivity to noxious heat are also well correlated among small-diameter sensory neurons in culture [83, 84]. Moreover, TRPV1 and native heat-evoked currents have a number of properties in common, including similar current-voltage relationships [79, 84, 85], selective permeability to cations [79, 84, 85], and, in some studies, sensitivity to vanilloid receptor antagonists [86]. At the same time, numerous discrepancies between native heat- and vanilloid-evoked responses have been reported, including differences in relative permeability to calcium and sodium ions [79, 84] and sensitivity to vanilloid receptor antagonists [84, 85]. Additionally, Nagy and Rang have recently shown that the amplitudes of the capsaicin- and heat-evoked responses among individual sensory neurons are not tightly correlated, contrary to the scenario expected if the same channel responds to both stimuli. Most significantly, capsaicin- and heat-evoked responses show poor cosegregation at the single-channel level in membrane patches excised from cultured rat sensory neurons [84]. That is, most patches were sensitive to capsaicin or heat, but only a few patches responded to both stimuli (although the frequency of dually responsive patches is significantly higher than one would predict for random inclusion of two independent channels in the same patch). From these findings, Nagy and Rang proposed that distinct ion channels respond to capsaicin and heat. These channels could consist of entirely different molecules or different functional isoforms of TRPV1 generated through alternative RNA splicing, post-translational modification, or association with other cellular proteins. Unfortunately, similar patch clamp analyses have not been carried out with TRPV1- expressing HEK293 cells. Further experiments may help to address these issues.

At least, some of the apparent discrepancies listed above may be accounted. The fact that sensory neurons express multiple forms of heat-activated channels may result from the difference in their biophysical or pharmacological properties. Indeed, a recently identified TRPV1 homologue (VRL-1) is insensitive to capsaicin or protons but does respond to high-threshold heat stimuli (>50^{o} C) when expressed in nonneuronal cells [87]. It have been proposed that VRL-1 accounts for the "high-

threshold" thermal sensitivity of this subset of nociceptors while TRPV1 detects moderate-intensity heat stimuli in small-diameter, unmyelinated (C fiber) nociceptors. However, this model is based largely on correlative evidence, owing to the paucity of selective and potent pharmacological agents with which to manipulate vanilloid receptors *in vivo*.

TRPV1 as a receptor for protons

Tissue damage, such as that associated with infection, inflammation, or ischemia, produces an array of chemical mediators that activate or sensitize nociceptor terminals to elicit pain and promote tenderness at the site of injury [88, 89]. Protons constitute one important component of this pro-algesic response, reducing the extracellular pH levels below the physiological normal of ~7.6. Extracellular protons elicit both transient and sustained excitatory responses in cultured sensory neurons, the latter of which is believed to account for persistent pain associated with local tissue acidosis [90]. Protons are capable of modulating the activity of a number of receptors and ion channels expressed by primary afferent nociceptors, including acid-sensitive channels of the degenerin family [91, 92, 93, 94], ATP-gated channels [95, 96], and vanilloid receptors [97, 98, 99]. The contribution of these entities to acid-evoked pain is presently unclear, but electrophysiological and genetic studies of native and cloned vanilloid receptors suggest that they play a significant role in mediating sustained proton responses *in vivo*.

Because protons have been proposed to act as modulators of native vanilloid receptors, there is significant interest in understanding the relationship between sensitivity to capsaicin and proton sensitivity at the cellular and molecular levels. It have been shown, using both TRPV1-expressing mammalian cells and *Xenopus* oocytes, that moderately acidic bath conditions augment capsaicin-evoked responses by increasing agonist potency (50% effective concentration 90 nM at pH 7.4 versus 36 nM at pH 6.4) without altering efficacy [73,74, 82]. Importantly, extracellular protons also potentiate heat-activated currents [73]. Temperature response curves generated in TRPV1-expressing oocytes and HEK293 cells show that a reduction in extracellular pH produces markedly larger responses at temperatures that are noxious to mammals (>43° C). Moreover, a reduction in pH dramatically lowers the threshold for channel activation, such that at pH 6.3, substantial currents can be seen at

temperatures as low as 35°C, conditions under which the channel is normally closed (at pH 7.6). This augmentation of TRPV1 thermal responsiveness by protons closely resembles the increase in nociceptor thermal sensitivity associated with inflammation [88]. In both cases, there is a significant decrease in the threshold for heat-evoked responses and an increase in response magnitudes at temperatures above the initial pain threshold. Importantly, TRPV1 shows especially dynamic modulation of heat-evoked currents between pH 6 and 8 [82], a sensitivity range that matches the extent of local acidosis attained during most forms of tissue injury. Below pH 6, sustained membrane currents can be observed in TRPV1-expressing HEK293 cells at room temperature (22°C), with a half-maximal effective pH of 5.4 [73]. Whether these proton-evoked responses result simply from a decrease in the channel's thermal response threshold or involve additional steps is not entirely clear, but recent structure-function studies suggest that proton-evoked channel activation and proton-mediated potentiation can be functionally uncoupled [82].

Plasticity of TRPV1

TRPV1 is responsive to multiple stimuli, such as heat and proton. Injury brings on many changes that affect the activity of the nociceptor, including local tissue acidosis and the production of pro-algesic agents, such as bradykinin, AYP, monoamines, and arachidonic acid metabolites. The result is that response thresholds to noxious stimuli are decrease, thereby contributing to the development of thermal and mechanical hypersensitivity. The capacity of TRPV1 to detect and integrate information from diverse physical and chemical inputs makes this channel potentially well suited for assessing the physiological environment of the primary afferent nerve terminal and for altering nociceptor excitability in the setting of tissue injury.

Nociceptin/Orphanin FQ (N/OFQ)

Nociceptin [100] or Orphanin FQ [101] (N/OFQ) is the endogenous ligand for orphan opioid receptor like-1 (ORL-1). N/OFQ and ORL-1 are widely distributed in the central nevous system (CNS). N/OFQ show significant similarities to dynorphin A in structure and distribution in CNS of rat (Figure 2.9) [102]

Figure 2.9 Sequence of the N/OFQ 1–17 peptide with other opioid peptides. Shaded areas represent homologous residues between N/OFQ 1–17 and other peptides (Tristan, 1998)

In the rat forebrain, N/OFQ peptide and mRNA are prominent in the neocortex, endopiriform nucleus, claustrum, lateral septum, ventral forebrain, hypothalamus, mammillary bodies, central and medial of the amygdale and hippocampal formation. In the brainstem, N/OFQ are prominent in the ventral tegmental area, substantial nigra, nucleus of the posterior commissure, central grey, vestibular nuclear complex, solitary nucleus, raphe complex, caudal spinal trigeminal nucleus, and reticular formation [102, 103]. In the spinal cord, N/OFQ mRNA and ORL-1 mRNA are expressed in the superfacial of dorsal horn (laminaeI and Π). Considerable similarity in the distribution of N/OFQ between rat and human CNS has been reported. In human, the concentrations of N/OFQ are demonstrated in the periaqueductal gray, the locus coeruleus, the ventromedial nucleus of hypothalamus, the septum, the dorsal horn of the spinal cord and spinal trigeminal nucleus [104]

Regarding trigeminovascular system, both N/OFQ and ORL-1 have been studied in the human trigeminal ganglionic cells. In 2003, Hou [105] first reported that about 70% of all neuronal cells in trigeminal glanglia express N/OFQ. Double immunostaining showed that N/OFQ in the human trigeminal glanglia colocaliz with calcitonin gene-related peptide (CGRP), substance P (SP), nitric oxide syntheses (NOS).

Above studies demonstrate that both N/OFQ and ORL-1 are expressed in high concentration in the trigeminal nociceptive system. Such localization reflects their physiological involvement in the mechanism of nociceptive control.

Role of N/OFQ in nociception

Several studies have shown that the action of N/OFQ in the nociceptive system. The nociceptive response to this peptide varies depending on the local of ORL-1. In an *in vitro* study using brain slice techniques, N/OFQ displays bidirectional effects on monosynaptic transmission with a potentiation at lower concentration (100-300mM) and a dose-dependent depression at higher concentrations (1-3mM). These responses do not depend on opioid receptor [106]. N/OFQ also attenuates the amplitude of glutamategic excitatory postsynaptic currents (EPSCs) which monosynaptically evoked by stimulating the A delta or C-fiber. This inhibitory effect is more prominent in unmyelinated (C-fiber).Such results indicate that N/OFQ suppresses excitatory but not inhibitory synaptic transmission [107]. This selective effect may underlie the anti-nociceptive property of N/OFQ.

In an *in vitro* study, N/OFQ (0.1-3nmol) dose-dependently inhibited the nociceptive behaviors as well as c-Fos protein expression in the superfacial dorsal horn induced by intrathecal administration of AMPA. The administration of the non-selective opioid receptor antagonist, naloxone (10mg/kg i.p.), do not modify the N/OFQ-induced inhibition of AMPA-evoked nociception [108]. The anti-nociceptive effect of N/OFQ is also supported by the observation in behavioral studies induced by intraplantar injection of SP. Several studies about the effect on trigeminal nociception indicated that N/OFQ plays an inhibitory role in the process of trigeminal nociception. Microiontophoretical or intracerebroventricular application of N/OFQ reduced the NMDA-evoked responses in 86% of TNC neurons, and the AMPA-evoked responses in 86% of neurons. Although this inhibitory effect is not specific to nociceptive stimulation, it is more pronounced on noxious stimulus-evoked responses than those evoked by non-nociceptive stimuli. These finding suggest that N/OFQ produces a predominantly inhibitory effect on noxious stimuli evoked responses as well as excitatory amino acid receptor-mediated transmission in TNC. Therefore N/OFQ

primarily produces an anti-nociceptive effect at the level of the dorsal horn of the medulla [109, 110].

More recent evidence demonstrates that the effect of N/OFQ on the trigeminal system can be both pronociceptive and anti-nociceptive effect. In the proestrous female rat, microiontophoretical application of N/OFQ onto TNC produces facilitation of the NMDA-evoked responses in 50% of nociceptive neurons, inhibition in 31% and biphasic effects in 19%. In contrast, in the female, N/OFQ inhibits the responses in 86%, and facilitated the responses in 14%. These finding may indicate that N/OFQ is primarily pronociceptive in the female and primary anti-nociceptive in the rat [111].

N/OFQ and primary headache

Plasma level of N/OFQ has been determined in two clinical headache syndrome namely migraine without aura and cluster headache. In migraine, the level of circulating N/OFQ is lower in headache-free migraine patients compared to that of the controls. N/OFQ level is reduced during the attacks [112], while it do not correlate with length of disease or episode length [113]. Lower level of N/OFQ is also observed in patients during the attacks of cluster headache, and may result in defective regulation of trigeminal activity to the headache attacks.

Although the effect of N/OFQ in the nociception and pain perception is not fully understood, accumulating evidences have indicated that N/OFQ may have a role anti-nociceptive effect. The mechanism underlying its anti-nociceptive effect may include inhibition of glutamate transmission, inhibition of cAMP formation, increase in K^+ conductance and decrease in Ca^{2+} conductance. In addition, several studies in migraine and cluster headache patients have shown that the level of N/OFQ in plasma is reduced. Low N/OFQ level during migraine attack and cluster headache period may have a defective regulation of trigeminal activity that may be important in the pain process of migraine and cluster headache. According to the spreading depression theory of migraine, a CSD-like event leads to the neurogenic inflammation, which excites unmyelinated sensory Aδ- and c-fibers that innervated to meninges on cortex. CSD in TG and TNC are altered with N/OFQ pretreatment. TRPV1-IR in TG and c-Fos expression in TNC are also altered.

c-Fos protein

The transmission and modulation of pain in the nervous system is complex. With the discovery in the mid-1970s of a series of oncogenes as metabolic markers of neuronal activation, mapping of the pathways and neuropeptides involved in pain transmission has been possible. Neurotransmitters, membrane electrical activity and neurotrophic growth factors are important modulators of neuronal gene expression. The first oncogenes, *FOS*, was identified and extensively used in multiple animal models of pain. Among these, and relevant to cephalic pain transmission is the activation of the trigeminovascular system, constituted by meningeal and superficial cortical blood vessels which contain sensory nerve fibers projecting mostly from the ophthalmic division of the trigeminal nerve. The immunocytochemical identification of c-Fos protein has been used as an indicator of activation of the nociceptive neurons [114].

C-fos expression can be induced within the trigeminal nucleus by applying different stimuli (mechanical, electrical or chemical) in either intra- or extracranial structures innervated by the trigeminal nerve. Of particular relevance to the study of migraine has been the observation that induction of spreading depression [115], an electrophysiologic wave of depolarization hypothesizes to underlie migraine aura, or stimulation of specific intracranial structures (meninges, trigeminal ganglion, superior sagittal sinus) induces c-Fos expression within cells in TNC. Cells in lamina I and II receive direct synaptic inputs from primary afferent fibers concerned with the transmission of nociceptive information from trigeminal receptive fields and second and higher order neurons which transmit nociceptive information to rostral centers [116].

Up-to-date, there is evidence that at least seven receptors (5-HT, NK-1, GABA, NMDA, AMPA, class III of metabotropic glutamate receptors, and opioids receptors) modulate c-Fos expression within TNC induced by activation of the trigeminovascular system. Drugs with clinically proven anti-migraine efficacy at 5-HT receptor, such as the triptans and the ergot alkaloids, inhibit c-Fos induction within TNC [117, 118]. Therefore, activation of the trigeminovascular system allows to study *in vivo* the molecular basis of cephalic pain and provides targets for the development of anti-migraine

The objective of this study

1. To study the effects of N/OFQ on cortical activity in CSD model.

2. To study the effects of N/OFQ on trigeminal nociceptive system in CSD model.