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Role of VPM thalamus in mechanical sensitivity and hyperalgesia

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SUMMARY

Many lines of evidence demonstrate that the thalamus plays an important role in gating and relaying pain sensation. However, the alteration of thalamic neuronal activity under a hyperalgesic state caused by peripheral or central tissue injury is less well understood. By using the topical application of capsaicin to produce hyperalgesia, electrophysiological studies in awake primates and psychophysical studies in both primates and humans were designed to investigate the role of VPM neurons in mechanical sensitivity and hyperalgesia.

Experiment 1: The first study tested a transient hyperalgesia model using capsaicin in awake monkeys performing an operant task and compared the results with the psychophysical data from human. One monkey and seven human subjects participated in this experiment. The monkey escaped more thermal and mechanical stimuli presented at the site of capsaicin application than stimuli presented at the site of vehicle application, suggesting of thermal and mechanical hyperalgesia. Human subjects reported higher pain intensity for similar stimuli after the application of capsaicin, in accordance with the monkey escape behavior. The procedure is repeatable and produces minor distress in monkey. Thus, it could provide a practicable method for studying neural mechanisms of hyperalgesia.

Experiment 2: The second study investigated the response characteristics of thalamic VPM neurons to punctate mechanical stimuli. Single-unit recording was performed within the thalamic nucleus in two awake behaving monkeys while applying innocuous and noxious mechanical stimuli to the receptive fields of the monkey's face. Thirty-seven neurons were studied in detail, nineteen of these were classified as low threshold (LT) in which the mechanically evoked activity between the innocuous and noxious level was not found to be significantly different. Eighteen neurons were classified as wide-dynamic-range (WDR), which showed graded responses maximally to noxious stimuli. Nociceptive-specific (NS) neurons were not found in this study. Both LT and WDR neurons demonstrated somatotopical distribution within the VPM nucleus. The size of receptive fields was not significantly different between two types of VPM neurons.

Experiment 3: The first and second studies illustrated an important animal model of hyperalgesia and demonstrated the response characteristics of VPM neurons to punctate mechanical stimuli, respectively. The third study further investigated the correlated VPM neuronal activity under hyperalgesia induced by capsaicin. Fifteen neurons (5 LT, 10 WDR) were fully characterized and evaluated for the effects of capsaicin. For the WDR cells, both spontaneous and mechanically evoked responses increased after capsaicin. In contrast, there was a non-significant decrease in both spontaneous and evoked

activity in LT neurons. These findings suggest that the VPM WDR but not the LT neurons could participate in neural processing underlying capsaicin-induced mechanical hyperalgesia. The decreased LT neuronal activity supports the hypothesis of the touch gate theory that low threshold somatosensory transmission can be suppressed in a state of pain or hyperalgesia. This observation is a corollary to the gate control theory statement that pain sensation could be suppressed by the activity of large fibers.

Experiment 4: Results of the third study showed that capsaicin increases spontaneous and mechanically evoked activity of WDR neurons of thalamic VPM nucleus in alert monkeys. However, neither the difference nor the ratio of mechanically evoked and spontaneous activity was altered significantly by capsaicin. Based on these findings, we predicted that these neuronal properties could result in an unaltered ability of human subjects to discriminate intensities of mechanical stimuli before and after the application of capsaicin. In this study, the effects of capsaicin on mechanical perception and discrimination were tested on the facial skin in eight human subjects. Ratings of mechanical sensation were increased after capsaicin in both innocuous (245mN) and near-noxious (882mN) baseline forces. The stronger baseline force (882mN) was found to induce mechanical hyperalgesia. In the discrimination test, two mechanical baseline forces (245 & 882mN) were each paired with two

incremental forces (ΔF) and forced-choice discrimination was tested. The results showed that mechanical discrimination was not altered by capsaicin. These results, combined with the electrophysiological findings in monkeys, suggest and show that : 1) thalamic WDR neurons participate in neural processes underlying capsaicin-induced pain sensation and mechanical hyperalgesia, 2) the difference and ratio between mechanically evoked and spontaneous activity does not alter significantly after the application of capsaicin, coincident with the unchanged discriminative ability in humans.

RÉSUMÉ

Plusieurs évidences existent démontrant l'importance du thalamus comme relais de la sensation de la douleur. Par contre, la modification de l'activité neuronale du thalamus lors d'états d'hyperalgésie causée par une lésion centrale ou périphérique est mal comprise. Nous avons réalisé des études électrophysiologiques chez le singe éveillé et des études psychophysiques chez le singe et l'humain lors de conditions d'hyperalgésie induite par l'application topique de capsaicine. Le but de notre projet était d'étudier le rôle des neurones du noyau ventro-postéro-médian (VPM) dans la sensibilité méchanique et l'hyperalgésie.

Expérience 1: Lors de la première étude, nous avons employé un modèle d'hyperalgésie transitoire créée par l'application topique de capsaicine chez le singe éveillé. Nous avons comparé les données du singe avec les données psychophysiques de l'humain. Un singe et sept humains ont participé à cette expérience. Le singe a évité plus souvent les stimuli thermiques et mécaniques au site d'appplication de la capsaicine qu'au site du véhicule suggèrant une hyperalgésie thermique et mécanique. Après l'application de capsaicine, les sujets humains ont rapporté des niveaux de douleur plus élevés pour des stimuli comparables appuyant ainsi les données comportementales obtenues chez le singe. Cette précédure est reproductible et n'entraîne pas de signe de détresse de la part du singe. Ainsi, ce modèle pourraît être une

méthode idéale pour l'étude des mécanismes impliqués dans l'hyperalgésie.

Expérience 2: La deuxième étude porte sur les caractéristiques de la réponse des neurones du thalamus VPM à des stimuli mécaniques. L'enregistrement unitaire dans le noyau thalamique a été réalisé chez deux singes éveillés. Pendant les enregistrements, des stimuli mécaniques indolores et douloureux sont appliqués au champ récepteur (CR) au niveau de la face du singe. Trente-sept neurones ont été enregistrés dont dix-neuf ont été classés comme mécanorécepteurs à seuil bas (SB). Chez ces derniers, l'activité évoquée par des stimulations mécaniques indolores n'est pas significativement diférente de celle évoquée par des stimuli douloureux. Dix-huit neurones ont été classifiés comme mécanonocicepteurs (MN) caractérisés par une réponse qui augmente graduellement avec l'intensité du stimulus. Dans cette étude, aucun nocicepteur spécifique (NS) n'a été trouvé. Autant les SB que les MN démontrent une distribution somatotopique à l'intérieur du noyau VPM. La superficie du CR n'était pas différente chez les deux types de neurones thalamiques.

Expérience 3: Les deux premières études ont révélé un modèle animal important d'hyperalgésie et ont démontré les caractéristiques des réponses des neurones du VPM à des stimuli mécaniques. L'étude présente approfondie la corrélation entre l'activité des neurones du VPM sous l'influence de l'hyperalgésie induite par la capsaicine. Quinze

neurones (5 SB, 10 MN) ont été entièrement caractérisés et évalués sous l'influence de la capsaicine.Pour les MN, l'activité spontanée et cell évoquée mécaniquement ont augmenté après l'application topique de capsaicin. Par contre, il y avait une diminution non-significative de l'activité spontanée et de l'activité évoquée mécaniquement chez les neurones SB. Ces résultats suggèrent que les MN mais non les SB du VPM pourraient participer dans le processus neuronal sous-jacent à l'hyperalgésie mécanique induite par l'application de capsaicine. La diminution de l'activité neuronale observée chez les SB sopportent l'hypothèse de la théorie du portillon voulant que la transmission somatosensorielle à seuil bas puisse être supprimée dans les états douloureux ou d'hyperalgésie.

Expérience 4: Dans la troisième expérience, nous avons démontré que la capsaicine augmente l'activité spontanée (AS) et l'activité évoquée mécaniquement (EMv) chez les neurones MN du noyau VPM thalamique chez le singe éveillé. Par contre, ni la différence (EMv moins AS) ni le ratio (EMv divisé par l'AS) de l'Emv et l'AS n'ont été modifiés par la capsaicine. D'après ces résultats, nous prévoyons que ces décharges neuronales inchangées pourraient entraîner une habilité inchangée chez des sujets humains à discriminer des intensités différentes de stimulations mécaniques avant et après application de capsaicine. Dans cette étude, les effets de la capsaicine sur la perception et la discrimination mécanique ont été testés au niveau du visage de huit

sujets humains. La sensation mécanique induite par la capsaicine a été augmentée pour les stimuli mécaniques indolores (245 mN) et aussi pour les stimuli pratiquement douloureux (882 mN). La force la plus élévée (882 mN) a produit une hyperalgésie. Dans le test de discrimination, les deux forces mécaniques (245 et 882 mN) ont été couplées avec deux forces plus élevées ($\triangle$F) et un test de choix forcé a été utilisé. Les résultats démontrent que la capacité de discrimination mécanique n'est pas modifiée par l'application de capsaicine. Ces résultats, combinés avec les données électrophysiologiques chez le singe suggèrent et démontrent que: 1) Les neurones MN du thalamus participent dans le processus sous-jacent à la douleur et l'hyperalgésie induite par la capsaicine. 2) l'augmentation de l'activité EMv-S et Emv/AS évoquées par les stimuli mécaniques et qui ne démontrent pas de différence après l'application de capsaicine coïncide avec l'habilité de discrimination inchangée observée chez l'humain.

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LIST OF ABBREVIATIONS

AMH	A- mechano-heat fiber
CL	Central lateral nucleus
CMH	C- mechano-heat fiber
CNS	Central nervous system
D	Difference of activity between MEv and S
DCML	Dorsal-column-medial-lemniscal pathway
LT	Low -threshold
MEv	Mechanically evoked activity
MIA	Mechanically insensitive afferents
NS	Nociceptive-specific
Pf	parafascicular
PNS	Peripheral nervous system
Pom	Medial part of the posterior thalamic complex
RA	Rapidly adapting
RF	Receptive fields
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
SA	Slowly adapting
SMT	Spinomesencephalic tract
STT	Spinothalamic tract
VB	Ventrobasal complex

Vmpo	Posterior part of ventral medial nucleus
VPI	Ventral posterior inferior nucleus
VPL	Ventral posterior lateral nucleus
VPM	Ventral posterior medial nucleus
WDR	Wide-dynamic-range

PREFACE

Although many important advances that have been achieved in the understanding of peripheral and spinal processing of somatosensory and pain information, thalamic mechanisms are less well known. This is particularly important because the thalamus not only serves as a relaying center, but also plays a role in the integration and gating of the convergent information. Further understanding of thalamic mechanisms is also an important step towards the treatment of chronic pain and hyperalgesia or allodynia which occurs in patients with central pain syndrome.

This research project examines the response of thalamic VPM neurons to punctate mechanical stimulation under normal and hyperalgesic states and compares these to behavioral and psychophysical data in monkeys and humans. The thesis consists of a general literature review concerning thalamic sensory processing, mechanisms of hyperalgesia and experimental pain models, four experimental chapters and a chapter of general discussion and conclusion.

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Part I. LITERATURE REVIEW

Chapter 1. Thalamus and the somatosensory processing

1.1 General Introduction of the thalamus

The thalamus is the major component of the diencephalon, bounded by the third ventricle, the hypothalamic sulcus, and the stratum zonale. In primates, the thalamus is oriented obliquely with the anterior poles towards the midline and the posterior poles laterally. The thalamus is made of many nuclei, some of which are very small and some primary functions are still not well known. The nomenclature of thalamic nuclei has not been consistent. For example, in describing homologous structures shared by humans and other primates, the nomenclature of motor-related thalamic nuclei has been used inconsistently among different groups of investigators and clinicians (Macchi and Jones, 1997; Percheron et al., 1996). In order to transfer our knowledge of the thalamus, which has mostly been derived from animal studies, to the human thalamus, it is important to use a common nomenclature.

All sensory impulses except those related to olfaction terminate in the nuclei of the thalamus. For example, the thalamus receives inputs related to audition, vision, touch, temperature and pain, and from there this sensory information is relayed to the related cortical regions and is perceived as a particular modality of somato-sensation in humans. Much evidence now indicates that the thalamus is not just a passive relay site, but also plays an important role in sensory gating that can be influenced

by the state of arousal (Casey, 1966; Coull, 1998; Morrow and Casey, 1992; Steriade et al., 1993).

In addition to processing sensory information, the thalamus also mediates motor functions by transmitting information from the cerebellum and basal ganglia to the motor regions of the frontal lobe (Darian-Smith, 1996; Macchi and Jones, 1997; Percheron et al., 1996). Moreover, the thalamus is also involved in autonomic reactions and the maintenance of consciousness (Delacour, 1997; Jasper, 1998; Tononi and Edelman, 1998). As well, because of the close relationship of the thalamus and cerebral cortex, the role of the thalamus in neuropsychiatric illness has also been investigated (Jones, 1997; Scheibel, 1997). Jones (1997) proposed that loss of certain thalamic neurons and related corticothalamic inputs could lead to the disintegration of thought processes in schizophrenic patients.

Traditionally, thalamic nuclei are classified into two functional groups: relay nuclei and diffuse-projection nuclei. Relay nuclei process a single sensory modality or relay distinct motor afferents, as well as project to and receive the reciprocal projections from specific regions of the cerebral cortex. These reciprocal connections presumably allow the cortex to modulate the input that it has received. Diffuse-projection nuclei have more widespread connections than do the relay nuclei, and are regarded as part of the system that governs the state of arousal.

Anatomically, the thalamus is divided into six groups of nuclei by the Y-shaped white matter of the internal medullary lamina, and these groups are: the lateral, medial, anterior, intralaminar, midline and reticular nuclei. The medial nuclei are relay nuclei, and are thought to be involved in limbic functions and affective-motivational aspect of pain (Albe-Fessard et al., 1985). The anterior nuclei may participate in emotion by relaying information from the hypothalamus to the cingulate cortex (Vogt, 1985). The intralaminar, reticular and midline nuclei are diffuse-projection nuclei, that is, they perform certain functions with the limbic system and modulate the thalamic activity. The largest among the intralaminar nuclei is the centromedian nucleus. The reticular nucleus is the only thalamic nucleus with an inhibitory output.

The lateral thalamus, which is involved in sensorimotor functions, is probably the most studied thalamic subgroup. Nuclei of the lateral thalamus receive restricted sensory or motor inputs from the periphery and the cerebellum respectively, and basal ganglion and relay to and receive projections from a specific cortical region. The lateral thalamic nuclei are relay nuclei that are further divided into the ventral and dorsal tiers. Within the ventral tier, the ventral anterior and ventral lateral nuclei play an important role in motor control, while the ventral posterior nucleus is particularly important for somatic sensation. The medial geniculate nucleus mediates information about hearing, and the lateral geniculate nucleus mediates information about vision. The dorsal tier, including the

lateral dorsal, lateral posterior and pulvinar nuclei, might work together to integrate sensory information and emotional expression.

Functional grouping of the lateral thalamus has been investigated by electrophysiological recordings of single neuron responses (Asanuma et al., 1985; Chung et al., 1986a,b; Jones, 1985; Macchi et al., 1986). The largest somatic sensory relay nucleus in the lateral thalamus is referred to as the ventrobasal complex. This nuclear complex is composed of two main portions: the ventral posterolateral nucleus (VPL), which receives somatic sensory inputs from the body; and the ventral posteromedial nucleus (VPM), which receives inputs from the facial areas. Neurons in VPL and VPM encode the timing, intensity and location of innocuous cutaneous mechanoreceptive stimuli from the dorsal-column-medial-lemniscal pathway (DCML) as well as the noxious and innocuous information from the spinothalamic (STT) pathway (Berkley, 1980; Bushnell et al., 1993; Bushnell and Craig, 1993; Bushnell and Duncan, 1987; Casey and Morrow, 1983; Casey, 1966; Chung et al., 1986a; Jones, 1990; Jones and Friedman, 1982; Jones et al., 1982; Kenshalo, et al., 1980; Morrow and Casey, 1992; Poggio and Mountcastle, 1960; 1963; Tremblay et al., 1993).

1.2 Ascending somatosensory pathways

The main subthalamic somatosensory inputs are conveyed from the spinal cord and trigeminal nuclei. There are two parallel ascending

pathways. In general, the DCML pathway transmits proprioceptive and tactile information from each type of mechanoreceptor afferents, such as Meissner, Merkel, and Pacinian (receptors or endings), while the trigemino- and spinothalamic tracts (STT) transmit thermoreceptive, nociceptive and mechanoreceptive information (Jones and Friedman, 1982; Jones et al., 1982, Poggio and Mountcastle, 1963; Willis and Coggeshall, 1991). In addition to the STT, several other pathways also transmit nociceptive information indirectly to the thalamus. These include the post-synaptic dorsal column, spinoreticular, spinomesencephalic and spinocervical tracts (Willis and Westlund, 1997; Willis and Coggeshall, 1991; Willis, 1985a,b).

1.2.1 Lemniscal Inputs

This pathway relays information about tactile and limb proprioception, which originates from larger diameter primary afferents and ascends ipsilaterally to the dorsal column nuclei. From the dorsal column (cuneate and gracile) nuclei, the tract decussates and projects to the thalamus as the medial lemniscus. The medial lemniscal input to thalamus conveys information from the contralateral dorsal column nuclei, principal trigeminal nucleus, and lateral cervical nucleus. This input enters into the thalamus and terminates particularly in the VP regions.

The cuneate and gracile nuclei project to the contralateral VPL in an organized manner that follows an overall somatotopic organization. They

receive the ascending fibers which have the cutaneous receptive fields and project to the central core of VPL (Berkley and Hand, 1978; Groenewegen et al., 1975; Hand and Van Winkle, 1977). The ventrocaudal region of the dorsal column nuclei, which responds to deeper stimuli, projects in a diffuse manner to the dorsal parts of VPL (Hand and Van Winkle, 1977).

Terminations in VPM come from the tactile trigeminal projections arising from the ventral two-thirds of the principal nucleus that join and ascend together with the medial lemniscus after decussating in the rostral pons. This pathway has been demonstrated in rats, cats and monkeys by electrophysiological and immunohistological studies (Burton and Craig, 1979a,b; Mizuno, 1970; Smith, 1975). An ipsilateral trigeminothalamic pathway from the principal trigeminal nucleus has also been reported (Smith, 1975). The ipsilateral trigeminal pathway ascends with the crossed trigeminal fibers in the medial lemniscus and terminates in VPM, dorsomedial to the part receiving the crossed fibers in monkeys (Smith, 1975). This observation is also confirmed in studies with cats using retrograde labeling techniques. However, studies using rats failed to report the same observation, thus there appears to be a significant species difference in this pathway.

In addition to conveying information about innocuous tactile stimuli from large primary afferents, the dorsal column also contains a system of postsynaptic fibres carrying nociceptive information (Bannatyne et al.,

1987; Bennet et al., 1983; Cliffer and Willis, 1994; Enevoldson and Gordon, 1989; Giesler and Cliffer, 1985). These post-synaptic-dorsal column (PSDC) fibers make synapses in the spinal dorsal horn and travel along the dorsal column. Neurons of dorsal column nuclei responding to noxious distension of the uterus and vagina in animals have been reported by some investigators (Berkley and Hubscher, 1995; Cliffer et al., 1992; Ferrington et al., 1988). Furthermore, the effectiveness of limited median myelotomy in reducing cancer pain in humans has also been demonstrated (Gildenberg and Hirshberg, 1984; Hirshberg et al., 1996). These findings provide evidence that the DCML also participates in transmitting nociceptive information, especially in relaying visceral pain (Al-Chaer et al., 1996a,b,1995; Hirshberg et al, 1996).

1.2.2 Spinothalamic Inputs

The spinothalamic tract is the main ascending pathway conveying sensory information about pain and temperature to the thalamus (Gybels and Sweet, 1989; Willis and Coggeshall, 1991; Willis, 1985a,b); it is also capable of transmitting tactile information. This observation has been confirmed by experimental spinal lesions in animal studies and clinical findings in humans (Vierck et al., 1990; Vierck and Luck, 1979).

The origin of the spinothalamic tract has been investigated in rats, cats, and monkeys (Willis and Coggeshall, 1991). The spinal neurons projecting to VPL are concentrated in certain laminae. In cats, they are

located in VI-VIII; in monkeys, larger fractions of STT neurons are located in the marginal zone (lamina I) and lamina V (Apkarian and Hodge, 1989b; Craig et al., 1989; Willis et al., 1979). Neurons that project to the medial thalamus are situated in the deeper dorsal horn and ventral horn (lamina VI-VIII) (Willis et al., 1979). Most of these projections are contralateral, although a small fraction projects ipsilaterally (Apkarian and Hodge, 1989a). According to the characteristics of responses evoked by peripheral stimulation, STT neurons have been classified as either nociceptive-specific (NS), wide-dynamic-range (WDR) or low-threshold (LT). NS neurons are specially concentrated in lamina I (Willis et al., 1974), WDR neurons are concentrated in lamina V (Chung et al., 1979; Kenshalo et al., 1979; Price et al., 1978), and LT neurons are found in all layers.

Projections of the spinothalamic tract have been traced to the thalamus in both human and non-human primates (Willis and Coggeshall, 1991). The spinothalamic afferents from the contralateral side of the spinal cord terminate throughout VPL; the fibers arising in the nucleus caudalis of the spinal trigeminal complex terminate in VPM of cats and monkeys (Burton et al., 1979). Medial lemniscal and spinothalamic axons originating from the same spinal segments are closely associated in VPL (Apkarian and Hodge, 1989a,b; Berkley, 1980,1983; Boivie,1979). Some investigators suggest that single VPL neurons may receive inputs from both medial lemniscal and spinothalamic axons (Ralston and Ralston,

1994). Using electron microscopic methods they have shown the convergence of medial lemniscal and spinothalamic tract input onto the proximal dendritic trees of thalamocortical neurons. This would account for the observation that thalamic neurons respond to input conveyed through both the dorsal column and the lateral funiculus (Al-Chaer et al., 1996a).

In additon to terminating in VPL, the STT also terminates in the following nuclei: the medial part of the posterior complex (Pom), the central lateral (CL) nucleus, and other intralaminalr and medial thalamic nuclei (Apkarian and Hodge, 1989c; Apkarian and Shi, 1994; Berkley, 1980; Boivie, 1979; Gingold et al.,1991; Kerr, 1975; Mantyh, 1983). By injection of the anterograde tracer PHA-L in the cervical or lumbosacral enlargement in cats and monkeys, the projection of laminae I has been traced to the posterior part of the ventral medial nucleus (VMpo) (Craig et al., 1994; Craig, 1992, 1991). The VMpo neurons were found to respond particularly to noxious and cold stimuli in anesthetized monkeys (Bushnell and Craig, 1993; Craig et al., 1994).

1.2.3 Spinoreticular tract

Neurons of the spinoreticular tract originate in the deeper layers of the dorsal horn and in laminae VII and VIII (Willis and Coggeshall, 1991). Recently, an additional source of spinoreticular tract neurons was also described from lamina I in cats and monkeys (Craig, 1995). In general,

the spinoreticular and spinothalamic tracts ascend together in the ventrolateral spinal cord and brainstem (Mehler et al., 1960). Some spinoreticular neurons are the collateral branches from spinothalamic tract cells (Giesler et al., 1981; Haber et al., 1982; Kevetter and Willis, 1983). The spinoreticular projections terminate in several nuclei at different levels between the medulla and mesencephalon, including the retroambiggus, superspinalis nucleus, lateral reticular nucleus, the nucleus gigantocellularis, the nucleus paragigantocellularis dorsalis and lateralis (Kerr, 1975; Mehler et al., 1960).

From the nucleus gigantocellularis, the spinoreticular tract projects to the central median and parafasicularis nuclei of medial thalamus. Since many reticular neurons respond preferentially to noxious stimuli (Casey, 1969; 1971; Foote et al., 1991; Guilbaud et al., 1973), the spinoreticular tract may convey nociceptive information and be involved in the affective-motivational component of pain (Peschanski and Besson, 1984).

1.2.4 Spinomesencephalic tract

Another indirect pathway that transmits nociceptive information to the thalamus is the spinomesencephalic tract (SMT). In primates, the cells of origin of the SMT are located in laminae I and IV-VI, while some are in the ventral horn and lamina X (Mantyh, 1982; Wiberg et al., 1987; Willis et al., 1979). The SMT neurons respond either exclusively to noxious stimuli or in a graded fashion to stimuli in the innocuous to

noxious range, and thus are said to be nociceptive (Willis and Coggeshall, 1991). The SMT projects to periaqueductal gray matter (PAG), deep layers of superior colliculus, the parabrachial nucleus, anterior and posterior pretectal nuclei, and the red nucleus. Some SMT neurons have collateral projections that terminate in the lateral thalamus (Yezierski et al., 1987; Zhang et al., 1990).

1.2.5 Spinocervical tract

The spinocervical tract originates from lamina IV and ascends to the lateral cervical nucleus in segments C1 and C2 in both cats and monkeys (Brown et al., 1980; Bryan et al., 1974; Craig, 1978; Willis and Coggeshall, 1991). From the lateral cervical nucleus, axons of the spinocervical tract then cross the midline at the spinobulbar junction and join the medial lemniscus to the contralateral VPL, VPM, and medial parts of the posterior complex (Augustin, 1985; Berkley, 1980; Dykes et al., 1981). Since nociceptive responsiveness of the neurons of the spinocervical tract has been demonstrated in cats (Cervero et al., 1977) and monkeys (Bryan et al., 1974; Downie et al., 1988), this tract is therefore a potential nociceptive pathway to the lateral thalamus. However, the comparable nucleus and functional significance is still unclear in humans.

1.2.6 Trigeminal sensory system

Somatosensory information from the face and oral cavity is principally conveyed by the trigeminal or fifth cranial nerve. This trigeminal innervation consists of three peripheral branches: the

ophthalmic, the maxillary, and the mandibular. The ophthalmic division innervates the superior third of the face and a part of the dura mater. The maxillary division innervates upper part of the oral cavity and cutaneous structures of the middle third of the face. The lower part of the oral cavity and inferior third of the face are innervated by the mandibular branch. The trigeminal nerve is functionally equivalent to a peripheral spinal nerve, in which the ophthalmic and maxillary branches are pure sensory nerves while the mandibular division carries both sensory and motor fibers.

Trigeminal sensory afferents synapse on the second-order cells located in the principal sensory trigeminal nucleus and the spinal trigeminal nucleus. Most of the large-diameter fibers that carry tactile information from the face travel to ipsilateral principal sensory nucleus. From there, these fibers decussate through the trigeminal lemniscus and join the spinal DCML, which in turn projects to the contralateral VPM nucleus of the thalamus. In addition, some afferent fibers conveying tactile information project to spinal trigeminal nucleus and proceed within the dorsal columns to the posterior nucleus of the thalamus.

Thinly myelinated and unmyelinated trigeminal afferents that carry information of pain and temperature terminate in the spinal trigeminal nucleus. According to Olszewski (1950), spinal trigeminal nucleus in human and monkey consists of three subnuclei: the nucleus caudalis,

nucleus interpolaris, and nucleus oralis. The nucleus caudalis is continuous with the dorsal horn of the cervical spinal cord and possesses similar anatomical structure. Gobel et al., (1977), therefore, introduced the term "medullary dorsal horn" to emphasize this similarity and suggested eight-layered nomenclature similar to Rexed's laminar subdivisions of spinal gray matter.

Due to the clinical findings that trigeminal tractotomy (Sjöqvist operation) could alleviate pain sensation and abolish the temperature sensibility in the orofacial region, the nucleus caudalis is considered to be the major component of the trigeminal nuclear system concerned with nociception. This hypothesis was supported by Price et al., (1976,1978) in studies on the anesthetized monkeys. They showed that orofacial nociceptive input is encoded by the WDR and NS neurons of nucleus caudalis. In awake monkeys, Hoffman et al., (1981) described the properties of nociceptive neurons that transmit information about noxious heat stimuli. They examined the neuronal properties of medullary dorsal horn while monkeys were performing the discrimination task of innocuous (37-43°C) and noxious (45-49°C) thermal stimuli applied to the face. They found that these neurons have restricted receptive fields and exhibit stimulus-response functions similar to those found in the anesthetized monkeys. The magnitude and latency of neuronal responses suggested that both WDR and NS neurons code sensory-discriminative information about noxious heat stimulation. Further investigation by the same group

indicates that the behavioral context (such as, warning signal preceding noxious stimuli) can modulate the responses of these nociceptive neurons (Hayes et al., 1981).

Based on the psychophysical findings that humans and monkeys can detect small increases in noxious heat applied to the face (Bushnell et al., 1983; Handerwerker et al., 1982; Kenshalo et al., 1989; Robison et al., 1983), the relationship between the activity of medullary dorsal horn nociceptive neurons and the ability to detect noxious heat stimuli was investigated in awake monkeys (Dubner et al., 1989). They demonstrated that a subgroup of WDR neurons showed greater discharge frequency on correctly detected trials. These findings indicate that nociceptive neurons of medullary dorsal horn have the capacity to precisely encode stimulus features in the noxious range; and that a subpopulation of WDR neurons is likely to participate in the encoding process by which monkeys perceive the intensity of such stimuli (Maixner et al., 1989).

1.3 Somatosensory processing in the lateral thalamus

Electrophysiological studies of the thalamic ventral posterior nucleus in animals demonstrate that the somatosensory information from the contralateral limbs and trunks is transmitted to the ventrobasal complex (VB) (Dykes, et al., 1982; Jones and Friedman, 1982; Jones et al., 1982; Mountcastle and Henneman, 1952; Poggio and Mountcastle, 1963, 1960,). Many studies also have shown that most of these VB

neurons are modality specific (Dykes, 1983; Dykes et al., 1981; Jones and Friedman, 1982; Jones et al., 1982; Loe et al., 1977; Poggio and Mountcastle, 1963; Rose and Mountcastle, 1954; Welker and Johnson, 1965). They receive different modalities of somatosensory inputs from the spinal cord, including cutaneous tactile, subcutaneous pressure, noxious and thermal (Jones, 1985).

1.3.1 Tactile or hair movement

A major part of the VP nucleus responds to cutaneous tactile stimulation. These neurons respond to hair movement and light stroking of the skin with modality and location specificity, which are the characteristics of lemniscal inputs (Poggio and Mountcastle, 1963, 1960). The receptive fields are usually small and localized in contralateral limbs and trunk. Neurons in VPL responding to deflection of body hairs have rapidly adapting discharges and no direction selectivity (Golovchinsky et al., 1981; Gordon and Manson, 1967). On the other hand, neurons that respond to light touch are found to have either slowly or rapidly adapting discharges. Neurons that respond to hair movement on the face are well represented in VPM. In addition, many neurons in VPM receive inputs from the mucosa of the lips, tongue, and mouth. The receptive fields are small and localized, and their responses are usually rapidly adapting. On the nose, lip, tongue and palate close to midline, the receptive fields are sometimes bilateral (Poggio and Mountcastle, 1963). Hayward (1975)

described the different types of discharge patterns to stimulation of several types of facial hair in unanesthetized monkeys' VPM. He demonstrated that neurons responding to movement of common hairs are rapidly adapting cells situated in the dorsal half of the VPM, and have slightly larger receptive fields than units responding to movements of the circumoral vibrissae or of long facial whiskers, which were situated in the ventral half of VPM. In addition, it was shown that circumoral vibrissa have slowly or rapidly adapting discharges, while facial whisker units have only slowly adapting discharges along with direction selectivity. (Cited from: Jones, 1985).

1.3.2 Light pressure

The pressure-sensitive receptors usually are located in the dermis, muscle, and joints and can be activated by different degrees of pressure. Therefore, the thalamic neurons may receive inputs from subcutaneous and deeper tissues as well, making it difficult to define the thalamic pressure-sensitive neurons.

In VPL, neurons which can be activated by a maintained light pressure have their receptive fields located in either hairy or glabrous skin (Gordon and Manson, 1967, Tsumoto, 1974). These neurons can be classified further as either slowly (SA) or rapidly adapting (RA). Rapidly adapting, light pressure-sensitive neurons of VPL probably receive their inputs from the Meissner receptors in glabrous skin (Gordon and Manson,

1967). Other rapidly adapting VPL neurons might receive inputs from Pacinian corpuscles. Dykes et al., (1981) described multi-unit responses in ventral posterior inferior (VPI) of squirrel monkeys when tapping the wrist and attributed these to Pacinian inputs. Slowly adapting VPL light-pressure-sensitive neurons that respond to the stimulation of glabrous skin are mediated by inputs from Merkel receptors (Iggo and Ogawa, 1977; Janig et al., 1968).

In VPM, some neurons respond to tapping of the teeth, usually with RA characteristics, and have small receptive fields confined to one or more teeth (Bombardieri et al., 1975; Poggio and Mountcastle, 1963). The teeth of the mandible and maxillae are somatotopically represented in VPM (Lisney, 1978).

1.3.3 Nociceptive information

The spinothalamic tract transmits nociceptive and thermal information from the contralateral side of the spinal cord to lateral, medial or posterior regions of thalamus, including the VPL, VPM, VPI, and POm nuclei in the lateral thalamus, CL in the medial thalamus and posterior part of ventral medial nucleus (VMpo). The postsynaptic dorsal column pathway projects from the dorsal column nuclei to the VPL and POm nuclei. Some of the brainstem neurons receiving inputs from the spinoreticular and spinomesencephalic pathways project to the central median and parafascularis nuclei of medial thalamus. The spinocervical tracts relay from the lateral cervical nucleus to the VPL and POm nuclei.

The most extensively studied thalamic nuclei receiving projection from ascending nociceptive pathways are those in the VP region (Applebaum et al., 1979; Carstens and Trevino, 1978; Kerr, 1975; Mehler et al., 1960; Perl and Whitlock, 1961; Willis et al., 1979). This area is particularly interesting due to its proposed specific role concerning the sensation of pain and temperature. Single-unit recordings of nociceptive responses have been investigated in the VP thalamus of monkey by many investigators (Apkarian and Shi, 1994; Bruggemann et al., 1994; Bushnell and Duncan, 1987; Bushnell et al., 1993; Casey and Morrow, 1987, 1983; Chandler et al., 1992; Chung et al., 1986a,b; Duncan et al., 1993; Kenshalo et al., 1980; Morrow and Casey, 1992; Tremblay et al., 1993; Yokota et al., 1988). However, there is no consensus about the proportion of VP neurons that are nociceptive. Some investigators report that only a few neurons can be activated by noxious stimuli (Casey, 1966; Kenshalo et al., 1980; Poggio and Mountcastle, 1963; 1960), others describe significant populations (Chung et al., 1986a,b; Yokota et al., 1988, 1985), while Chung et al (1986a,b) reported a large proportion of nociceptive neurons in VPL of anesthetized monkeys. This difference may result from the possible application of more intensive stimuli in the anesthetized animal or from sensitization of nociceptive neurons by repeated noxious stimuli.

Although both NS and WDR neurons have been reported in the primate lateral thalamus, most investigators report a predominant

proportion of WDR neurons in this region of the thalamus (Bushnell and Duncan, 1987; Casey and Morrow, 1987,1983; Chung et al., 1986a,b; Kenshalo et al., 1980). The WDR neurons of VP nuclei respond to innocuous mechanical stimulation, and show maximal or preferential response to noxious mechanical and sometimes noxious thermal stimulation. These neurons appear to be scattered among those responding to innocuous mechanical stimuli. They generally have small contralateral receptive fields and are somatotopically organized, capable of encoding the intensity of the stimuli (Bushnell et al., 1993; Bushnell and Duncan, 1987; Casey and Morrow, 1983; Morrow and Casey, 1992; Tremblay et al., 1993). The receptive field and response properties of these neurons in the lateral thalamus suggest that they play an important role in sensory-discriminative aspects of pain perception.

Recordings of neuronal activity have also been studied in the human VP thalamus (Lenz et al., 1994b; 1993a,b). Lenz and his colleagues reported that stimulation in VPL often evoked pain and even could induce anginal pain (in a patient with a history of angina pectoris) without the accompanying cardiovascular changes (Lenz et al., 1994a). These findings imply that VPL may also be involved in visceral and referred pain.

As mentioned above, in addition to the VPL and VPM nuclei, noxious information is also relayed to other thalamic nuclei. These nuclei include VPI of lateral thalamus (Apkarian and Shi, 1994; Morrow and

Casey, 1992), VMpo of posterior thalamus (Craig et al., 1995,1994), and the parafascicular (Pf), centrolateral, medial dorsal and central median nuclei of the medial thalamus (Bushnell and Duncan, 1989; Casey, 1966; Dong et al., 1978; Dostrovsky and Guilbaud, 1988; Kawakita et al., 1993). Other findings from Craig et al. (1994) demonstrated that the neurons in VMpo nucleus in monkeys respond preferentially to noxious and cold stimuli. Studies have shown that these neurons project to the insula, suggesting its role in the motivational-affective aspect of pain sensation.

Human studies involving electrical stimulation of discrete thalamic sites indicate functional differences between the medial thalamus and the ventrocaudal (corresponding to the VPLc in monkeys) nucleus in their contributions to pain mechanisms. Studies of Hassler (1982,1970b) support the hypothesis that the VcPc nucleus in humans is involved in the localization and discrimination of the sensory aspects of pain. He found that stimulation of this area in conscious humans resulted in localized pain. Other investigators confirmed this result and reported that stimulation in nucleus ventrocaudalis parvocellularis resulted in well-localized, unpleasant burning or sharp painful sensation (Davis et al., 1996; Dostrovsky et al., 1992; Halliday and Logue, 1972; Lenz et al., 1993a.b, 1990a,b).

The effects of thalamic lesions or electrical stimulation to relieve pain have been examined in human clinical treatments and studies. Lesions that are directed toward the entire VB complex show poor results

in pain relief and are accompanied by the undesirable side effect of mechanoreceptive deficit (Sano et al., 1966; White and Sweet, 1969). This deficit did not occur when the lesion was limited to the caudal area, which is approximately equivalent to VPLc in primates. Temporary reduction in the perception of acute pain was noted in ventrocaudal lesions. Better and longer relief was noted in the lesions of VPM for facial pain, possibly because such lesions may also interrupt the projection to the intralaminar thalamic nuclei (Mundinger and Becker; 1977; White and Sweet, 1969)

In addition, the excitability of the thalamus is controlled by the brain stem, and relates to arousal and conscious states. Morrow and Casey (1992) reported that thalamic somatosensory responses in awake monkeys could be modulated by the state of arousal. Thus, thalamic functions should include the relay function and the state-dependent function.

The thalamus serves as a passageway for somatosensory information to the cerebral cortex. A model of organization of thalamocortical projections to the sensory cortex shows thalamic nuclei with unique subthalamic inputs that in turn project to a particular region of the cortex (Darian-Smith and Darian-Smith, 1993; Darian-Smith et al., 1990; Jones, 1985). Antidromic stimulation showed that the VPL and VPM neurons project to the SI cortex (Dong and Chudler, 1995; Kenshalo et al., 1980). Double-labeling studies demonstrated that WDR neurons in

VPL project to SI (Gingold et al., 1991) while most NS neurons in VPI project to SII (Stevens et al., 1993). These findings provide the evidence that the lateral thalamus mediates the sensory-discriminative aspect of pain perception. Evidence is now accumulating that MDvc and Pf project to anterior cingulate cortex (Musil and Olson, 1988; Vogt et al., 1987) and that VMpo projects to the insular cortex (Craig, 1994; Craig et al., 1994), implicating that in pain processing, MDvc/Pf and VMpo are important for emotional or behavioral reaction to pain.

Chapter 2 Hyperalgesia and Allodynia

2.1 Introduction

Hyperalgesia is defined as an increased response to painful thermal or mechanical stimuli as a result of tissue injury and inflammation (Merskey, 1986). Most definitions of hyperalgesia include allodynia, pain which is caused by a stimulus that does not normally provoke pain. Sensitization is defined as an increase in neuronal responsiveness after tissue damage and underlies hyperalgesia and allodynia. Hyperalgesia and allodynia can result following peripheral tissue injury or damage to the peripheral and central nervous systems (Bonica, 1979; Sunderland, 1978). Hyperalgesia can occur to both heat and mechanical stimulation. This phenomenon was described by Déjérine and Roussy (1906) in the patients with thalamic lesions. Déjérine and the subsequent researchers, Head and Holmes (1911), and Riddoch (1938) described "central pain", which included spontaneous pain and hyperalgesia following various lesions of the spinal cord, brain stem and brain. Similar alteration in sensitivity was also reported in patients with herpers zoster (Bauman, 1979), or associated with bone injury (Houghton et al., 1997). Clinically, hyperalgesia is characterized by a decrease in pain threshold and an increase in pain perception, and is often accompanied by a spontaneous pain sensation. Hyperalgesia seems to serve as a protective function to prevent further injury and therefore may ultimately promote healing.

Primary and secondary hyperalgesia

Two distinct types of hyperalgesia were described earlier this century. The first one is primary hyperalgesia, which occurs at the site of the injury. The other one is secondary hyperlagesia, and it occurs at the surrounding or remote undamaged site (Hardy et al., 1950; Lewis, 1935). Primary and secondary hyperalgesia differ in their sensory characteristics and probably in underlying mechanisms. Primary hyperalgesia is characterized by increased pain sensations to heat and mechanical stimuli (Lewis, 1935, 1942; Hardy, 1953). On the other hand, it is believed that secondary hyperalgesia is restricted to mechanical stimuli but not heat (Ali et al., 1996; Dahl et al., 1993; Raja et al., 1984). For instance, in one experiment, cutaneous injury produced by 53°C heat application on human glabrous skin induced hyperalgesia to mechanical stimuli in both injured and undamaged areas; however hyperalgesia to heat stimuli was found only in the primary injured site. Similar results were observed by using capsaicin and mustard oil in humans and animal studies (Ali et al., 1996). This dissociation of mechanical and heat hyperalgesia in the primary and secondary areas suggests that the neural mechanisms for these altered sensations might be different.

However, some investigators report that heat hyperalgesia also occurs in the secondary area when induced by capsaicin (Arendt-Nielsen et al., 1996; Wallace et al., 1997) or heat burn (Pederson and Kehlet, 1998). They argue that the absence of heat hyperalgesia in the secondary

area seen in earlier studies may be attributed to the small size of the stimulator and insufficient stimulus intensity.

Different experimental models such as thermal heat and various chemical substances have been used to induce hyperalgesia. Due to its accessibility, most investigations of hyperalgesia were focused on the cutaneous tissue. The characteristics of cutaneous hyperalgesia have been intensively studied with heat injury and the application of capsaicin. Using the model of heat burn, the stimulus-response function for heat pain applied to the injured site is shifted to the left, indicating a decrease in pain threshold and increased pain sensation to suprathreshold stimuli (Lynn, 1977; Raja et al., 1984). However, hyperalgesia to heat was not observed in the undamaged skin. In contrast to heat hyperalgesia, mechanical hyperlagesia was found in both primary and secondary areas. Similar sensory changes were observed in the model of capsaicin application in humans (LaMotte et al., 1991; Simone et al., 1989a,1987). Therefore, this literature review will focus on the mechanisms of cutaneous hyperalgesia after tissue injury induced by heat or capsaicin.

2.2 Neural mechanisms of pain in normal skin

To understand the mechanisms of hyperalgesia, it is necessary to know how the primary nociceptive afferents and the central nociceptive neurons are involved in transmitting pain sensation in normal skin. For many years, intensive neurophysiological studies have been searching for

the primary nociceptive afferents in animals and humans, as well as for the psychophysical comparisons in humans. In the central nervous system, nociceptive neurons located in the spinal cord, thalamus and somatosensory cortex were found to be able to encode noxious stimuli. These central nociceptive neurons include NS and WDR types.

2.2.1 Primary nociceptive afferents

There are two groups of primary nociceptive afferents that innervate the skin, namely the thinly-myelinated A-δ fibers and the unmyelinated C fibers. Many unmyelinated C-fiber nociceptors respond to mechanical and heat stimuli and therefore are called the C-fiber mechano-heat nociceptor (CMH) (Meyer et al., 1994; Raja et al., 1988). In some cases, responses to cold stimuli or chemical stimuli may also be evident and are referred to as polymodal nociceptors. The responses of CMHs to heat stimuli ranging from 41 to 49 °C in monkeys were found to be comparable with pain ratings in human subjects (Meyer and Campbell, 1981). This provides evidence that CMHs are responsible for heat pain sensations from normal skin. Other findings in which heat pain sensation is not decreased following the selective block of A fibres, also support the conclusion that C-fibers are responsible for heat pain (Torebjörk and Hallin, 1973). Furthermore, Torebjörk and Ochoa (1980) demonstrated that painful sensations could be induced by micro-neurographic stimulation of single C-fiber nociceptors in humans.

Most thinly-myelinated A-δ fiber nociceptors, on the other hand, are

responsive to mechanical stimuli but are normally insensitive to heat. However, these A-δ fibers can become sensitized to heat after injury (Fitzgerald and Lynn, 1977), and therefore are referred to as type 1 A-fibre mechano-heat nociceptors (type 1 AMH). This type of nociceptor exists in both hairy and glabrous skin and may be responsible for the continuous pain caused by prolonged heat stimuli (Meyer and Campbell, 1981). A second type of Aδ-nociceptor (type 2 AMH) has been identified in monkeys and in humans (Adriaensen et al., 1983; Dubner and Bennett, 1983; Meyer et al., 1991a,b, 1985; Treede et al., 1990,1984). This type 2 AMH fiber has heat response properties similar to those of CMHs but with shorter activation latency, and they seem to exist only in hairy skin (Treede et al., 1990).

For the primary nociceptive afferents, prolonged application of a constant stimulus leads to adaptation, while repeated application of brief stimuli leads to fatigue (Meyer and Campbell, 1981; Handwerker et al., 1987). Therefore, spatial and temporal summation of nociceptive inputs at central levels is needed to evoke pain under normal circumstances.

The afore-mentioned nociceptors that encode both noxious thermal and mechanical stimuli do not respond well to chemical irritants such as capsaicin. The pronounced pain provoked by such a chemical irritant suggests the existence of other receptor types in the skin. These fibers are not responsive to mechanical stimulus in normal tissue and are referred to as mechanically insensitive afferents (MIAs) (Davis et al., 1993;LaMotte et

al., 1988; Meyer and Campbell, 1988; Meyer et al., 1991a,b). Some of these MIAs were found to respond to chemical stimuli and therefore may be chemoreceptors (Davis et al., 1993).

2.2.2 Central nociceptive neurons

Two classes of neurons possibly involved in pain sensation have been described in the central nervous system (Willis, 1985). One class is called NS neurons. These types of neurons have high thresholds for cutaneous stimuli that are transmitted only by nociceptors. NS neurons in the spinal cord were found in lamina I and V (Christensen and Perl, 1970).

Another type of central nociceptive neuron also encode the intensity of noxious stimuli, and are referred to as WDR neurons. This type of neuron responds to both noxious and low-threshold mechanoreceptive inputs, and in fact, they may better account for the sensory discriminative aspect of pain (Dubner et al., 1989). They can be activated by mechanical and heat stimuli, and receive convergent inputs from A- and C-fibres. Most WDR neurons are located in lamina V.

As described in the previous chapter, most spinal nociceptive neurons (NS and WDR) send projecting axons into the spinothalamic tract (STT) and terminate in the VP, VPI or VMpo of the thalamus. In the ventral posterior lateral nucleus (VPL), most nociceptive neurons were reported as WDR (see chapter 1). Furthermore, recordings from human thalamic principal sensory nuclei have demonstrated that some of these thalamic neurons are able to encode noxious heat (Lenz et al., 1993a,b) and

mechanical stimuli (Lenz et al., 1994a).

At the cortical level, both NS and WDR neurons were found in areas 3b and 1 of the primary somatosensory cortex in monkeys (Chudler et al., 1990; Kenshalo et al., 1988; Kenshalo and Isensee, 1983). These neurons respond to both mechanical and thermal stimuli and generally have small contralateral receptive fields. Brain imaging studies revealed contralateral SI activation during the application of painful stimuli (Coghill et al, 1995; Duncan et al., 1994; Iadarola et al., 1993; Talbot et al., 1991). However, some other studies failed to observe such activation (Jones et al., 1991).

2.3 Neural Mechanisms of Primary hyperalgesia

The underlying mechanisms of primary hyperalgesia may be attributed to the combination of peripheral and central sensitization. (Kilo et al., 1994; LaMotte et al., 1992,1991; Torebjörk et al., 1992; Schmelz et al., 1996). Experiments designed to study the peripheral neural mechanisms of primary hyperalgesia logically focused on the responses of nociceptors in the injured area. In this section, the sensitization of primary afferent nociceptors that account for primary hyperalgesia to heat stimuli will be described first, then the role of primary afferent nociceptors in mechanical hyperalgesia will be discussed. In addition, the possible central mechanisms of primary hyperalgesia will also be summarized.

2.3.1 Peripheral mechanisms of primary heat hyperalgesia

Nociceptor sensitization Since primary hyperalgesia occurs at the site of injury, the correlated sensitization of nociceptors was proposed to be the essential peripheral neural mechanism of the primary hyperalgesia. Sensitization of nociceptors is characterized by 1) the decrease in threshold, 2) an increase in response to suprathreshold stimuli, and 3) occasional spontaneous activity. These properties correspond to the characteristics of hyperalgesia in human subjects.

The nociceptors' sensitization to **heat** stimuli has been studied extensively in primates and humans with mild heat injury (Beitel and Dubner, 1976a; Campbell et al., 1979; Campbell and Meyer, 1983; Croze et al., 1976; Kumazawa and Perl, 1977; LaMotte et al., 1984, 1983a,b, 1982b; Meyer and Campbell, 1981; Thalhammer and LaMotte, 1982; Torebjörk et al., 1984; Torebjörk and Hallin, 1978). The results suggest that the types of sensitized nociceptors are different in the glabrous and hairy skin.

On **glabrous skin**, a correlated psychophysical investigation in human and neural response of Type I AMH and CMH nociceptors in anesthetized monkeys was conducted by Meyer and Campbell (1981). They applied heat stimuli ranging from 41 to 49 °C to the glabrous skin of hands in humans and monkeys before and after a burn with 53°C for 30 seconds. For the AMHs, the heat threshold greatly decreased and the

response to suprathreshold stimuli was increased after the burn. Similar observations of sensitization of AMHs by heat were also reported by other investigators (Burgess and Perl, 1967; Dubner et al., 1977; Fitzgerald and Lynn; 1977). In contrast, the CMHs showed a decreased response to suprathreshold stimuli following the burn. This lack of sensitization of CMHs innervating monkey glabrous skin was supported by other investigators (LaMotte et al., 1983a). These data suggest that AMHs, but not CMHs, participate in the primary heat hyperalgesia of the glabrous skin.

On **hairy skin**, in addition to the AMHs, the CMHs can also be sensitized by heat after burn (Beital and Dubner, 1976; Bessou and Perl, 1969; Campbell and Meyer; 1983; Fitzgerald and Lynn, 1977; LaMotte et al., 1983a,b; 1982; Lynn, 1977; Perl, 1976; Thalhammer and LaMotte, 1982; Torebjörk et al., 1984). Therefore, both C-fibre and A-fibre nociceptors are likely to play a role in heat hyperalgesia on hairy skin. These observations indicate that the distribution of CMHs varies with skin type (Campbell and Meyer, 1983) and respond differently to heat following a burn.

The use of chemogenic materials to produce tissue damage in studying hyperalgesia was also reported. Results from psychophysical experiments with topical application of capsaicin (Culp, 1989; Koltzenburg et al., 1992) or mustard oil (Koltzenburg et al., 1992) indicate that sensitization of C-nociceptors accounts for heat hyperalgesia in human hairy skin and is linearly dependent on the log of capsaicin dose between 2

x 10^{-4} M and 2 x 10^{-1} M (Culp et al., 1989). Similar studies with topical capsaicin (1%) or freezing skin to -28°C have also shown that heat pain threshold was lowered by 5 – 9 °C and was probably due to nociceptor sensitization. (Kilo, et al., 1994).

These data provide evidence that the primary hyperalgesia to heat stimuli is due to sensitization of primary nociceptive afferents. Nevertheless, Baumann et al., (1991) failed to demonstrate this peripheral mechanism of neurogenic hyperalgesia. They injected 100 ug capsaicin and recorded in the primary afferents that innervated the arm, hand, or leg of an anesthetized monkey. They found that both CMHs & AMHs responded too weakly to account for the level of pain that was measured in the human subjects. They suggested a novel type of chemonociceptive primary afferent that subserves the heat and mechanical hyperalgesia at the site of injection of capsaicin, and that the sensitization that contributed to the neurogenic hyperalgesia was in the CNS. However, it was observed in the same study that the topical application of capsaicin enhanced the responses of CMHs and AMHs to heat.

2.3.2 Peripheral mechanisms of primary mechanical hyperalgesia

Mechanical hyperalgesia can be produced at both primary and secondary areas related to the injured skin. Different types of mechanical hyperalgesia can be distinguished according to the stimulus modalities and qualities. One type of mechanical hyperalgesia is induced by stroking the

skin and has been called stroking hyperalgesia, dynamic hyperalgesia, or allodynia (Hardy et al., 1950; LaMotte et al., 1991; Lewis, 1942; Simone et al., 1989a; Torebjork et al., 1992). The second type of mechanical hyperalgesia is evident using punctate stimuli and has been termed punctate hyperalgesia (LaMotte et al., 1991). The other type of mechanical hyperalgesia is evident in tonic blunt probes (Culp et al., 1989; Koltzenburg et al., 1992). The peripheral mechanisms of mechanical hyperalgesia are likely to be different from the heat hyperalgesia.

Nociceptor sensitization Primary hyperalgesia to mechanical stimuli has been observed in human psychophysical studies (Raja et al.,1984). It was presumed that the sensitization of primary nociceptive afferents could account for this mechanism. A study conducted by Bessou and Perl (1969) showed that the mechanical threshold of CMHs decreased 33% in cats. Reeh et al., (1987) also reported that the mechanical threshold decreased after a mechanical injury in the A-fibres of rats. Therefore, sensitization of primary nociceptive afferents to mechanical stimuli might be expected to parallel the sensitization to heat stimuli. However, such sensitization to mechanical stimuli has not been observed in primates or humans either for AMHs (Campbell et al., 1979) or CMHs (Campbell et al., 1988; LaMotte et al., 1987; Simone et al., 1986; Thalhammer and LaMotte, 1982). Thus, the sensitization of primary nociceptors can not completely account for the primary mechanical hyperalgesia. Other possible mechanisms might also be involved.

Expansion of the receptive field The expansion of the receptive field of nociceptors into an adjacent area may be an alternate peripheral mechanism for primary mechanical hyperalgesia. Investigations into changed receptive fields of nociceptors were conducted in monkeys' hairy skin (Thalhammer and LaMotte, 1982). The results demonstrated that most of AMHs and some CMHs expanded their receptive fields after a heat injury. Consequently, a stimulus will activate more nociceptors than it would have before injury, resulting in increased sensation without the presence of lowered mechanical threshold. This spatial summation could be one of several possible peripheral mechanisms underlying primary mechanical hyperalgesia.

Mechanically Insensitive Afferents (MIAs) Other possible explanations for primary mechanical hyperalgesia have also been proposed (Davis et al., 1993; 1990; Meyer et al., 1991a,b). Schaible and Schmidt (1985) described "sleeping nociceptors" in knee joints, which are normally unresponsive to mechanical stimuli but become responsive after inflammation. Similar primary afferents were described in the skin and are referred to as mechanically insensitive afferents (MIAs) (Meyer et al., 1991b). The role of these MIAs in pain sensation is still unclear. However, a large percentage of thinly-myelinated A-fibers and C-fibers were found to belong to this group (Meyer et al., 1991b). According to their study, these afferents can be sensitized to mechanical stimuli after injection of an artificial inflammatory solution (contains bradykinin, histamine, serotonin,

and prostaglandin E1) in the hairy skin of anesthetized monkeys.

Decreased inputs from low-threshold mechanoreceptors Another possible mechanism underlying primary mechanical hyperalgesia is a change in peripheral inputs from the large mechanoreceptors. The suppression of activity of large mechanoreceptors after injury was observed in cats (Beck et al., 1974). It was proposed that a decreased response of low-threshold mechanoreceptors could lead to a reduction in the concurrent inhibition of inputs into the dorsal horn. This disinhibition of primary afferents could therefore enhance the nociceptive inputs in the dorsal horn and thus contribute to primary mechanical hyperlagesia.

2.3.3 Central neural mechanisms of primary hyperalgesia

Persistent activity in primary nociceptive afferents results in increasing responsiveness of dorsal horn neurons in the spinal cord (Cervero et al., 1992; Cook et al., 1987; Dubner and Ruda, 1992; Hylden et al., 1989a,b; Laird and Cervero, 1989; Simone et al., 1989b). After a cutaneous injury within the receptive fields, neural sensitization to heat stimuli has been reported at the level of the spinal cord (Ferrington et al., 1987; Keshalo et al., 1982, 1979; Simone et al., 1991b), thalamus (Guibaud et al., 1987; Kenshalo et al., 1980; Peschanski et al., 1980) and cortex (Kenshalo and Isensee, 1983). However, these enhanced central activities may be due solely to the increasing inputs from peripheral

sensitized nociceptors and do not necessarily indicate a change in central processing.

Changes of receptive fields of central neurons after peripheral tissue injury could provide a possible central mechanism of primary hyperalgesia. The expansion of receptive fields of dorsal horn neurons after an injury produced by a burn (McMahon and Wall, 1984) or by a central action of chemicals (Sherman et al., 1997a,b) has been observed in rats. Intrathecal administration of strychnine could alter the receptive fields and sensory modalities of VPL neurons (Sherman et al., 1997a) or altered response properties of NS neurons in medial thalamus (Sherman et al., 1997b).

Furthermore, mechanical hyperalgesia may result from central alteration such that the input from low-threshold mechanoreceptors causes pain sensation. In cats, capsaicin could induce rapid reorganization of receptive fields of cuneate nuclei (Pettit and Schwark, 1996). This phenomenon has been investigated in secondary hyperalgesia and will be discussed below.

2.4 Neural mechanisms of secondary hyperalgesia

Secondary hyperalgesia is characterized by the presence of enhanced sensitivity to mechanical stimuli in the surrounding undamaged tissue after injury. This altered sensitivity to mechanical stimuli generally includes an increased magnitude of pain sensation to noxious stimuli

(hyperalgesia) and a change in the modality of the sensation, e.g. from touch to pain (allodynia).

Two different mechanisms have been proposed to explain how hyperalgesia spreads from the injured site to the surrounding undamaged tissue. Earlier studies by Lewis (1942) emphasized a peripheral mechanism, while Hardy (1950) proposed a central mechanism. Lewis (1942) suggested that the release of substances from the activated nociceptive afferent terminals could in turn sensitize other nearby primary nociceptive afferents (axon reflex). Conversely, Hardy (1950) described a central mechanism that suggested the involvement of a spreading sensitization within the spinal cord. Both hypotheses emphasize the importance of initial sensitization of primary nociceptive afferents but differ in whether the conduction was toward the CNS or just a local spread to the periphery. To verify these hypotheses, experiments have been designed to block the proximal or distal propagation, but no consensus has been achieved in the results.

2.4.1 Peripheral mechanisms of secondary hyperalgesia

Role of nociceptors To develop secondary hyperalgesia, the initiation of nociceptive activity is necessary. Several lines of evidence support this hypothesis. For example, capsaicin, which only activates nociceptors without affecting low-threshold mechanoreceptors, can produce secondary hyperalgesia. This is contrasted with innocuous stimuli

such as touch or warm temperature, which do not produce secondary hyperlagesia. Furthermore, it was shown that cooling or anesthetizing the injured site can suppress secondary hyperalgesia, while rewarming the skin could bring it back (LaMotte et al., 1991). This indicates that activity in sensitized nociceptors at the injured site is also required to maintain the secondary hyperalgesia.

Peripherally spreading sensitization Cutaneous injury may develop a flare response in a broad surrounding area. This phenomenon involves several factors: a) excitation of nociceptors, b) the effector ending near an adjacent blood vessel, c) the release of vasodilator agents, and d) the dilatation of arterioles. The most accepted hypothesis proposes that the flare spreads via the cutaneous axon reflexes (Lewis, 1927). This hypothesis states that the branching of primary afferent neurons supplies both the sensory ending and the effector ending on blood vessel. Lewis described secondary hyperalgesia as resulting from the sensitization of nociceptors located in the injured area, which leads to the propagation of action potentials in other branches of the afferents, thus resulting in the release of chemicals such as substance P in the adjacent area. He also demonstrated that electrical stimulation of the peripheral nerve caused hyperalgesia along the distribution of the nerve. This axon-reflex mechanism that accounts for the flare response was also reported by Lembeck (1983). Thus, substance P could participate in the phenomenon of widespread vasodilatation (flare) on the injured site. Carpenter and

Lynn (1981) were able to demonstrate that depleting nerve terminals of substance P by long term application of capsaicin could block the effector side of the axon reflex and diminish the flare size. Fitzgerald (1979) reported that in the intact area adjacent to an injured site, antidromic electrical stimulation in rabbits' peripheral nerves could result in sensitization of C-fiber nociceptors to heat. However, several other authors have not succeeded in replicating this observation in studies using rats and primates (Meyer et al., 1988; Reeh et al., 1986; Thalhammer and LaMotte, 1983).Szolcsányi et al., (1992) proposed that C-polymodal nociceptors and their varicosities serve as receptor-effector function for the spread of flare. Furthermore, using infrared thermography, Serra et al., (1998) demonstrated that after cutaneous injection of capsaicin, the flare response reflects a multifocal dilatation of underlying arterioles around the injury site. In addition, the area of flare was found to coincide with the area of mechanical and heat hyperalgesia.

Other neurophysiological studies focused on the change in mechanical sensitivity following an injury. In monkey studies, A-fiber and C-fiber nociceptors failed to demonstrate a change in mechanical thresholds following a heat injury adjacent to the receptive field (Thalhammer and LaMotte, 1982). However, in studies using rats A-fiber nociceptors showed a lowered mechanical threshold after an adjacent mechanical injury (Reeh et al., 1987). These controversial observations

suggest that peripheral sensitization may not account sufficiently for secondary hyperalgesia in primates.

It was shown by injection of xylocaine that secondary hyperlagesia induced by intradermal injection of capsaicin does not spread beyond the xylocaine area (Simone et al., 1985). This observation provides indirect evidence for a peripheral mechanism for secondary hyperalgesia.

2.4.2 Central mechanisms of secondary hyperalgesia

A central mechanism for secondary hyperalgesia was postulated by Hardy and his colleagues (Hardy et al., 1950). According to their hypothesis, inputs of sensitized nociceptors from the injured areas to the dorsal horn produce a "sensitization" of dorsal horn neurons that also receive inputs from the regions surrounding the injury. This sensitization could be due to facilitation of nociceptive neurons or to enhanced synaptic links between central nociceptive neurons and low-threshold mechanoreceptors (e.g. stroking hyperalgesia or allodynia).

Neurophysiological evidence for central mechanisms of secondary hyperalgesia have been reported in some studies using rats and monkeys. In these studies, it was shown that a burn injury inside the receptive fields (RF) of monkey STT neurons led to an increased response while applying the mechanical stimuli outside the injured skin area (Kenshalo et al., 1982). McMahon and Wall (1984) reported a decreased mechanical threshold of

the RF of rat lamina I neurons when small burns were caused outside the RF.

To support the central mechanism for secondary hyperalgesia, LaMotte and his colleagues (1991) performed a psychophysical study in humans in which a proximal nerve block was performed to prevent the development of secondary hyperalgesia. Three hours after the recovery from the proximal blockade of lateral antebrachial nerve with 1% xylocaine, secondary hyperalgesia did not occur. Therefore, it seems that the sensitized neurons in the CNS are necessary for the existence of secondary hyperalgesia.

Nevertheless, the central mechanism for stroking hyperalgesia still remains inconclusive. Treede and Cole (1993) presented a report of a patient with large fiber neuropathy, showing that an intradermal injection of capsaicin produced only punctate but not stroking hyperalgesia. By using different woolen fabrics to produce different prickle sensations to normal and hyperalgesic skin, Cervero et al., (1994) suggested that stroking hyperalgesia is due to central sensitization to input from low-threshold mechanoreceptors and that punctate hyperalgesia is due to central sensitization to input from nociceptors. Recently, Cervero and Laird (1996a,b) proposed a new mechanism using a capsaicin pain model in humans. They suggested that the low-threshold A-β mechanoreceptive fibers depolarize the central terminals of nociceptive primary afferent neurons via interneurons.

As described above, an increase in receptive field size is more evidence for a central mechanism. In rat dorsal horn neurons, an expansion of the RF could be induced by applying mustard oil outside the RF (Woolf and King, 1990). Mechanical injury within the RF of dorsal horn neurons led to the expansion of the RF into uninjured skin (Laird and Cervero, 1989). In addition to the spinal cord, a supraspinal center may also be involved in central mechanisms. Thalamic neurons in rats exhibit enhanced responsiveness to mechanical and heat stimuli whose RF is remote to the injury site (Guilbaud et al., 1986). In primates, a thalamic correlation with secondary hyperalgesia has not yet been documented.

2.5 Summary

Primary heat hyperalgesia can be accounted for by the sensitization of primary nociceptive afferents. In glabrous skin, AMHs become sensitive to heat after the injury. In hairy skin, both AMHs and CMHs are involved in this heat sensitivity after injury. Regarding primary mechanical hyperlagesia, it has been observed that central mechanisms may also be necessary to account for the change in mechanical sensation in addition to the changes in threshold and receptive fields. In the spinal cord, changes in threshold to heat and mechanical stimuli have been described. Increases in the size of the receptive field of dorsal horn neurons also indicate an important sensory integration in primary

hyperalgesia at both spinal and supraspinal levels.

An additional mechanism that may contribute to primary hyperalgesia without lowering the nociceptor threshold is the recruitment of silent nociceptors and of previously insensitive branches of nociceptors, leading to the expansion of receptive fields (Thalhammer and LaMotte, 1982; Schmelz et al., 1996,1994; Schmidt et al., 1995).

Secondary hyperalgesia is characterized by enhanced sensitization to mechanical stimuli adjacent to the injured area. The proposed peripheral mechanism consists of an axon reflex, a phenomenon which spreads peripherally by the coupling of C-fibers or via the release of neuropeptides. However, neurophysiological evidence for the peripheral mechanism of secondary hyperalgesia is not convincing. Substantial evidence suggests that central sensitization is the principal mechanism of secondary hyperalgesia. This central sensitization may occur in the spinal cord or at higher levels. Dorsal horn neurons demonstrate changes in stimulus-response functions and receptive field size from a remote injury. Secondary mechanical hyperalgesia may result from the enhanced nociceptors' input and from low-threshold mechanoreceptors as well. Recently, Pederson and Kehlet (1998) found that there is no difference in pain response to mechanical stimuli in the primary and secondary zone. They suggested that central mechanisms might be responsible for the punctate mechanical hyperalgesia regardless of whether it is in the primary or secondary zone.

Chapter 3 Experimental methods in the study of pain and hyperalgesia

3.1 Introduction

3.1.1 General consideration

When using animal models to assess pain, it is understood that they will be exposed to different intensities of stimuli ranging from innocuous to noxious. For this reason ethical issues should be taken into account to minimize any pain the animals may suffer (Zimmerman, 1987,1986,1983). In general, experimental animals should not be exposed to pain situations that humans could not tolerate. Furthermore, in order to avoid unexpected tissue damage, verification of the noxious stimuli should be performed before applying them to the animals. In experiments involving the use of an anesthetized animal, sufficient anesthetics and systemic monitoring should be provided throughout the procedures. Adequate post-operative pain control is also indicated in some cases. Experiments such as the ones that are involved in the investigation of pain mechanisms, or the ones that correlate the psychophysical and neurophysiological results, require only minimal anesthesia in order that the animals subject to the same type of stimulation as experienced by humans. In these types of experiments, animals can be trained to perform an operant procedure **by initiating the trials** and are able to escape intolerable noxious stimulations.

To be considered as an ideal behavioral animal model for assessing pain, the following criteria have been suggested by Dubner (1989): 1) It

should distinguish between responses to innocuous versus noxious stimuli, 2) The responses measured should vary in magnitude over a range from threshold to tolerance, 3) Threshold and suprathreshold measures should be taken into account together, 4) The model should be susceptible to behavioral and pharmacological manipulation, 5) Sensitivity to other non-sensory factors, such as attention, motivation and motor performance should be distinguished, and 6) There should be little or no tissue damage with repetitive stimulation. In addition, the data obtained from animal models should present reliable parallel comparisons with that of humans (Watkins, 1989).

3.1.2 Types and properties of stimuli

For experimental models of pain assessment, selecting an appropriate type of stimulus is important. The following are the most commonly used types of stimuli in human and animal pain studies: thermal (cold and heat), mechanical, electrical stimuli and chemogenic agents. For neurophysiological investigations, certain potentially tissue-damaging noxious stimuli may be adopted. For psychophysical studies however, reversible transient noxious stimulation is preferred.

Heat Thermal injury has long been used to mimic the effects of clinical pain. The radiant heat technique (Hardy et al., 1950; Raja et al., 1984) and the contact thermode probe (Darian-Smith et al., 1973; Dubner et al., 1975; Kenshalo et al., 1967; LaMotte et al., 1991,1982a) are the most

commonly used methods to apply heat stimuli. In the radiant heat method, the light from a projection lamp is focused through a lens and directed to a part of the skin blackened to enhance heat absorption. A thermocouple measures the skin temperature and sends feedback to the device. For behavioural studies, however, this method does not change the temperature fast enough and therefore an animal could, through expectation, respond to the innocuous temperature before the stimulus reaches the target level. Laser devices, which allow a very rapid change in skin temperature, can overcome this disadvantage (Mor & Carmon, 1975).

A contact thermode probe with a Peltier device and a thermistor for measuring the temperature of the junction between the skin and the thermode have been used extensively in pain studies (Bushnell et al., 1993; Darian-Smith et al., 1973; Dubner et al., 1975; Kenshalo et al., 1967). With this technical improvement, the rate of temperature change can be as fast as 20°C/s. However, contact thermodes have the disadvantage of activating the mechanoreceptors innervating the area of skin under the thermode probe.

Different degrees of heat temperature have been used to produce cutaneous injury: it could be induced by 53°C for 30 s(Raja et al., 1984) or by 49-50°C for 5-7 minutes in humans (Dahl et al., 1993; Møiniche et al., 1993). This could result in skin damage equivalent to a second-degree burn and primary heat hyperlgesia as well as mechanical hyperalgesia in the secondary area for up to 24 hours (Møiniche et al., 1993). Similar burning

effects have been reported in human studies even when using a lower heat temperature of 47°C for seven minutes, (Ilkjœr et al.,1996; Petersen et al., 1997). LaMotte et al (1982b) reported that heat injury in hairy skin caused a greater degree of hyperalgesia than that in glabrous skin. In primate normal hairy skin, heat stimuli between pain threshold and tolerance are believed to activate CMH nociceptors, whereas the AMHs are only activated by temperatures above 51°C (LaMotte et la., 1982b; Campbell et al., 1988).

Cold Noxious cold temperatures are not often used to produce an inflammatory state. This method was first introduced by Lewis in 1936. Recently, some investigators have demonstrated a freeze injury model by freezing the skin to -28 ° C in humans (Kilo et al., 1994). Moderate pain with itching and burning that lasted for two hours were reported. Punctate mechanical hyperalgesia was found in the secondary area and heat hyperalgesia occurred prominently in the primary area. These observations remained present even 22 hours post-injury. Punctate hyperalgesia persisted throughout an A-fiber block, suggesting peripheral mechanisms are being mediated by C fibers or by thinly myelinated A fibers (Kilo et al., 1994).

Chemical agents Many chemical substances have been used to investigate pain and hyperalgesia in animals and humans. Formalin has been injected in the footpads of rats and cats to mimic clinical human pain (Dubuisson & Dennis, 1977; Tjølsen et al., 1992). Injection of carrageenan

or complete Freund's adjuvant (CFA) results in an acute and more persistent pain that mimics postoperative pain or arthritis (Guilbaud et al., 1992a; Schaible et al., 1987). Enhanced neuronal responses in rat thalamus and SMI after the injections of carrageenan were also observed (Guilbaud et al., 1992a). Mustard oil injection or topical application on human hairy skin have been used to investigate the mechanisms of pain and hyperalgesia (Cervero and Laird, 1996a; Cervero et al., 1993; Koltzenburg and Handwerker, 1994; Koltzenburg et al., 1992; Schmidt et al., 1995). Sharp burning pain and hyperalgesia were reported after application.

In addition to the chemical agents mentioned above, capsaicin is probably the most common chemical irritant used in the study of pain. The topical application or low-dose intradermal injection of Capsaicin, the pungent substance found in hot chili peppers, produces burning pain and heat or mechanical hyperalgesia in humans (Carpenter and Lynn, 1981; Cervero et al., 1994; Culp et al., 1989; Kilo et al., 1994; Koltzenburg et al., 1992; LaMotte et al., 1992,1991; Morris et al., 1997; Simone et al., 1991,1989, 1987; Torebjörk et al., 1992; Treed and Cole, 1993). Whereas low doses of capsaicin activate C-fibers, higher or repeated doses lead to desensitization of C-fibers. The mechanisms by which capsaicin induces pain, hyperalgesia and desensitization will be discussed in detail.

3.1.3 Types of experimental pain models

Two types of animal models have been developed to mimic human clinical pain in order to assess their characteristics and mechanisms. The

first method is comprised of tissue injury models, in which tissue damage is produced by applying noxious stimuli (heat, cold and chemicals) to create acute or chronic pain and sequential hyperalgesia. The second method is the neuropathic model, which involves surgical intervention to mimic chronic or central pain syndromes in humans by creating nerve injuries or lesions in the peripheral or central nervous systems.

Tissue injury models As mentioned earlier, injection or topical application of chemicals (formalin, mustard oil, capsaicin, carageenin, Freund's adjuvant solution) has been used to produce tissue injury and inflammation in animal models. Due to the absence of adequate verbal communication in animals, their behavioral responses, such as limb withdrawal reflexes, tail flick reflex or vocalization, are considered as the criteria when assessing pain sensation. These methods usually measure the latency of response after the application of noxious stimulation to the testing areas.

In the learned operant experiments, animals acquire the knowledge to avoid or escape the noxious stimuli (Vierck and Cooper,1984). Some investigators have trained animals to press a bar to reduce the intensity of an electrical stimulus (Weiss & Laties, 1963) or to release a lever in response to detecting changes in thermal or mechanical stimuli (Bushnell et al., 1993). However, animals in the operant experiments have the tendency to avoid the trials rather than to escape, and consequently the

threshold of pain tolerance is difficult to determine. Therefore, caution should be taken when developing an escape animal model.

The human experimental models of pain induced by chemical or thermal injury were first introduced by Lewis (1936) and Hardy et al., (1950). A similar model of neurogenic inflammation by intradermal injection or topical application of capsaicin in humans was demonstrated by other investigators (Koltzenburg et al., 1992; LaMotte et al., 1991; Morris et al., 1997; Simone et al., 1987; Torebjörk et al., 1992). Capsaicin was found to introduce primary hyperalgesia along with a flare reaction in the surrounding area as characterized by mechanical hyperalgesia. This capsaicin model was also used in anesthetized monkey to investigate the response of spinal dorsal horn neurons (Chung et al., 1993; Dougherty et al., 1994b; Simone et al., 1991) and the reorganization of DCN in cats (Pettit and Schwark, 1996). A detailed description of the studies will follow.

Neuropathic pain model Based on pain resulting from a lesion of the peripheral or central nervous system, a chronic neuropathic pain model was developed by doing a partial ligation of a peripheral or a spinal nerve in rats (Attal et al., 1990; Bennett and Xie, 1988; Kim and Chung, 1992; Seltzer et al., 1990). In these methods, the abnormal limb posture and the response latency are used to assess pain and hyperalgesia. While thermal hyperalgesia was reported in all cases, mechanical hyperalgesia was found by Attal et al. (1990) but not reported by Bennett & Xie (1988). A model that

involves the complete section of the sciatic nerve in a rat also has been employed (Coderre et al., 1986; Rodin & Kruger, 1984; Sweet, 1981). With this model, autonomy was observed about two weeks after the complete section of the sciatic nerve. Nerve blockage by lidocaine eliminated this self-mutilation, suggesting that the autotomy was a response caused by pain and not due to the limb's lack of sensation (Blumenkopf and Lipman, 1991).

3.2 Capsaicin and pain mechanisms

3.2.1 General information

Humans have used hot chili peppers as food additives and as an herbal medicine for hundreds of years. Oral consumption of hot peppers can provoke a burning sensation and induce sweating that ultimately leads to heat loss. Although we are all familiar with this spicy substance in daily life, the major pungent ingredient of hot pepper, capsaicin (8-methyl-N-vanillyl-6-nonenamide), was not investigated systemically until the middle of this century.

In the late '50s and '60s, Hungarian pharmacologist Nicolas Jancsó and his colleagues started a series of investigations on the pharmacological effects of capsaicin. They demonstrated that the application of capsaicin could initially evoke vigorous irritation followed by a refractory state to further stimuli (Jancsó, 1968;1960) which can last weeks after systemic administration in rats (Jancsó, 1968). Subsequent experiments conducted by his son and colleagues using systemic administration of capsaicin in

neonatal rats demonstrated that the destruction of small neurons in the dorsal root ganglion resulted in their permanent insensitivity to chemical irritants (Jancsó et al., 1977). Since then, with the characteristics of selective effects on the primary afferents (i.e. unmyelinated C and thinly myelinated A-delta fibers), capsaicin is probably one of the most commonly used chemical irritants in the study of pain and hyperalgesia. Extensive studies have been conducted by different authors through different routes of administration to investigate the effects of capsaicin on somatosensory systems as well as the mechanisms of its actions and its therapeutic potential in analgesia.

Furthermore, Jancsó also postulated that capsaicin exerts its actions by interacting at a specific recognition site, and that capsaicin-sensitive nerves contain inflammatory mediators, later identified as substance P and calcitonin gene-related peptide (CGRP) (Buck and Burks, 1986; Holzer, 1988). In addition to its specific action on somatosensory neurons, capsaicin also has biological effects in a variety of neural and non-neural tissues, such as the liver, heart and uterus, respiratory and thermoregulatory systems (Castle, 1992; Nagy, 1982; Smith et al., 1970; Zering et al., 1983). However, the present section will concentrate only on the effects of capsaicin in the somatosensory system.

3.2.2 Capsaicin receptors

The painful burning sensation caused by capsaicin-induced sensory

neuron excitation indicates that capsaicin-sensitive neurons may include the same primary afferents that innervate the nociceptors, namely the unmyelinated C-mechano-heat and A delta mechano-heat fibers (Baumann et al., 1991; Buck and Burks, 1986; Szolcsányi et al., 1992,1988). Since Janscó postulated that capsaicin can exert its activity at specific binding sites, and that the biological effects of capsaicin are dose dependent, efforts have been made to demonstrate the existence of capsaicin receptors. However, due to the high lipophilicity of capsaicin, identification of specific capsaicin receptors by means of radiolabeling (Miller et al., 1982a) or capsaicin-like photoaffinity methods (James et al., 1988) were unsuccessful until resiniferatoxin (RTX) was isolated from Euphorbia resinifera. RTX is an extremely potent irritant and has been used in cancer research to promote tumor formation (zur Hausen et al., 1979). During their studies, instead of identifying the receptors that mediate the biological action of tumor promoting, they found that RTX turns out to have an unusual potent inflammatory effect. Structure-activity comparisons revealed that RTX and capsaicin share a common homovanillic acid constituent essential for inflammatory activity. Based on these similarities in structure-activity relations, Szallasi and Blumberg assumed that RTX and capsaicin might bind to the same receptors named vanilloid receptors (Bevan et al., 1992; Szallasi and Blumberg,1990a,b, 1989). Further studies have suggested that the receptor complex has a molecular weight of 270kD (Szallasi and Blumberg, 1991). Other evidence for the existence of capsaicin receptors

comes from capsazepine, a potent selective competitive antagonist of capsaicin, which can suppress the activity of capsaicin both in vivo and in vitro (Bevan, et al., 1992).

The molecular characteristics of capsaicin action and its relationship to endogenous pain signalling can also be achieved by cloning of a gene encoding capsaicin receptor. Julius and his colleagues (Caterina et al., 1997) isolated a cDNA clone in rat sensory ganglion which reconstitutes capsaicin responsiveness in non-neuronal cells. They reported that this cloned vanilloid receptor (VR1) seems to be expressed exclusively by small-diameter sensory neurons and thus provides a molecular explanation for the selectivity of capsaicin action. They also demonstrated that this cloned receptor is a cation channel that is also activated by noxious heat. Further study by the same group suggested that heat gates VR1 directly and that protons decrease the temperature threshold for VR1 activation (Tominaga et al., 1998). These findings propound that the vanilloid receptor functions as a polymodal signal detector whose activity reflects the combined status of multiple physiological stimuli.

3.2.3 Mechanisms of actions of capsaicin

Capsaicin interacts with a specific recognition site similar to RTX and capsazepine. Since these three substances share a common homovanillic acid structure, this receptor was named the vanilloid receptor (Szallasi and Blumberg, 1990b). Upon binding to this receptor, capsaicin opens a cation

channel which in turn causes an increase in the permeability of Ca^{++} and Na^{+} ions (Bevan et al., 1987). However, it still remains unknown whether there is an endogenous ligand for this receptor.

Excitation As mentioned above, capsaicin opens the cation channel, causing an increase in the permeability of both Ca^{++} and Na^{+}. This in turn induces depolarization and results in the immediate effects of excitation. (Bevan and Szolcsányi, 1990; Bevan et al., 1987; Lynn, 1990; Winter and Bevan, 1995; Wood et al., 1988). Capsaicin-induced accumulation of Ca^{++} ions or the flux of radiolabelled ions have been investigated in an attempt to quantify the effects of capsaicin-like molecules. This membrane ion channel is unique and is insensitive to conventional calcium and sodium ion-channel blockers such as dihydropyridines and tetrodotoxin (Bevan and Geppetti, 1994; Maggi, 1991; Wood et al., 1988). Thus as expected, capsaicin-induced activation of peripheral nociceptors was found to be unaffected by calcium channel blocking agents (Dray et al., 1990). The resulting cation influx leads to impulse generation (afferent action) and to the release of neuromediators (efferent function) (Bevan and Szolcsányi, 1990; Holzer,1991).

Desensitization Excitation by capsaicin is followed by refractory period which can be either reversible (traditional pharmacological desensitization to further application of capsaicin) or irreversible (functional desensitization to other noxious stimuli as well) (Buck and Burk, 1986; Dray

et al., 1990; Holzer, 1991). These two phenomena often occur together but can be distinguished at low concentration.

The mechanism of the desensitization is not clear at this moment; it appears to require external Ca^{2+} (Amann, 1990) and it may involve a subsequent activation of Ca^{2+}- dependent phosphatases, such as calcineurin (Yeats et al., 1992).

More is known however about the long-term functional desensitization; it is clear that capsaicin can block axonal conductance (Baranowski et al., 1986) and stop the intra-axonal transport of molecules (Miller et al., 1982b; Taylor et al., 1985, 1984). This block of axonal flow can deprive somata of nerve growth factor (NGF) (Miller et al., 1982b). The absence of NGF results in the loss of the DRG's ability to respond to capsaicin (Winter et al., 1988), as well as the quantitative loss of their expressed RTX binding sites (James et al., 1992).

Degeneration and neurotoxicity

The neurotoxicity caused by capsaicin is likely due to a combination of mitochondrial impairment caused by excessive accumulation of Ca^{2+} (Jancsó et al., 1984) and the osmotic swelling from the intracellular NaCl formation (Bevan and Szolcsanyi, 1990;Wood et al., 1988). Axonal block and NGF deprivation also appear to play an important role in neurotoxocity. In addition, neurotoxicity depends on an age factor, the route of administration, and the dosage. While systemic injection of capsaicin (35 mg/kg) into neonatal rats can cause the death of numerous DRG neurons,

large doses of systemic injection into adult rats revealed the survival of most of the cell bodies as well as the tendency of axonal regeneration (Chung et al., 1990,1985; Winter et al., 1993,1990).

Therapeutic potential

The selective actions of capsaicin on unmyelinated C fibers and thinly myelinated Aδ primary afferents have resulted in the potential therapeutic value of capsaicin and capsaicin analogues. Efforts have been made to seek ways to retain its analgesic activity due to prolonged desensitization and selective action on primary afferents while reducing its irritant effects. In animal studies, the analgesic effect is transient, lasting only for a few hours, with systemic administration of 1 to 10 mg/kg in adult rats. At higher doses, ranging from 50 mg/kg to cumulative dosages up to 950 mg/kg s.c., the antinociceptive effect can persist for several months or even be life-long in neonatal or adult rats (Jancsó et al., 1977). However, different groups have reported inconsistent results from studies using adult rats receiving capsaicin neonatally. In these studies, analgesia to noxious mechanical or chemical stimuli was reported, but conflicting results were obtained using noxious thermal stimuli (Faulkner and Growcott, 1980; Hayes et al., 1981; Nagy and van der Kooy, 1983). In addition, while some groups reported an increase in thermal nociceptive threshold (Nagy and van der Kooy, 1983), others failed to obtain the same result (Cervero and McRitchie, 1983; Hayes et al., 1981). At high doses of systemic administration,

however, the narrow window between its effects and toxicity limits its potential therapeutic action in humans.

3.3 Capsaicin and studies of pain and hyperalgesia

Capsaicin has been used to investigate the characteristics of chronic and acute pain in humans by producing various sensory effects including pain, hyperalgesia, and hypoalgesia (Buck and Burk, 1986). The response of primary afferent fibers to heat and mechanical stimuli was investigated by electrophysiological recordings in monkeys and rats (Bauman et al., 1991; Konietzny and Hensel, 1983; Szolcsányi et al., 1988) and humans (LaMotte et al., 1991; Torebjork et al., 1992) using intradermal injection and topical application of capsaicin. Psychophysical studies in normal human subjects showed that topical application of capsaicin at low doses can produce a burning pain sensation as well as thermal and mechanical hyperalgesia (Carpenter and Lynn, 1981; Cervero et al., 1994; Culp et al., 1989; Kilo et al., 1994; Koltzenburg et al., 1992; LaMotte et al., 1991). Pain and hyperalgesia after intradermal injection of capsaicin in humans was shown to be dose-dependent from 0.01 to 100ug (Simone and Ochoa, 1991; Simone et al., 1991,1989a,1987; Torebjörk et al., 1992). Following intradermal injections of 100 μg of capsaicin, mechanical allodynia and hyperalgesia in the primary and secondary areas can last up to two hours (Koltzenburg et al., 1992; LaMotte et al., 1991, 1992; Simone et al., 1991; Torebjörk et al.,1992).

In peripheral neuropathy or rheumatoid patients, the characteristics and mechanisms of hyperalgesia produced by capsaicin demonstrated the same alteration as found in normal subjects (Morris et al., 1997;Treede and Cole, 1993). In subjects with large-sensory fiber neuropathy, hyperalgesia to punctate stimuli was observed in healthy subjects, whereas hyperalgesia to light touch never occurred (Treed and Cole, 1993). In rheumatoid patients, areas of mechanical hyperalgesia significantly enlarged after topical application of 20 ul of capsaicin (Morris et al., 1997).

Some investigators have recorded from spinothalamic neurons in anesthetized monkeys and parallel psychophysical study in humans (Simone et al., 1991). Neurobiological mechanisms were also investigated. STT neurons showed enhancing responses to excitatory amino acids (EAA) after intradermal injection of 0.3% capsaicin in monkey (Dougherty and Willis, 1992). It has also been shown that repeated application of high dosage of capsaicin can block the peripheral C-fibres, resulting in the reorganization of receptive fields in the cunate nucleus, thalamus, and cortex in cats (Petit and Schwark, 1996).

Part II. Aims of the present studies

In order to investigate how thalamic VPM neurons participate in pain and mechanical hyperalgesia, we designed a series of experiments with the following goals: 1) to establish a hyperalgesia model which can be applied repeatedly without causing permanent tissue damage. 2) to characterize the normal baseline responses of thalamic VPM neurons to quantitative mechanical stimulation; 3) to compare the thalamic activity in the normal and hyperalgesic states; 4) to correlate the neurophysiological data in animals with the psychophysical results in humans.

Chapter 4 A transient hyperalgesia model in awake primates: A psychophysical study

Note: This chapter has been published as:

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4.1 Summary

1. The aim of this study was to develop a transient hyperalgesia model in the awake monkey performing an operant task. One monkey and seven human subjects participated in this experiment.

2. The monkey was trained to perform a visual detection task to receive a reward or to escape mechanical or thermal stimuli by pressing a response lever. Innocuous and noxious thermal (43, 47 and 51°C) or mechanical (245, 490, 736 and 1472 mN) stimuli were presented on the face. The animal was tested before, 1 h and 24 h after topical capsaicin application (0.3ml; 0.004M).

3. Human subjects were tested with these similar procedures and rated the intensity of thermal and mechanical stimuli on a numerical scale. The intensity of the spontaneous pain caused by the capsaicin was also rated.

4. At the site of capsaicin application, the monkey escaped more thermal and mechanical stimuli, suggestive of thermal and mechanical hyperalgesia. Human subjects reported higher pain intensity for similar stimuli after capsaicin application, in accordance with the monkey escape behavior.

5. The procedure is repeatable and produces no overt sign of distress. Thus, it could provide an important tool for studying neural mechanisms of hyperalgesia and for testing analgesic treatments in primates.

4.2 Introduction

Allodynia and hyperalgesia to mechanical and thermal stimuli are often observed after skin injury, inflammation and nerve lesions (Lewis, 1936; Willis, 1992). In recent years, several animal models have been developed in which partial lesions were inflicted to a peripheral or spinal nerve, ultimately leading to behavioral signs of thermal and mechanical allodynia and hyperalgesia (Bennett and Xie, 1988; Seltzer et al., 1990; Kim and Chung, 1992). Although most of these models were developed for use in rodents, some investigators have also applied them to monkeys (Carlton et al., 1994). These models have significantly improved our knowledge of the mechanisms underlying allodynia and hyperalgesia. However, they have the disadvantage of being invasive and leading to long-term somatosensory changes, lasting for weeks or even months. Consequently, the use of these models, particularly in primates, may pose serious ethical problems. Therefore, the aim of this study was to develop a model of transient hyperalgesia in the primate.

Many substances have been reported to produce allodynia or hyperalgesia after cutaneous or subcutaneous application. Among these, capsaicin, the pungent ingredient of hot chilli pepper (8-methl-N-vanilly-6-noneamide), is one of the best studied both in human psychophysical and animal electrophysiological studies. Psychophysical studies in humans have shown that intradermal injection or topical application of capsaicin

produces a painful syndrome by causing burning pain and mechanical and thermal hyperalgesia (Culp et la., 1989; Baumann et al., 1991; LaMotte et al., 1991, 1992; Koltzenburg et al., 1992; Kilo et al., 1994). LaMotte and colleagues were the first to use capsaicin in monkeys to study the neurophysiological mechanisms underlying hyperalgesia. These authors intradermally injected capsaicin in anesthetized monkeys and recorded from primary afferent fibers and spinothalamic tract neurons. The observed neural changes obtained during the recordings in these anesthetized animals were correlated with both psychophysical and electrophysiological measurements of pain and hyperalgesia obtained in humans submitted to identical stimuli (Simone et al., 1991). This approach has the disadvantage that no direct correlations can be made between the behavioral consequences of capsaicin application and the induced electrophysiological changes. In this report we present a model of capsaicin-induced hyperalgesia in the awake monkey performing operant tasks. This method produces thermal and mechanical hyperalgesia as assessed in an operant escape paradigm. Since the procedure is minimally stressful to the animal, it can be used in conjunction with awake animal neuronal recording or to study the effect of analgesic treatments in primate. A preliminary report of these data has been presented in abstract form (Kupers et al., 1996).

4.3 Materials and methods

4.3.1 Animal studies

One adult male monkey (Macaca mulatta) was studied. Throughout the experiment, the animal's weight and food and fluid intake were monitored daily to insure their stability. To motivate the animal to participate in the behavioral tasks, food intake was reduced to 80% of the animal's normal daily consumption. On training and experimental days, the monkey received liquid banana-flavored food (Bioserve Liquidiet@) as reward for correct responses in the behavioral tasks. Additional food was provided after each session, and water was provided ad libitum.

Both training and experimental sessions lasted between 2 and 3 h and were conducted with the monkey sitting in a primate chair. A response lever and a cue light were positioned in front of the animal. A tube for delivering liquid food reward was mounted on the chair, close to the mouth of the animal. During the training period, which lasted about 5 months, the monkey acclimated to the primate chair, learned to press a response lever for obtaining food reward (detection task) and then learned to press the lever to immediately terminate undesired stimuli (escape task; see below).

4.3.2 Apparatus

Mechanical stimulator

A special mechanical stimulator applying a wide range of innocuous and noxious forces was designed for this experiment (Ten Bokum et al., 1996). This computer-controlled stimulator advances a mechanical probe against the skin until a target force is achieved and maintains that force until retracting the probe at the end of stimulation. The comparator receives a voltage/force signal from the control computer and compares it with the voltage from the load cell amplifier. The comparator sends the results of the comparison to the motor control logic which translates this into motor control signals, i.e., ADVANCE, RETRACT, STOP. The motor logic also controls the number of steps/sec (speed) and the step or half-step mode. A single load cell (resolution 9.81; range 2452 mN) monitors the probe force and provides feedback to a servo-controlled amplifier which adjusts probe displacement (maximum of 25mm), thus maintaining the target force in spite of variable skin compliance. The probe rapidly advanced and retracted (1472mN/s) in 5-mN steps. When the stimulus was turned on, the probe advanced at a constant rate until it reached the specified pressure and then stopped, with no detectable overshoot. The stimulator had a changeable circular probe tip (0.2 mm^2) that was made of low thermal conductivity material (Delrin). The mechanical probe was positioned orthogonal to the skin at a distance of about 5 mm from the skin surface.

Thermal stimulator

Thermal stimuli were delivered via a 1x1cm Peltier contact thermode (Bushnell et al., 1993). A thermistor measured the temperature of the junction between the skin and the thermode and provided precise feedback control of heating and cooling. Both the thermal and the mechanical stimulators were controlled by a PC.

4.3.3 Visual detection task

Visual stimuli were presented using a 3-cm-diameter translucent light located 35-50 cm in front of the animal. This task consisted of pressing the response lever when a white cue light was illuminated to obtain food reward. At irregular intervals (interstimulus intervals of 5-12 s), the cue light was illuminated for 1.5 s. A lever press during this period was immediately followed by a liquid food reward. If the animal pressed the response lever when the cue light was not illuminated, a buzzer sounded and no reward followed.

4.3.4 Escape task

A contact thermode was positioned on one side of the monkey's face in the maxillary region, and the mechanical stimulator was placed on the other side at the homologous site. To insure consistent contact of the stimuli, the monkey's head was stabilized during the sessions using a custom-fit acrylic loop around the muzzle and behind the head. Thermal and mechanical stimuli of 4.5-s duration, in both the noxious and

innocuous range, were alternately presented. Pressing the response lever during the presentation of any of these stimuli resulted in the immediate interruption of the stimulation. No food reward was given for pressing the lever in the escape task.

On heat trials, the thermode temperature rapidly increased (20°C/s) from a baseline of 34°C to one of three pre-determined levels: 43, 47, or 51°C. When the animal did not escape the stimulus, the temperature returned to the 34°C baseline after 4.5s. On mechanical trials, the plastic probe touched the skin with one of four forces: 245, 490, 736 or 1472 mN. If the animal did not respond after 4.5 s, the probe was automatically withdrawn from the skin.

4.3.5 Capsaicin application

After stable baseline data were obtained, i.e., after several months of training, capsaicin experiments were begun. Capsaicin (Sigma) dissolved in 70% ethanol (0.004 M; 0.3ml) or the vehicle alone was topically applied to the maxillary region via a gauze pad (1x1 cm). The hairs were clipped a few days before application of the patch. The gauze pad was covered by a self-adhesive plastic film to insure contact and prevent evaporation. The patch was applied for 30 min. After removal of the patch, the skin of the area of capsaicin administration was marked with ink.

4.3.6 Testing protocol (Shown in Table 1a, 1b)

Each testing session began with 40 `warm-up' trials, which were not included in the data analysis. The capsaicin or vehicle was then applied for 30 min, during which time the monkey performed the visual detection task. After the 30-min capsaicin application, the patch was removed from the face, and the head restraint, response lever and stimuli were removed from the chair. The monkey was then given a 30-min rest period, since human data showed that spontaneous pain completely disappears by this time (see human test results below). After the rest, the experimental testing began, with the thermal stimulus positioned on the site of capsaicin application and the mechanical stimulus positioned on the contralateral homologous site. Detection and escape trials were presented for 140 trials, the probe sites were interchanged, and another 140 trials were presented. Trials were grouped in blocks of 14, each block consisting of seven visual detection trials, the four different mechanical stimuli and the three thermal stimuli. The sequence of the trials within each block was pseudo-random with the sole restriction that two mechanical or two thermal stimuli were never successively presented. Interstimulus intervals ranged between 5 and 12 s.

Control sessions with vehicle application were conducted at 1 day before and after each capsaicin session. In the present experiments, capsaicin was applied six times in consecutive weeks. The experimental protocol was approved by the ethical committee of the University and was

in accordance with the guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain.

Table 1a. Weekly schedule

Day 1	Vehicle patch	
Day 2	Capsaicin patch	
Day 3	Vehicle patch	

Table 1b. Daily protocol

Sections	Parameters	Contents
Warm-up	visual, mechanical, thermal	rewards, escape
Patch on site	visual	Rewards
Rest	none	None
Testing 1	visual, mechanical, thermal	rewards, escape
Testing 2 (reverse the stimulators)	visual, mechanical, thermal	rewards, escape

4.3.7 Human psychophysical studies

The effect of capsaicin on pain intensity ratings was tested in seven human volunteers (age range: 29-48 years). Testing site, dosage and

route of application were the same as in the monkey studies. Subjects were asked to rate the intensity of thermal (43, 47 and 51°C) and mechanical (490, 1472 and 1962 mN) stimuli on a numerical scale, where '0' represented no sensation and '200' the most intense pain imaginable. Subjects were instructed to use numbers greater than 100 if the stimulus was felt as painful. If a stimulus was felt as just noticeably painful, subjects were asked to use a number near 100. Every stimulus intensity was presented five times, both at the ipsilateral and contralateral side. In addition, subjects were also asked to rate the intensity of the spontaneous pain caused by the application of the capsaicin.

4.3.8 Statistics

Data are represented as means ± SE. Analysis of variance (ANOVA) for repeated measurements or a paired Student's *t*-test were used for statistical analysis. The Wilcoxon signed rank test was used for non-parametrical data (withdrawal latencies). *P* values of <0.05 were considered statistically significant.

4.4 Results

4.4.1 Monkey studies

4.4.1.1 General behavior during capsaicin application

Within 2-3 min of the application of the capsaicin patch, the monkey displayed some signs of agitation, such as facial grimaces and attempts to

reach up and remove the patch. These behaviors occurred intermittently during a period of approximately 10 min and then diminished. However, no signs of distress, such as vocalization, large body movements, or disruption of task performance or eating behavior, occurred. In fact, the monkey continued to perform the visual task through the entire period of capsaicin application, with no disruption to performance.

4.4.1.2 Effects of capsaicin on heat escape behavior

Fig.1 shows the percent of heat stimuli escaped during the test days preceding capsaicin application (bef), 1 hour after capsaicin application (1 h) and 24 hours after capsaicin application (24 h). In this and the following figure, data points represent the mean ± SE of eleven control (bef) and six capsaicin (1h and 24h) sessions in which each stimulus intensity was presented 10 times. During all tests, higher stimulus intensities produced more (escaping) responses, indicating that the animal was escaping, and not avoiding, the stimuli. Before capsaicin administration, the monkey rarely escaped the 43°C and 47°C temperatures. One hour after capsaicin, the monkey escaped significantly more thermal stimuli (ANOVA; $P<0.0001$): the number of responses to the 43°C and 47°C temperatures increased from 1.4 ± 1.0% to 46.7 ± 11.7% and from 2.9 ± 1.4% to 75 ± 9.6%, respectively. The number of escape responses to the 51°C temperature increased from 60.0 ±7.7% to 98.3 ± 1.7%. Reaction times for the escape responses were also significantly faster: whereas before

capsaicin the mean reaction time to the 51°C stimulus was 3.07 ± .13 s, 1h after capsaicin the reaction time decreased to 2.0 ± .09 ms ($P < 0.001$; Wilcoxon signed ranks test). Twenty-four hours after capsaicin administration, there was still a significant increase in the number of escapes to the 47°C and 51°C stimuli (ANOVA; $P < 0.01$). No significant changes were observed at the contralateral site (data not shown).

4.4.1.3 Effect of capsaicin on mechanical escape behavior

Fig. 2 shows the percent of mechanical stimuli escaped during the various conditions. During all conditions, the monkey responded in a graded fashion to increasing forces. One hour after capsaicin administration, the monkey escaped significantly more mechanical stimuli than before (ANOVA; $P < 0.05$). The values obtained 24 h after capsaicin application were not statistically different from baseline values. There was no significant effect on the percentage of escapes to mechanical stimuli at the contralateral side (data not shown).

4.4.1.4 Effect of capsaicin on appetitive visual detection task

Capsaicin did not affect the performance of the visual detection task. The reaction time to respond to the visual stimulus for food reward was not different before and after capsaicin application, with the mean latencies before and after being respectively 649 ± 13 and 664 ± 12 ms ($P >0.05$). Neither was there a significant increase in the number of failures to

respond or in premature responses (before the light was illuminated) after capsaicin administration. These findings show that the capsaicin-related effects on escape behavior are not the result of a general behavioral disruption and provide additional evidence that the procedure of topical capsaicin application is not too stressful for the animal.

4.4.2 Human psychophysical studies

4.4.2.1 Spontaneous pain

Fig.3 shows the ratings of spontaneous pain during and after capsaicin application. Within 1 min of the application of the patch, subjects reported a mild spontaneous pain. Pain intensity ratings reached a maximum after 10 min (mean intensity: 25 ± 6.2). Thereafter, there was a gradual decline in pain intensity ratings, although the patch was still on the face. The patch was removed at 30 min, and 20 min later (50 min from application onset), only three of seven subjects still reported a weak pain. At 60 min, all subjects were pain-free. All subjects described the pain as 'burning' and 'stinging' or 'pricking'.

4.4.2.2 Heat ratings

The effect of capsaicin on the heat intensity ratings is shown in Fig.4. One hour after capsaicin application, subjects reported significantly higher intensity ratings for the three different heat stimuli ($P < 0.01$; ANOVA followed by post hoc comparisons between means). Before capsaicin, only

the 51°C stimulus was perceived as painful, whereas 1 h after capsaicin, the 47°C stimulus was also rated as painful. Twenty-four hours after capsaicin, intensity ratings were no longer significantly different from baseline ratings. No effects were observed at the contralateral side.

4.4.2.3 Pressure rating

Fig.5 shows that the perceived intensity of mechanical stimuli was also increased by capsaicin. One hour after capsaicin application, subjects reported significantly higher pressure intensity ratings than before capsaicin (ANOVA; $P < 0.05$). Post hoc comparisons between means revealed that the difference between the conditions reached statistical significance only for the 490-mN stimulus. Twenty-four hours after capsaicin, intensity ratings were no longer different from baseline values. No effects were observed at the contralateral side.

4.5 Discussion

The results of the present study show that topical application of capsaicin in the maxillary region of the monkey produces changes in escape behavior suggestive of a transient mechanical and thermal hyperalgesia. After capsaicin application the monkey escaped more frequently at all stimulus intensities, including intensities that did not lead to escape behavior before capsaicin treatment. This interpretation is supported by our findings that human subjects submitted to the same

procedures report painful stimuli as more painful and previously non-painful stimuli as painful (hyperalgesia and allodynia). These findings are consistent with earlier reports that topical application of capsaicin produces mechanical and thermal hyperalgesia in human subjects (Culp et al., 1989; Kilo et al., 1994).

The data for the thermal escape task are particular convincing. Before application of capsaicin, the monkey frequently escaped the 51°C stimuli, but almost never the 43°C and 47°C temperatures. This behavior corresponds to the reports of human subjects who received the same treatments: 43°C was described as slightly warm, 47°C as distinctly warm but non-painful and 51°C as clearly painful. The parallel findings that after capsaicin application the monkey escaped the 43°C and 47°C stimuli and that humans reported these temperatures as painful strongly indicates that thermal allodynia had developed in the monkey. The observation that the monkey also escaped more 51°C stimuli and with faster latencies implies that thermal hyperalgesia had also developed.

The monkey also demonstrated increased escape behavior to mechanical stimuli after capsaicin, consistent with the human subjects' increased pain ratings. Nevertheless, these data were somewhat less consistent than those for the thermal stimuli. First, before capsaicin treatment the monkey sometimes escaped stimuli that human subjects never reported as painful (e.g. 245 and 490 mN). Since the mechanical stimulator was advanced and retracted, in contrast to the stationary

thermal stimulus, these results may reflect a general disturbance of the monkey by being suddenly touched. Second, after capsaicin treatment, the increase in mechanical escape behavior was modest. Even for the highest force used, 1472 mN, the withdrawal rate after capsaicin application did not surpass 60%. This modest increase in escape behavior was, however, paralleled by similar small increases in human pain ratings. There are several possible explanations which might account for this reduced effect. First, it might be that the concentration of capsaicin required to produce mechanical hyperalgesia is higher than that required to produce thermal hyperalgesia. Experimental support for this hypothesis can found in the study by Culp et al., (1989) where it was shown that a dose of 6.6×10^{-3} M of capsaicin produced a marked effect on heat pain thresholds but only had a minor effect on mechanical pain thresholds. A second possible explanation for our smaller effect on mechanical trials has to do with the duration of the mechanical stimuli. Kilo et al., (1994) showed that the sensory ratings to tonic pressure after capsaicin application are largely affected by the stimulus duration. Whereas forces of 2 and 4 N did not produce an initial painful sensation, after sustained application (within 20s) they were able to provoke pain sensations. Thus longer stimulus durations might have resulted in larger capsaicin effects on mechanical trials.

For both thermal and mechanical perception, our data suggest that the hyperalgesia is transient. Ratings by human subjects for both thermal

and mechanical stimuli had returned to normal values by 24 h post-capsaicin. For the monkey, mechanical escape rates returned to normal by 24 h, but thermal escape rates were still somewhat elevated. Nevertheless, at 48 h post-capsaicin, the monkey's escape behavior was no longer significantly different from pre-capsaicin values (data not shown). Culp et al., (1989) reported decreased pain thresholds for periods up to 24 h after topical capsaicin administration, indicating that the increased sensitivity, although temporary, may be of variable duration, depending on such factors as skin region, dosage, and duration of application.

It has been shown that repetitive applications of capsaicin may produce long-lasting damage of nerve terminals. Carpenter and Lynn (1981) found that topical application of capsaicin initially reduces pain thresholds but after several applications increases thermal pain thresholds (skin becomes hypoalgesic to thermal stimulation). We took several measures to prevent such desensitization. First, we changed the site of capsaicin application for each experiment so that the same area of skin did not receive capsaicin twice. Second, we used a much longer time interval between successive capsaicin applications. Whereas Carpenter and Lynn made seven repetitive capsaicin application within a 24 h period, we waited at least 1 week between applications. In our study, we observed no systematic change in escape behavior across the pre-capsaicin test days, indicating that in fact no nerve damage occurred. However, in future applications of this model that might require shorter interstimulation

intervals or repeated stimulation to the same skin region, it might be advantageous to use an algesia agent that has less potential for creating long-term nerve damage, such as mustard oil.

In our study we chose topical application of capsaicin over intracutaneous injection, as has been used in some human psychophysical and neurophysiological studies. Human psychophysical studies reveal that intracutaneous injection of capsaicin produces an immediate and extremely severe burning pain, which is inappropriate for awake monkey experimentation (Simone et al., 1989; LaMotte et al., 1992). The findings that after topical capsaicin application the monkey showed no signs of distress and continued to perform the appetitive visual detection task without disruption suggests that our procedure is minimally stressful.

It is well documented that two zones of hyperalgesia develop after capsaicin application. The area where capsaicin is applied is referred to as the area of primary hyperalgesia, while the surrounding uninjured skin is referred to as the area of secondary hyperalgesia. Using topical capsaicin application, this secondary hyperalgesia has been reported to occur for light brushing mechanical stimulation but not for heat or tonic pressure, as used in our experiment (Koltzenburg et al., 1992; Kilo et al., 1994; Schmelz et al., 1996). Thus, in this study we limited our tests to the area where the capsaicin was applied and did not test for secondary hyperalgesia. Further studies could be designed adapting our model for

the study of secondary hyperalgesia by using a computer-controlled brush stimulus.

The present model of acute, transient hyperalgesia may be particularly suitable for neurophysiological studies (Chen et al., 1996) and offers several advantages over previously reported approaches. First, operant responses are used in the present model. These are at a hierarchically higher level than reflex-type responses that are used in many studies. Moreover, we found a good correlation between the animal's operant responses to the graded stimuli and psychophysical ratings of painfulness of these stimuli in human volunteers. Second, awake behaving animals were used so that a direct correlation between behavior and neuronal discharge could be studied. Third, the obtained results were not influenced by the administration of anesthetics. Fourth, the present approach allows the study of dynamic changes in single neurons that cannot be studied with models of peripheral nerve ligation that produce long-duration or permanent hyperalgesia. Finally, the present approach posed fewer ethical problems than models involving permanent lesions. A drawback, however, is that this model requires an extensive period of laborious training of the monkey in order to get reliable and stable data.

Fig.1. Percentage of escapes to three intensities of heat stimuli (43, 47 and 51 °C) before, 1h after and 24 h after topical capsaicin application in the monkey maxillary region. The animal escaped significantly more stimuli 1 h (ANOVA; $p<0.001$) and 24 h (ANOVA; $p<0.01$) after capsaicin application.

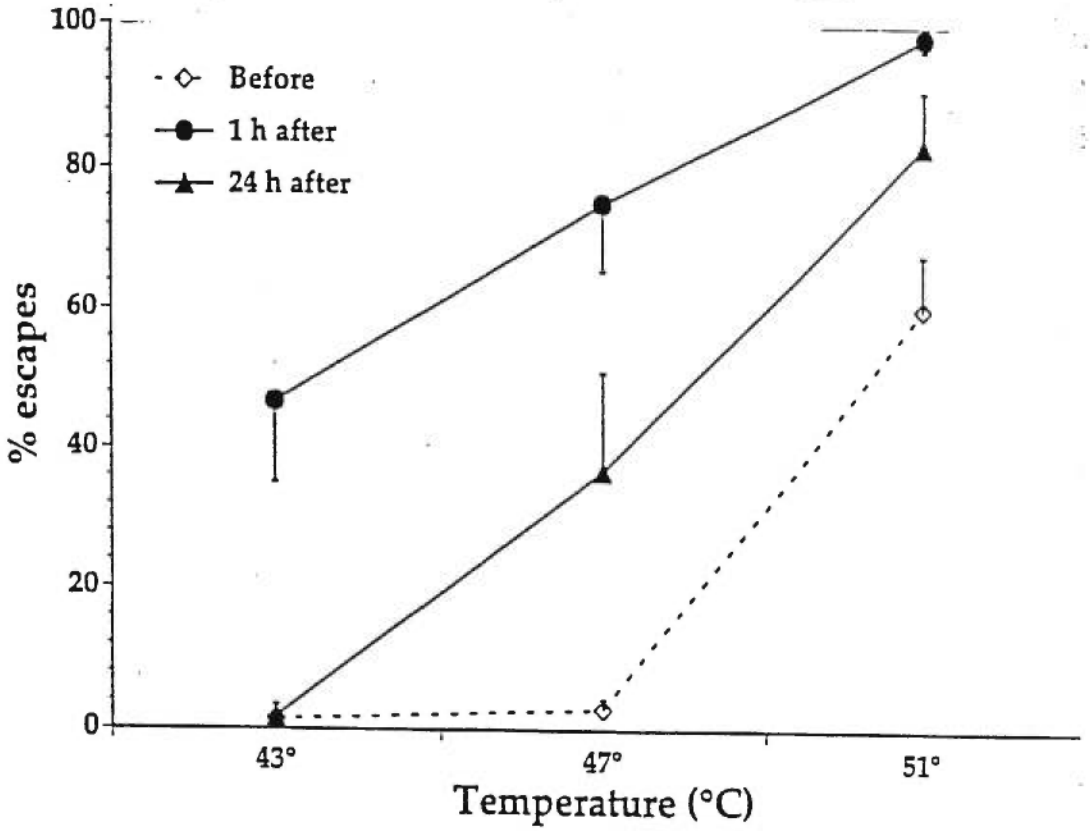

100
80
60
40
20
0
% escapes
Before
1 h after
24 h after
43°
47°
51°
Temperature (°C)

Fig. 2. Percentage of escapes to four intensities of mechanical stimuli (245, 490, 736, and 1472 mN) before, 1h after and 24h after topical capsaicin application in the monkey maxillary region. The animal escaped significantly more stimuli 1 h after capsaicin application (ANOVA; $p<0.05$). Values at 24 h after capsaicin were not significantly different from baseline values (ANOVA;$p>0.05$).

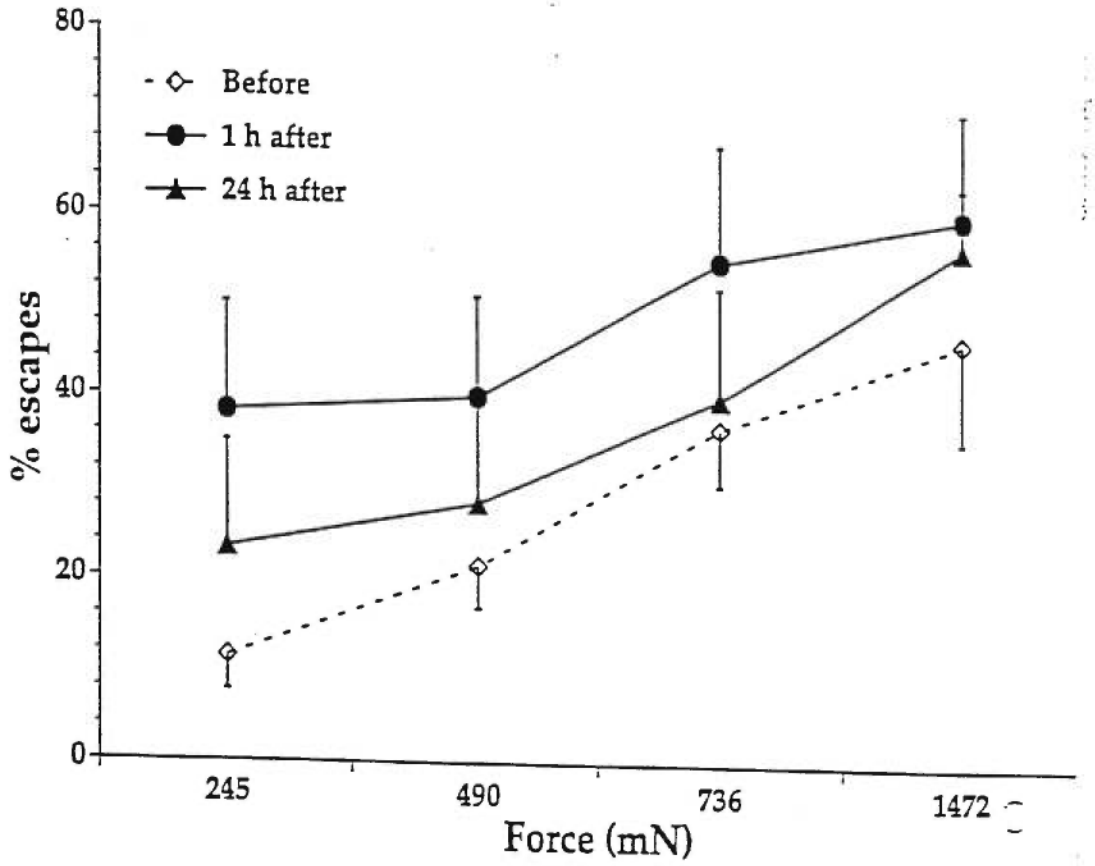

80
60
40
20
0
% escapes
Before
1 h after
24 h after
245
490
736
1472
Force (mN)

Fig. 3. Spontaneous pain ratings in seven human volunteers during and after topical capsaicin application. Spontaneous pain was scored on a scale of 0 to 100, "0" meaning no pain and "100" the most intense pain imaginable. The capsaicin patch was removed from the skin at 30 min. Within 1 min of capsaicin application, subjects reported a slight pain. The pain reached its maximum at 10 min, after which it gradually diminished. One hour after application, all subjects were pain-free.

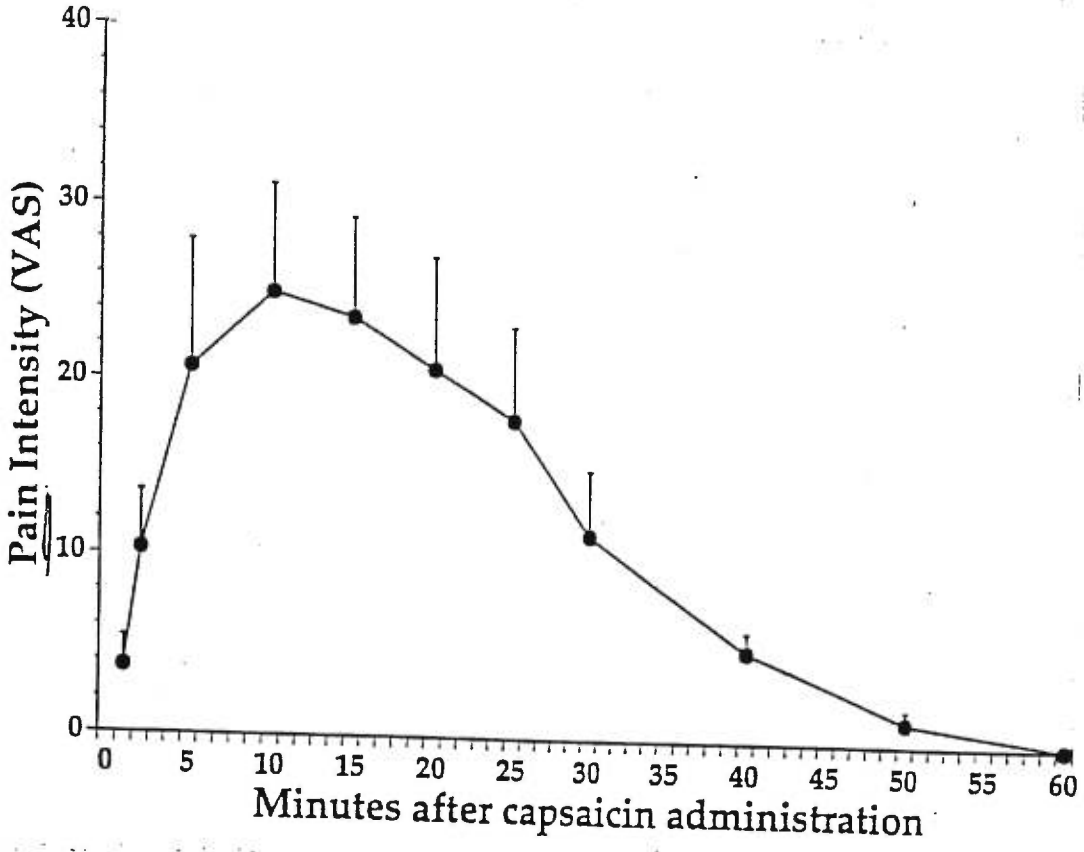

Pain Intensity (VAS)
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
55
60
Minutes after capsaicin administration

Fig. 4. Effect of capsaicin on intensity ratings of innocuous and noxious thermal stimuli in seven human volunteers. Ratings below 100 represent non-painful heat intensity, values greater than 100 (dotted line) represent pain. "0" and "200" correspond with no sensation and the most intense pain imaginable respectively. One hour after capsaicin application, subjects reported significantly higher intensity ratings than before capsaicin (ANOVA; $p<0.01$). The value observed at 24 h was not significantly different from baseline value.

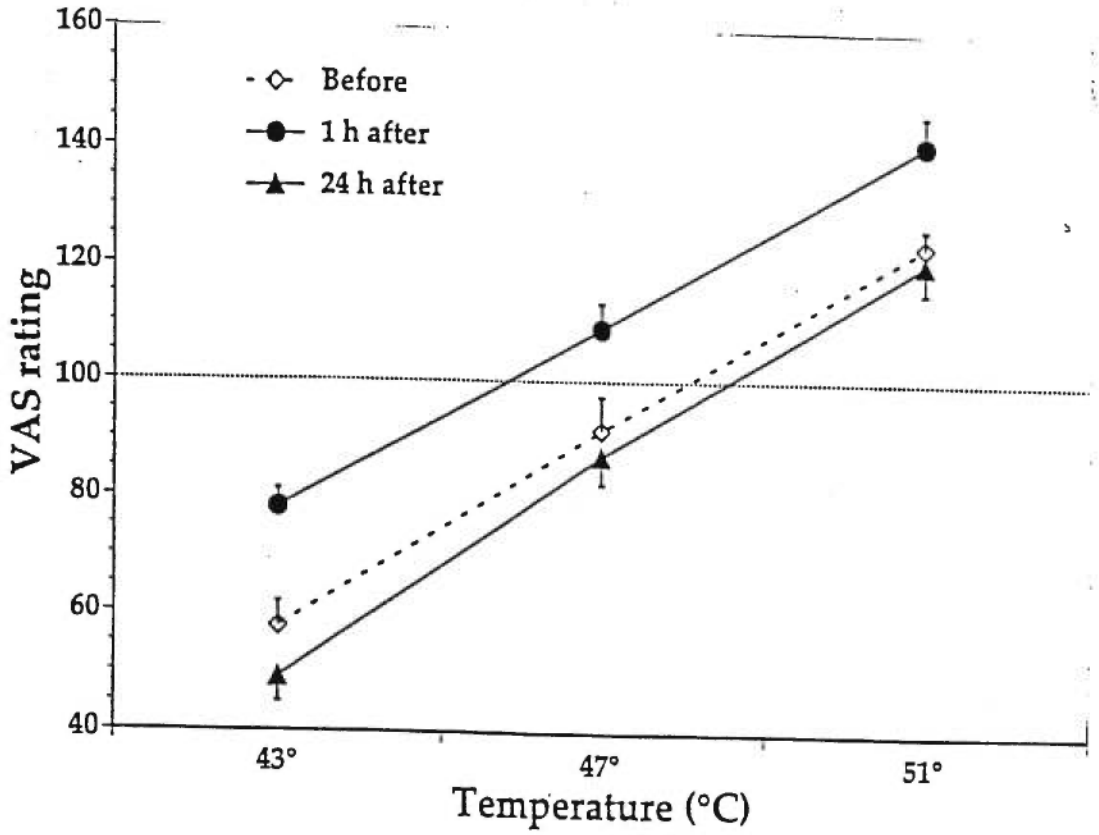
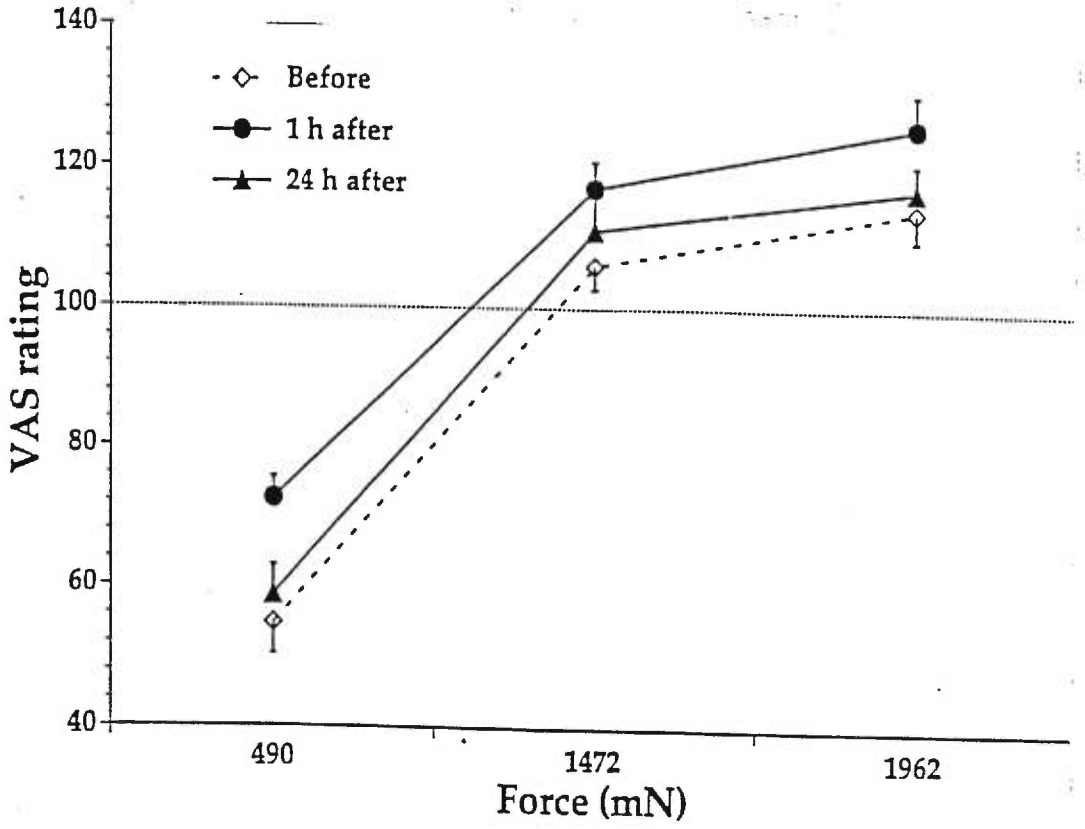

160
140
120
100
80
60
40
VAS rating
Before
1 h after
24 h after
43°
47°
51°
Temperature (°C)

Fig. 5. Effect of capsaicin on intensity ratings of innocuous and noxious mechanical stimuli in seven human volunteers. The rating scale for pressure was similar to that for heat (see legend to Fig. 4). One hour after capsaicin application, subjects reported significantly higher intensity ratings than before capsaicin (ANOVA;$p<0.05$). At 24 h post-capsaicin, ratings were not significantly different from baseline values.


140
120
100
80
60
40
VAS rating
Before
1 h after
24 h after
490
1472
1962
Force (mN)

Chapter 5 Response characteristics of thalamic ventral posterior medial neurons to quantitative mechanical stimulation in awake monkeys

5.1 Summary:

1. This study investigated the responses of thalamic VPM neurons to punctate mechanical stimuli in primates. We performed single-unit recordings within the thalamic VPM nucleus in two awake behaving monkeys (Macaca Mulatta). The intensity of a mechanical stimulus applied to the monkey's face ranged from innocuous (245mN) to noxious (1472mN), and was controlled by a computer. Thirty-seven neurons with contralateral cutaneous receptive fields on the face were collected. Neurons that responded to the stimulation of deep or intra-oral structures were excluded.

2. All neurons showed spontaneous activity in the absence of stimulation. Both the spontaneous and mechanically evoked activities were recorded, while the monkey performed visual tasks and received the mechanical stimuli. The difference of activity was calculated by subtracting the spontaneous activity from the mechanically evoked activity. The ratio of spontaneous and mechanically evoked activities was also measured to verify the characteristics of neuronal responses.

3. We classified the neurons as low-threshold (LT; 19/37) if the mechanically evoked activity was not significantly different between the innocuous and noxious stimuli, or wide-dynamic-range (WDR, 18/37) if the neurons showed a significant and maximally graded response to noxious stimuli. Nociceptive-specific neurons were not found in this study. LT neurons were further classified as having slowly adapting (SA) or rapidly adapting (RA) properties

according to their responses at the onset or offset of the stimuli. Fourteen of them were SA and five were RA.

4. The size of the receptive fields was not significantly different between LT and WDR neurons (averaged receptive fields were 44.9, 36.2cm^2, respectively). The distribution of LT and WDR neurons within the VPM nucleus was somatotopically organized.

5. This study provides a quantitative analysis of the responses of thalamic VPM neurons to innocuous and noxious mechanical stimulation of the skin in the awake monkey. This information about normal cutaneous processing is necessary for understanding the interaction between noxious and low-threshold mechanical pathways especially under pathological conditions, such as mechanical hyperalgesia or allodynia in central pain syndrome patients.

5.2 Introduction

The ventral posterior medial nucleus (VPM) of the thalamus is the region of the ventrobasal complex (VB) which receives somatosensory projections from areas innervated by the trigeminal nerve. Neurophysiological and anatomical studies in anesthetized and awake primates demonstrate that VPM nucleus receives projections from the principal sensory nucleus and spinal trigeminal nucleus, which convey tactile and noxious information respectively, (Apkarian and Shi, 1994; Bushnell and Duncan, 1987; Bushnell

et al., 1993; Duncan et al., 1993; Hayward, 1975; Jones et al., 1986; Kaas et al., 1984; Koyama et al., 1998; Price et al., 1976; Yokota, 1985). Termination of these trigeminal projections in VPM have been reported to be either in clusters of SA and RA neurons (Bushnell and Duncan, 1987; Hayward, 1975; Kaas et al., 1984), or in the form of a nociceptive shell region surrounding a central core of LT neurons (Koyama et al., 1998; Yokota, 1989). Studies of thalamocortical projections in monkeys also demonstrate that the cortical projections of the VB complex (mainly in SI and SII areas) are somatotopically organized (Friedman and Murray, 1986; Friedman et al., 1986; Gingold et al., 1991; Krubitzer and Kaas, 1992; Mayner and Kaas, 1986; Rausell et al., 1998; Rausell and Jones; 1991; Shi et al., 1993; Steven et al., 1993; Whitsel et al., 1978). Furthermore, Morrow and Casey (1992, 1988) have reported that in the somatosensory thalamus of the awake monkey, the spontaneous activity and neuronal responses to thermal and mechanical stimuli could be modulated by the state of arousal. All of these observations indicate that the thalamic ventrobasal complex plays an important role in conveying somatosensory and pain information, which transmits the peripheral stimuli into conscious awareness.

Using electrophysiological methods, the characteristics of thalamic VPM neurons have been investigated, with most of the descriptions of VPM neurons incorporated within the surveys of the ventrobasal complex. Among these, a majority of low-threshold (LT) mechanosensitive neurons has been

reported (Bushnell and Duncan, 1987; Darian-Smith, 1966, 1964; Dykes et al. 1981; Golovchinsky et al. 1981; Jones & Friedman, 1982, Jones et al., 1982; Kaas et al. 1984; Loe et al. 1977; Morrow & Casey, 1992, 1990; Pollin & Albe-Fessard, 1979; Tremblay et al., 1993; Yokota et al. 1985). These studies showed that the thalamic LT neurons responded best to innocuous tactile stimulation (touch or hair displacement). According to the neuronal responses to the onset and offset of the mechanical stimuli, these LT neurons could be classified as slowly adapting (SA) or rapidly adapting (RA) (Baker, 1971; Bushnell and Duncan, 1987; Darian-Smith, 1966; 1964; Dykes et al., 1981; Golovchinsky et al., 1981; Iwamura and Inubushi, 1974; Jones and Friedman, 1982; Kaas et al., 1984; Loe et al., 1977; Perl and Whitelock; 1961). However, no consensus has been reached regarding relative proportion of these population of RA and SA neurons.

The VB complex has also been investigated for neurons with nociceptive properties in both anesthetized and awake monkeys (Kenshalo et al., 1980; Apkarian and Shi, 1994; Bushnell et al., 1993; Bushnell and Duncan, 1987; Casey and Morrow, 1987, 1983; Chung et al., 1986a; Duncan et al., 1993; Koyama et al., 1998; Morrow and Casey, 1992). These results show that thalamic neurons of the VB complex are capable of encoding spatial and temporal features of noxious stimuli in addition to the tactile sensation. In these studies, most nociceptive neurons were reported as WDR, with responses that are graded in intensity as the stimulus

increases from innocuous to noxious. Only a few nociceptive-specific neurons, which responded exclusively to the noxious stimulation, were reported in anesthetized monkeys (Casey and Morrow, 1983; Koyama et al., 1998).

Among these studies, most address heat-evoked neuronal activity, while only a few studies investigated the response properties of VPM to mechanical stimuli. In the latter studies, most investigators employed non-quantitative mechanical stimuli such as brush, touch, pressure, and pinch (Bushnell and Duncan, 1987; Darian-Smith, 1964; Kenshalo et al., 1980); thus, there is little information concerning quantitatively measured mechanically evoked responses.

The current study is designed to investigate the response characteristics of mechanosensitive VPM neurons to quantitative mechanical stimuli ranging from innocuous (245mN) to noxious (1472mN).

5.3 Materials and methods

Prior to beginning the behavioral training, the monkey's food and water intake, as well as body weight, were monitored for two weeks to determine the ad libitum values. During the training period, food intake in the home cage was restricted for several hours before each daily training session. The monkey was rewarded with a banana-flavored liquid diet

(Bioserve Liquidiet) during the experiments, and was given food in the home cage after each session. Throughout the experiments, the body weight was monitored daily to ensure the animal's weight remained at least 90% of ad libitum values. Such monitoring continued throughout all stages of training, as well as the behavioral experiments and neuronal recording.

5.3.1 Apparatus

The apparatus used for the mechanical stimulation has been described in detail in the previous chapter (Kupers et al., 1997; Ten Bokum ET al., 1996, and in Chapter 4). In short, a thermally neutral mechanical probe (tip diameter: 0.2mm) delivered three different computer-generated mechanical forces (245, 736, 1472 mN) to the face. The mechanical probe was positioned about 5mm from the surface of the skin. A pressure transducer was used to measure the force at the skin/probe junction and provided precise feedback control of the mechanical stimulation. Visual stimuli were presented with a 3-cm diameter translucent light located 35-50 cm in front of the monkey. A computer controlled the intensity and duration of the mechanical and visual stimuli. A response lever, placed in front of the monkey, was used for initiation and termination of each trial by the animal, itself. To insure consistent application of stimuli, the monkey's head was stabilized during the training session with a custom-fit acrylic loop around the muzzle and behind the head. These stabilizing devices were replaced

by two stainless-steal posts which could attach to the primate chair during the recording sessions.

5.3.2 Experimental Tasks

We designed two experimental tasks for the training of two awake behaving monkeys. In the first task, the monkey had to perform a simple light detecting trial to obtain the liquid rewards. In addition, the animal received an alternative mechanical stimulation, which could be terminated by the animal by pressing the response lever (escape task). In the second task, another monkey was trained to discriminate the change of illumination in the visual trial, with simultaneous application of a constant period of mechanical stimulation on the face (constant-stimulation task).

Escape task: One adult male monkey (macaca mulatta, M-33) was trained to press the response lever either to receive a reward during visual trials or to escape from a mechanical stimulus applied to it's face during mechanical trials. During visual trials, the monkey could receive a liquid reward for pressing the response lever whenever a white light was illuminated. During mechanical trials, when a mechanical stimulus was presented to the monkey's face, the animal had the choice of either tolerating the stimulus or escaping from it by pressing the lever. The maximum duration of the stimulus was 4.5 seconds if the monkey did not press the lever. In contrast

to the visual trials, no reward was offered when presenting the mechanical stimuli.

Constant-stimulation task: Using the same apparatus, a second adult male monkey (Macaca mulatta, M-10) was trained to do a visual discrimination task. This task required the animal to press a lever to initiate a trial when presented a red cue light. A white light (L1) appeared for 3.5 seconds and then the luminance was increased (L2). Reward was offered if the monkey was able to detect the change of luminance. This visual task ensured that the animal could receive a certain number of rewards and that the animal would be motivated to continue the task. Mechanical stimulation was applied simultaneously during the light trials, independent of the monkey's performance.

5.3.3 Surgical procedures

Following the training period, a recording chamber was implanted stereotaxically on the right side of the skull under standard surgical procedures and general anaesthesia. The monkey was initially prepared using Ketamine anesthesia (10-mg/kg i.m.) and atropine sulfate (0.05mg/kg) to control the salivary secretions. The trachea was intubated with an endotracheal tube lubricated with local anesthetics (Xylocaine 2%, Astra). After the intubation, isoflurane gas (1.0-2.0%) was used for the

induction and maintenance of general anesthesia. Ophthalmic ointment was applied to the eyes to prevent corneal drying, the head furr was clipped, and the head was positioned in a stereotaxic frame. An anterior-posterior incision of approximately 3 inches was made on the scalp; the skin and underlying connective tissue were retracted to expose the skull bone. A 20-mm-diameter circle was drawn on the skull, and a dental drill was used to remove the bone, leaving the dura intact. The recording chamber was placed over the hole and 5-10 small size titanium screws were implanted around the chamber. The exposed area then was cleaned and covered with Codman sterile neurosurgical acrylic, thus attaching the recording chamber to the skull, with stabilization provided by the titanium screws.

Thalamic coordinates were chosen (A6.0, L6.0) according to the monkey stereotaxic atlas of Olszewski (952), so that electrodes passing through the center would reach the ventrobasal complex of the thalamus. A recording chamber and "X-Y" manipulator base were fixed perpendicular to the surface of the dura. This permitted us to replace a recording grid in the same position during each recording session. An extending slot was designed to allow a 5-degree angle forward, thus doubling the number of penetrations. For stabilization of the head, two stainless steel posts were placed on top of the neurosurgical acrylic and fixed with additional dental acrylic, these were used for attaching the head to stabilizing equipment

during recording experiments. Antibiotic ointment (Neosporin) and sterile saline (0.9% NaCl) were placed in the chamber, which was then sealed with a plastic cap.

After the surgery, the monkeys returned to the home cage and were under close observation until they could stand freely. Antibiotics (Septra, pediatric formulation) and analgesics (Buprenorphine) were given postoperatively for a week. Cleaning around the skull implant was done daily, and polysporin ointment and sterile saline were replaced in the chamber at that time. When extensive cleaning was required, the animal was tranquilized with a low dose of Ketamine HCL (5mg/kg) during the procedures. This experimental protocol was approved by the ethical committee of McGill University and Université de Montréal, and was in accordance with the guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain.

5.3.4 Experimental protocol for single-unit recording

Recording experiments began 10 days after the implantation of the recording chamber and head-holding post. The first week was dedicated to re-acquainting the monkeys with the behavioral tasks that they had previously learned. After the animals had performed the tasks reliably and consistently, recording of extracellular single-unit activity was initiated. Recordingswere made using gold and platinum plated Tungsten electrodes

(impedence 0.5MΩ, at 1KHz). The electrode was introduced into the brain by a micro-drive through a sterile stainless steel guide-tube. At the end of the recording session, the electrode was withdrawn, a new antibiotic solution was placed in the cylinder, and the skin around the skull implant was cleaned. During each recording day, the monkey sat in a primate chair with the head restrained. The side of the face to be tested was shaved on a regular basis more than 24 hours before the experiments. A 3-cm diameter translucent light and a response lever were attached 35-50 cm in front of the animal. In every recording session of the experiment, each mechanical intensity was applied 10 times pseudo-randomly. Spontaneous and mechanically evoked activities were recorded during each trial. The animals received rewards as they had during the training session; experimental sessions usually lasted 2-3 hours.

At the beginning of a recording session, the VPL nucleus was initially targeted in order to serve as a reference point for localizing VPM (Kaas, et al., 1984). For each penetration, tapping or touching was performed until a cutaneous region was found capable of driving the neurons. In this experiment, neurons were selected only if they responded to punctuate stimulation (i.e. a sharpened pencil) on the contralateral facial skin. Neurons that were driven by stimulation of non-cutaneous tissues or related to mouth movements were excluded. The VPM neuronal discharge was recorded while the monkey performed the tasks. Extracellular potentials of single

neurons were used to trigger a window and only those neurons whose action potentials could be reliably separated in amplitude from the activity of neighboring cells were studied. The signals were amplified (5-10 K), filtered (300 Hz, low pass and 5-10 kHz, high pass), and digitized (32 kHz) (High-gain, differential microelectrode AC amplifier, Model 1800, A-M system, Inc). Then, the amplified signals were fed into the window discriminator (Model DIS-1, Bak electronics, Inc.) and displayed on the oscilloscope screen. Cell responses to mechanical stimulation were recorded using a histogram program (1 s bins). The data then were recorded and stored using a customized program and a PC computer for off-line analysis.The signal was amplified and discriminated on the basis of the signal waveform and amplitude. An input oscilloscope, a trigger oscilloscope and a storage oscilloscope were used to monitor the signal-noise ratio, the isolated spikes, and to verify the consistency of the amplitudes and waveforms of spikes. Both monkeys performed visual trials only during the process of searching and isolating a single neuron; no mechanical stimulation was applied during this period.

5.3.5 Histology

Unfortunately, after three months of recording, one monkey (M-33) experienced a seizure and died. The tissue was not adequately perfused,

and the histological result was not optimal and will not to be used in this report.

For the other monkey (M-10), histological verification of the locations of the electrode tracks was carried out after 5 months of recording. Electrolytic lesions were made along two micro electrode tracks (one within the recording track and one lateral to the recording track) by passing 10-15 uA anodal DC current for 10 seconds. Serial 50 μm coronal sections stained with cresyl-violet were made in order to identify the subdivisions of the thalamus and the recording tracks.

5.3.6 Data acquisition and Analysis

Receptive field: When a single unit had been isolated, the receptive field was evaluated with a sharpened pencil and marked with a coloured pen in order to be consistent in the presentation of mechanical stimuli. Several anatomic landmarks (anterior border of the mouth, ear lobe, inferior orbital ridge) were used as references to transfer the location and the size of the receptive field to a schematic drawing of the monkey's face.

Neuronal discharges: In each trial, spontaneous activity was recorded for three seconds before applying mechanical stimulation. During the escape tasks, neuronal responses evoked by the mechanical stimulation were collected before the escape or the termination of the stimulus. During the constant-stimulation tasks, neuronal activity was recorded for 3.5

seconds of mechanical stimulation for ten applications of each intensity of mechanical force. The recorded data were then converted into on-line peristimulus histogram and stored in a PC for further off-line analysis.

In this study, ***spontaneous activity*** was defined as the number of neuronal discharges that occurred without the presence of either tactile nor thermal stimuli in the receptive field. The spontaneous activity was collected before each application of the mechanical stimulus. Mean spikes per second were calculated for further analysis. Neuronal responses recorded while mechanical stimuli were present on the receptive fields were defined as ***mechanically evoked activity.*** All the neurons presented here showed significant responses to the mechanical stimuli, when compared with the preceding spontaneous activities (ANOVA, repeated measure, $p<0.005$). These response discharges were also used to categorize the neurons (see below). The spontaneous activity subtracted from mechanically evoked activity was defined as the ***difference of the activity***. The mechanically evoked activity divided by the spontaneous activity was defined as the ***ratio*** of the activity. These data were used to confirm the characteristics of neuronal responses to mechanical stimulus.

Definition and Classification of neuronal activity

The responses to mechanical stimuli were used to categorize the recorded neurons. The mean spikes per second of spontaneous activity and

mechanically evoked activity were calculated. By using repeated measure ANOVA and a post-hoc comparison, those neurons showing a significant difference between mechanically evoked and spontaneous activities were selected for further analysis. In this study, VPM neurons were classified as LT or WDR according to their responses to graded intensities of mechanical stimulation ranging from innocuous to noxious. Neurons that responded maximally to innocuous stimulation (245, 736mN) (no statistical difference between 736 and 1472mN) were defined as LT, whereas neurons which responded in a graded fashion and increased maximally to noxious stimulation (1472mN) (statistics showed significant difference among 245, 736, and 1472mN) were defined as WDR. According to the correlation with presence of the mechanical stimuli, LT neurons were further sub-grouped as slowly adapting property which showed a tonic response to maintained stimulation or rapidly adapting property which showed the activity to the "on" and "off" of the stimulator. Neurons which responded exclusively to noxious stimuli (nociceptive-specific) was not found in this study.

5.4 Results

Thirty-seven thalamic VPM neurons with contralateral receptive fields on the face were studied in two awake monkeys. Five of these were classified as RA, 14 as SA and 18 as WDR neurons. Correlation of the classification and the thalamic recording location of these neurons is shown

in Table 1. Response characteristics of these neurons to mechanical stimuli are described, and for 27 of these cells, receptive field size and related thalamic schema of one monkey (M10) are also presented.

5.4.1 Neuronal sample

The neurons selected in this study were those that responded to contralateral cutaneous punctate mechanical stimulation. Neurons that responded to mouth movements or only to touch or hair displacement but not punctate stimuli, or those that had bilateral receptive fields were excluded from this study. In total, thirty-seven thalamic VPM neurons with contralateral receptive fields on the face of two monkeys (10, 27 neurons, respectively) are presented here. Of these 37 neurons, nineteen were classified as LT and 18 as WDR. LT neurons responded maximally to innocuous stimulation and there was no statistical difference between 736 and 1472mN intensity. Whereas, the responses of WDR neurons showed significant difference to 245, 736, and 1472 mN intensity. The majority of LT neurons (14 of 19 LTs) exhibited slow adapting (SA) properties, i.e. a tonic response to maintained stimulation. Others showed rapid adapting (RA) characteristics with a burst at the onset and offset of the mechanical stimulation. The location and the size of receptive fields of twenty-seven neurons in one monkey (M-10) are shown in Figure 1. The average size of the receptive fields was slightly larger in the WDR group than in the SA and

RA groups but was not significantly different (44.9 mm^2 , 28.8 mm^2, and 38.9 mm^2 p=0.5).

5.4.2 Thalamic distribution

Microelectrode penetrations performed in this study were advanced from dorsal to ventral through the thalamus. Since most of the recorded neurons were not collected on the same experimental day, by using the histological findings and reconstruction procedure, we tried to determined the anteroposterior and mediolateral arrays as accurately as possible. The locations of the twenty-seven units in one monkey were mapped according to a thalamic schema after histological study as shown in Figure 2A, B. In this sample, various neurons seemed to have an even distribution. Different types of neurons could be found in the same recording track.

Location of the recorded neurons: According to stereotaxic comparisons, all the recorded neurons were located in the posterior part of the VPM. In one monkey (M-10), most neurons were found in the recording tracks of A+11.0-12.0 with mediolateral arrays between L7.5-9.0. Some neurons were isolated in the penetration tracks of A+9.0-10.0 with mediolateral arrays between L8.5-9.5.

AP +9, +10 with Mediolateral arrays between L+8.5 ~ +9.5

The reconstructed penetrations shown in Fig.2A illustrate one laterally located WDR neuron with the receptive field on the zygomatic area,

and two medially located neurons (one LT & one WDR) with the receptive fields in the anterior maxillofacial area.

AP +11, +12 with Mediolateral arrays between L +7.5 ~ +9.0

Twenty-four neurons were recorded at AP levels between +11 and +12. There were 15 LTs and 12 WDRs (Fig. 2B). These two types of neurons were evenly distributed in VPM. A somatotopic organization clearly exists; VPM neurons with receptive fields on oral and anteromedial facial areas were located more medially than that of the posterolateral facial areas.

5.4.3 Characteristics of mechanical responses

The neuronal activity of a typical LT neuron, located at the mediodorsal border of VPM, is shown in Figure 3. The neuron responded maximally to innocuous stimuli (245, 736mN). A WDR neuron, located in the lateral border of VPM, is shown in Figure 4. Its neuronal responses showed graded increases to stronger mechanical stimuli, especially in the noxious range (1472mN).

The individual neuron stimulus-response curves for innocuous and noxious stimulation are shown in Figure 5. For all the neurons, spontaneous activity recorded during the pause between mechanical stimuli showed constant activity throughout each recording session. Mechanically evoked

activity of LT neurons reached a plateau during the application of the innocuous stimuli (245, 736 mN). During noxious stimulation, the neuronal activity did not show further increases, which is consistent with the definition of LT type neurons. Activity of WDR neurons usually increased during the innocuous stimulation, and showed further increases to the noxious stimuli (1472mN). This response characteristic was also noted consistently when spontaneous activity was subtracted from the evoked activity (i.e., the difference of the activity, Figure 5C).

The average stimulus-response functions for LT and WDR neurons are shown in Figure 6. The mean spontaneous activity of LT neurons was 12.0 ± 2.5 spikes/sec (mean ± SE, n =19) and for WDR was 11.9 ± 1.6 spikes/sec (mean ± SE, n =18). For LT neurons, the stimulus-response functions for graded mechanical stimulation demonstrate significantly higher stimulus-evoked activity compared with their spontaneous activity (ANOVA, $p=0.028$). This evoked activity reached a maximum at 736mN, and no difference was found between evoked responses to mechanical intensities of 736 and 1472mN (ANOVA, repeated measure, and post-hoc, $p<0.001$ between 245 and 736mN; $p=0.152$ between 736 and 1472mN). In contrast, significant differences were found in the activity of WDR neurons among the different intensities of mechanical stimuli (ANOVA, repeated measures, $p<0.001$). Figure 6b shows an ascending response to increased mechanical intensity for WDR neurons.

A direct comparison of neuronal activity observed in LT and WDR neurons is presented in Figure 7. Both types of neurons showed similar spontaneous activity (Figure 7A). In terms of the mechanically evoked activity, both LTs and WDRs showed similar responses to the lowest mechanical stimulus ($p=0.95$ at 245mN). These mechanically evoked increased discharges tended to become obviously different between the two groups when the mechanical intensity was increased into the noxious range ($p=0.36$ at 736mN and $p=0.09$ at 1472mn level, respectively) as shown in Figure 7B. These increased discharges were further verified by calculating the difference of the activities which represented the net activity of mechanically evoked activity subtracted from the spontaneous activity (Figure 7C). A significant difference between groups at the noxious level (1472mN) was noted ($p=0.006$). The ratio of evoked and spontaneous activities also confirmed this response characteristic in the noxious range between the two types of neurons ($p=0.04$) as shown in Figure 7D.

5.5 Discussion

Thalamic VPM neurons of two alert monkeys that showed punctate mechanical sensitivity were classified as low threshold neurons (n=19), in that they responded best to the innocuous stimuli (245 and 736 mN) on the facial skin. Of these, 5 had rapid adapting and 14 had slowly adapting properties. In addition, there was a population of 18 neurons showing wide-

dynamic-range properties that responded best to noxious stimulation (1472mN).

Mechanical stimulus

Adequate mechanical stimulation is especially important for studies of somatosensory sensation and pain. Traditional methods use brush, pinch or von Frey hairs to investigate the mechanosensitivity of neurons in electrophysiological and psychophysical studies. These methods have a disadvantage that the mechanical forces applied to the receptive area might not be constant throughout the experiment, thus making a comparison of neuronal activity among different experiments difficult. In addition, the size of the mechanical probe and the angle of stimulus could effect perception as indicated by Greenspan and McGillis (1991). They reported that with a probe size of 0.01mm, the sharpness and pressure threshold are very close. Our studies used a 0.02mm probe tip and computer controlled forces, and the results were consistent with the findings in animal behavioral observations and human psychophysical data (see Chapter 4; Kupers et al., 1996,1997;). In these studies, 245mN was barely perceptible, 746mN was reported to evoke a strong sensation but not pain, and 1472mN was almost always reported as painful in human subjects. In the behaving monkey, the animal escaped least frequently from the lowest stimuli but did escape most of the 1472mN stimuli. These results indicate that the mechanical stimuli

were consistent and the evoked neuronal responses were reliable. Special attention was also given to the angle when applying the stimulus. Greenspan and McGillis (1991) described that a stimulus angle larger than 15 degrees could result in the change of perception and pain thresholds. In this report, the mechanical probe applied to the receptive fields of all the selected neurons was always positioned perpendicular to the skin area so that the applying angle was never larger than 5 degrees (90°±5). Thus, by using this method, the mechanical forces could be consistently applied and were easily quantified in animal electrophysiological studies, allowing them to be reliably related to human psychophysical studies.

Properties of low threshold neurons in VPM

Although some investigators describe substantial populations of slowly adapting (SA) neurons in thalamic VPM and VPL (Baker, 1971; Darian-Smith, 1966., Iwamura and Inubushi, 1974; Loe et al., 1977; Perl and Whitlock, 1961), they report a predominate population of rapid adapting (RA) neurons in anesthetized cats and monkeys. In the present study, approximately 74% (14/19) of low threshold neurons were found to exhibit slowly adapting properties. This finding is consistent with the high incidence of SA neurons in awake monkeys described by Bushnell and Duncan (1987), in which they found about half of low-threshold neurons exhibited slowly adapting properties.

The reported discrepancies of SA and RA neuronal populations might be due to experimental parameters effecting the animals' consciousness level. In neurophysiological studies, anesthesia and the state of arousal have a potentially confounding influence on the interpretation of the experimental results. It was reported that anesthetics increased the receptive field sizes and the responses of low-threshold thalamic neurons, whereas the activity of WDR neurons was suppressed in rats (Guilbaud et al., 1981) and in monkeys (Dougherty et al., 1997). In somatosensory cortical neurons, Duncan et al., (1982) also demonstrated that anesthetics decreased the spontaneous activity and receptive field size in monkey. In addition, it has been shown that strong arousal increases responses in cortical primary somatosensory neurons in awake rats, and that a higher spontaneous activity is observed in awake animals than in anesthetized ones (Chapin and Woodeard, 1981; Chapin et al., 1981;). Similar observations were reported in awake primates (Morrow and Casey, 1992, 1988), in which it was shown that the spontaneous and evoked discharges of thalamic VP neurons could be modulated by the state of arousal. Taken together, these data provide evidence that the state of arousal and anesthesia may change the response characteristics of low threshold neurons in the thalamus.

Previous studies have shown that the neuronal distribution of SA and RA neurons was found to be aggregated in clusters within the thalamic VP

nucleus of anesthetized (Dykes et al., 1981; Kaas et al., 1984;) or alert monkeys (Bushnell and Duncan, 1987; Hayward, 1975). Similar observations in the somatosensory thalamus of humans were also reported (Lenz et al., 1998a; 1988). In this study, the SA neurons showed a tendency to form clusters, however, the sample size was too small to allow a detailed analysis.

Nociceptive responses in VPM

In addition to the low-threshold VPM neurons, we also found a population of nociceptive neurons (18/37). Nociceptive neurons in the spinal cord and medullary dorsal horn have been demonstrated to project to the ventrobasal complex in monkeys (Apkarian and Shi, 1994; Applebaum et al., 1979; Bushnell et al., 1984; Chung et al., 1986a,b; Giesler et al., 1981; Price et al., 1976; Willis et al., 1974). These results provide strong evidence implying that nociceptive neurons should exist in the ventrobasal complex. However, this inference was not proved in some studies, in that they failed to find such neurons in monkeys (Loe et al., 1977; Poggio and Mountcastle, 1963), nor in human ventral thalamus (Jasper and Bertrand, 1966; Ohye et al., 1972). Nevertheless, other investigators have reported a small population of nociceptive neurons in the VPM and VPL of monkey (Apkarian and Shi, 1994; Bushnell and Duncan, 1987; Casey and Morrow, 1983; Duncan et al., 1993; Kenshalo et al., 1980; Perl and

Whitlock, 1961; Pollin and Albe-Fessard, 1979; Price et al., 1976). In contrast Chung and his colleagues reported a large population of nociceptive VPL neurons (54/110) in anesthetized monkeys (Chung et al., 1986a). The majority of these nociceptive neurons were WDR, with a small percentage of neurons being nociceptive-specific in monkey (Apkarian and Shi, 1994; Chung et al., 1986a; Kenshalo et al., 1980; Perl and Whitlock., 1961) or in raccoons, (Simone et al., 1993). In our study, about 48.6% (18/37) of VPM neurons showed graded responses to increasing mechanical stimuli into the noxious range and were classified as WDR neurons. All these data suggest the predominance of WDR over NS neurons in VPL and VPM of monkeys; however, the absence of NS neurons in unanesthetized monkeys may have resulted from the difficulty in presenting the noxious stimuli to the awake animal. In order to be able to further test quantitatively the punctate mechanical stimulation, we excluded neurons that respond only to hair displacement but not to punctate stimuli. This, however may influence the percentage of neurons grouping.

Species differences of VPM nociceptive neurons

In contrast to the predominance of WDR neurons in monkeys and raccoons, studies of VB in anesthetized cats showed a different result (Yokota et al., 1985; Yokota and Matsumoto, 1983a, b). In the studies of Yokota and colleagues, most of the nociceptive VPM neurons in cats were

NS with only a small population of WDR. They also demonstrated that the anatomical distribution of the VPM nociceptive neurons in cats was different from that found in monkeys: these neurons were found in a "shell" arrangement, rather than the "clustering" in the nuclei proper (Yokota and Matsumoto, 1983a, 1983b; Yokota et al., 1985), a finding reported also by others (Honda et al., 1983; Kniffki and Mizumura, 1983). Recently, a similar shell arrangement of WDR and NS neurons in the periphery of VB complex of monkey was also reported by Koyama et al., (1998). Our study and those of other in monkeys and rats showed a mixture of both nociceptive neurons and low threshold neurons in the VPM nuclei proper rather than the peripheral nociceptive shell arrangement .

Receptive field characteristics

Most of the WDR neurons observed in the medullary dorsal horn have a receptive field that includes parts of two or three trigeminal nerve divisions (Hoffman et al., 1981). Bushnell and Duncan (1987) reported that in the VPM all but one WDR neuron had receptive fields restricted to one division in the alert monkey. In the present study, we confirmed this observation by showing that all neuron types had their receptive fields restricted to one trigeminal division. Honda et al., 1983; Kniffki and Mizumura, 1983; VPM neurons with bilateral receptive fields near the midline have been reported in monkeys by some investigators (Bushnell and

Duncan, 1987; Darian-Smith,1964;1966); and VPM neurons with ipsilateral receptive fields have also been described (Bushnell and Duncan, 1987; Jones et al., 1986). In addition, there is also a species difference in the mechanosensory projection from the mouth to the ventrobasal thalamus (Bombardiei et al., 1975). In their study, they reported that cats and raccoons have larger ipsilateral projections from the mouth to the VB complex, than do monkeys. In the current experiment, we did find some neurons having bilateral receptive fields; however, we exclusively selected only neurons with a contralateral receptive field for further study.

The receptive fields of thalamic VB WDR neurons have been reported to be smaller than those found in the spinal and medullary dorsal horns in anesthetized monkeys (Chung et al., 1986; Kenshalo et al., 1980) or in the VPM of awake monkeys (Bushnell and Duncan, 1987) or anesthetized cats (Yokota et al., 1985; Yokota and Matsumoto, 1983b). In our study, we found similar results; however, there is no quantification in the previous studies to allow a direct comparison. We found that there was no systematic difference between the averaged receptive field size of LT and WDR neurons (36.24 and 44.9 mm^2; respectively; p=0.28).

Conclusion

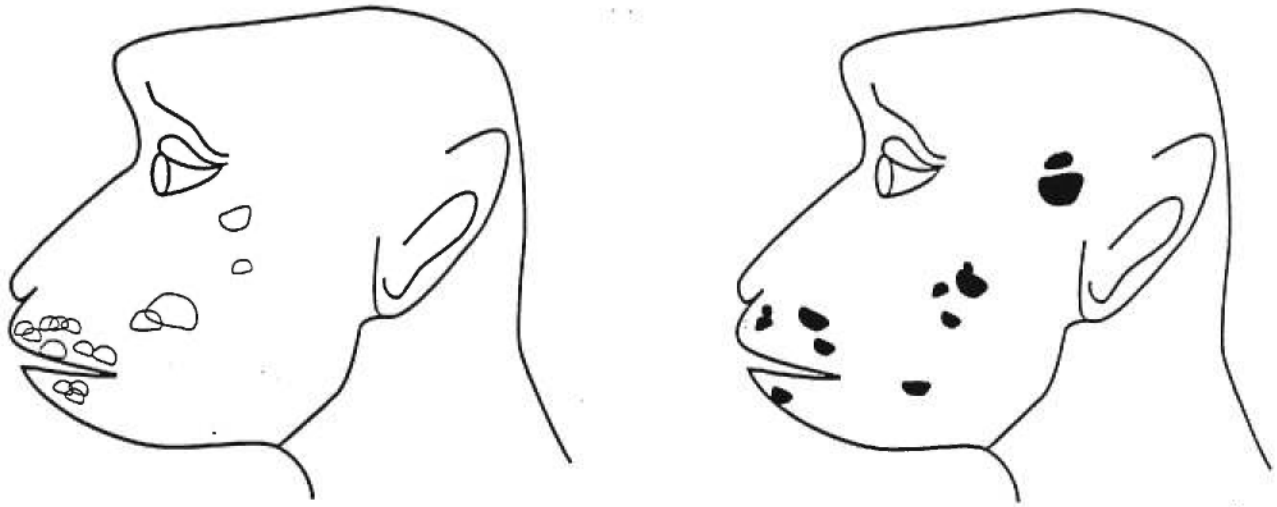
By using alert primates, the functional interpretation of VPM neurons could be drawn. The response characteristics of the thalamic VPM neurons

in this study suggest that the thalamic VPM neurons could consistently encode peripheral mechanical stimuli in both the innocuous and noxious ranges. The decreased size of the receptive fields of WDR neurons, compared to those in the dorsal horn, and their somatotopic organization demonstrate that the role of VPM in the processing of somatosensory information probably is more complicated than just a simple relay station. The intermixture of LT and nociceptive neurons in the ventrobasal complex may play an important role in peripheral or central neuropathies. This issue will be investigated in the next study.

Figure 1. Receptive fields of two types of thalamic VPM neurons in one monkey (M-10). (A) Receptive fields of LT neurons (N=15) and WDR neurons (B, N=12). (C) Average RF size of RA, SA, and WDR neurons, the mean area is 44.9 mm^2, 28.8 mm^2, and 38.9 mm^2, respectively, $p=0.5$). For all neurons, the receptive fields were small and confined to one trigeminal division.

(A)
LT

(B)
WDR



[C]

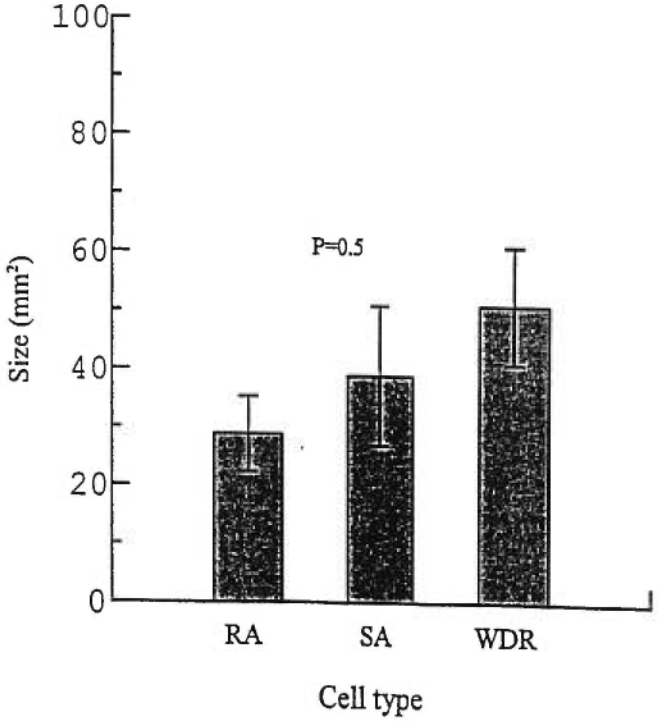


Figure 2. Relationship between the location of the recorded neurons within the thalamic VPM and receptive fields that responded to punctate stimuli. (A) Two WDR (solid circle) and one LT (open circle) neurons were recorded at A+9.0~10.0 with mediolateral arrays between L8.5~9.5, (B) 10 WDR and 14 LT neurons located at A+11~ 12 with mediolateral arrays between L7.5~9.0 and their receptive fields. The medial VPM represents somatotopically the anterior maxillofacial area and the lateral VPM represents the posterior facial area.

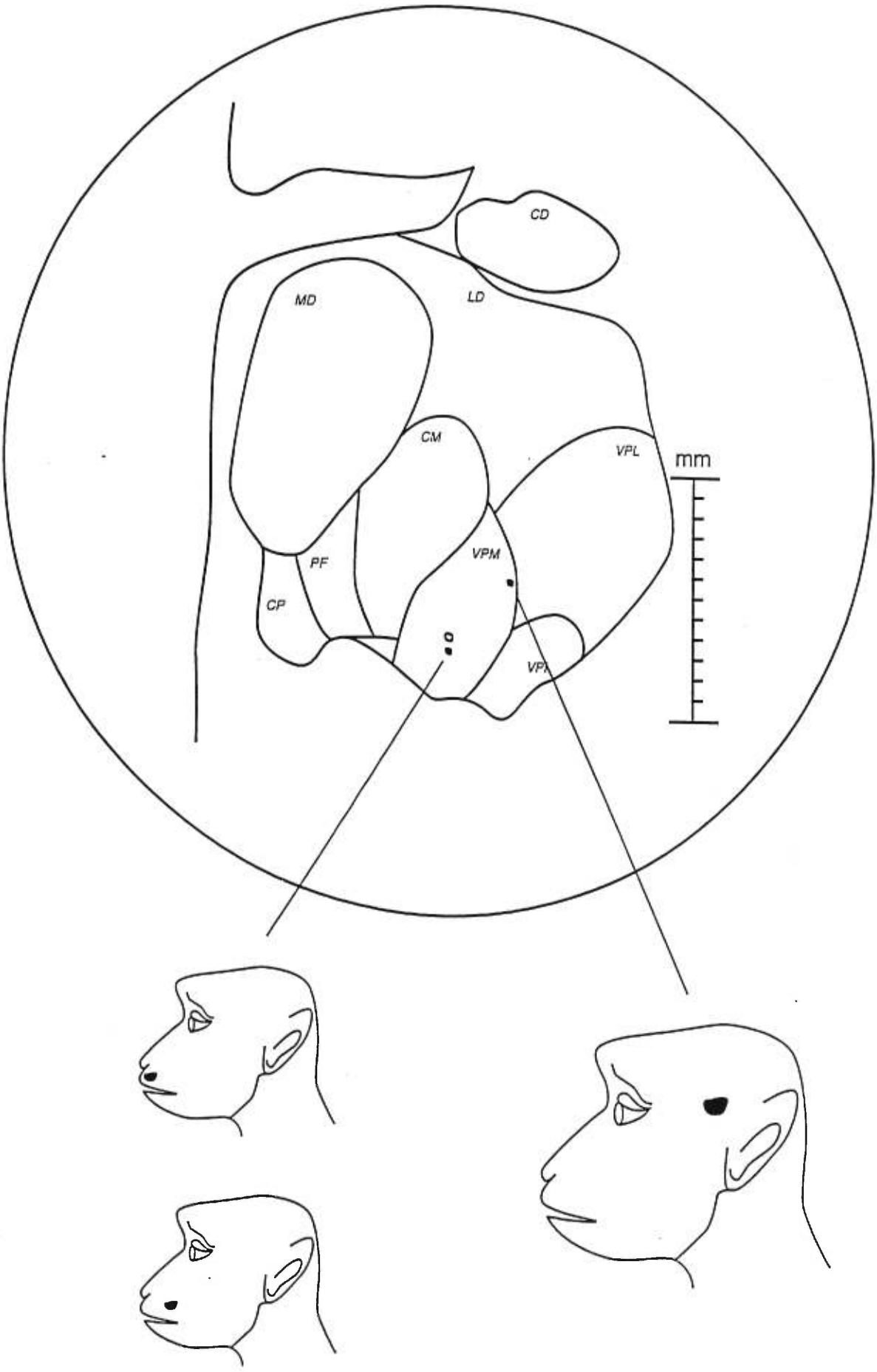
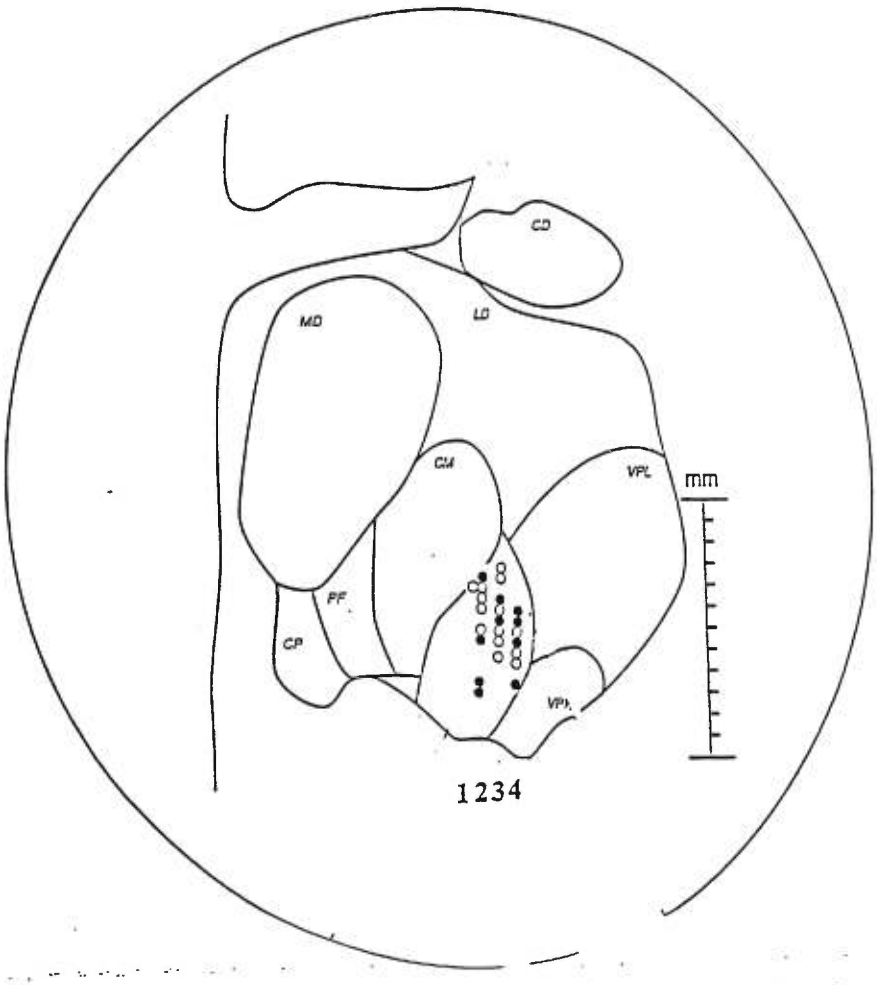


Fig. 2A



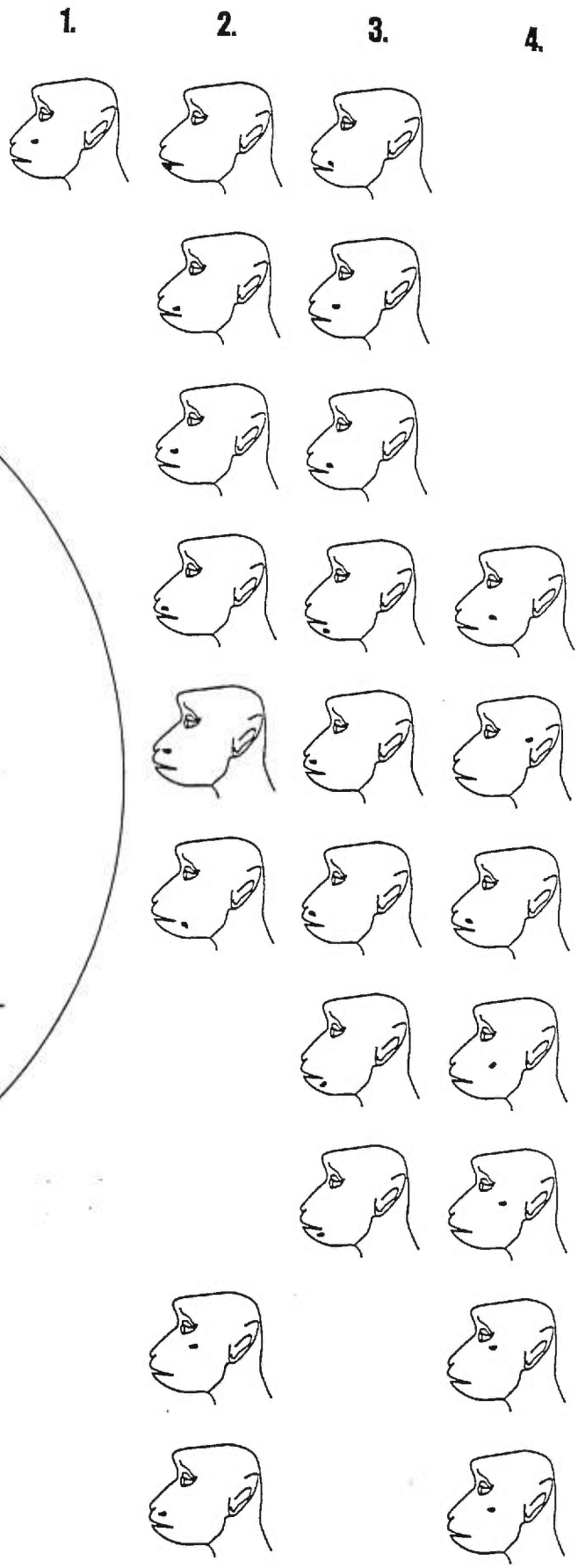
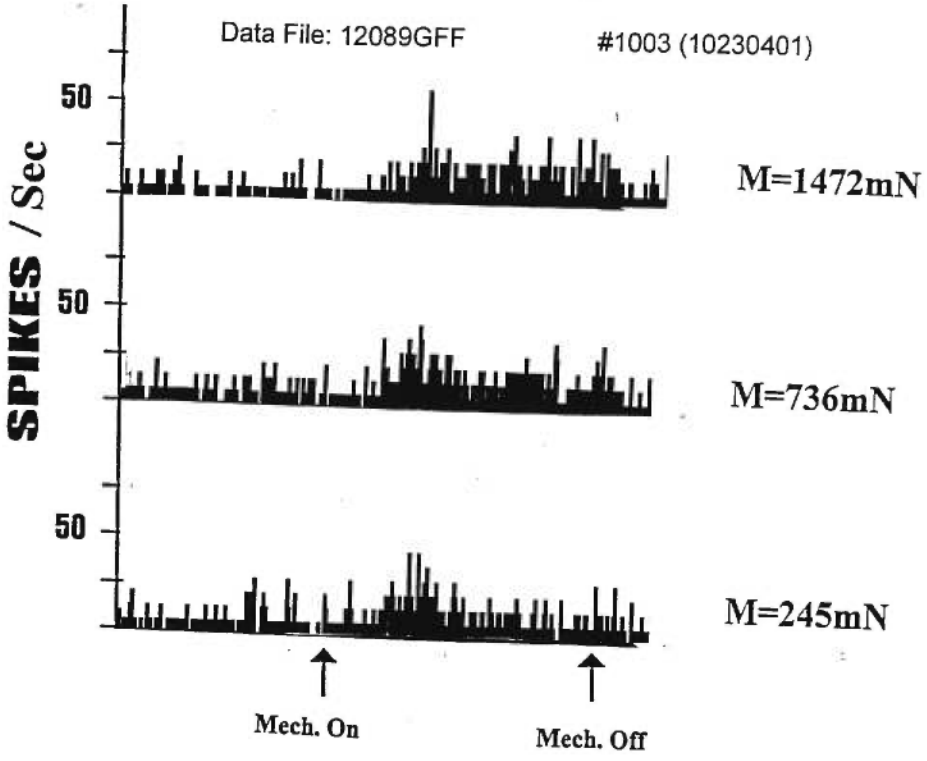


FIG. 2B

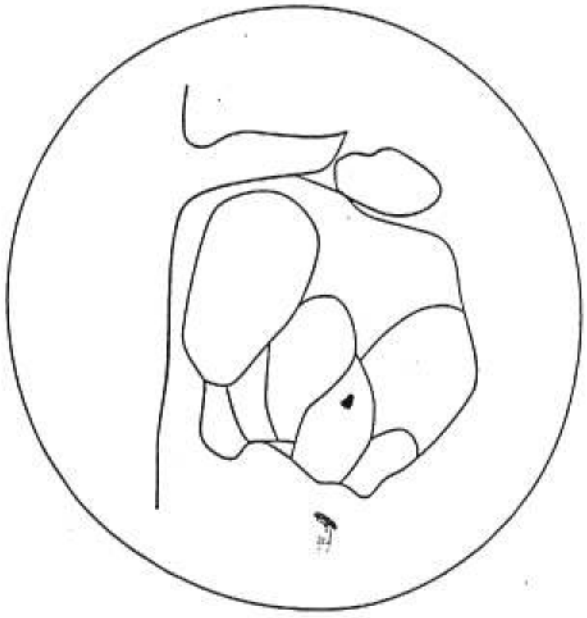
Figure 3. Typical responses of a low-threshold VPM neuron (M1018) to punctate mechanical stimuli. The peristimulus time histogram in (A) shows the spontaneous discharge and mechanically evoked responses. The mechanical intensities were 245, 736 and 1472 mN. Each stimulus was applied 10 times in each recording session. This neuron had a maximal response to innocuous mechanical force. The average number of spikes /s was 5.3, 7.4 and 7.6 accordingly for forces of 245, 736 and 1472 mN. The neuron was located at the mediodorsal border of VPM as shown in (B). The receptive field was 15x10mm on the maxillary area as shown in (C).

(A)


Data File: 12089GFF
#1003 (10230401)
SPIKES / Sec
50
50
50
M=1472mN
M=736mN
M=245mN
Mech. On
Mech. Off

(B)

A+11, L9 +5°



(C)

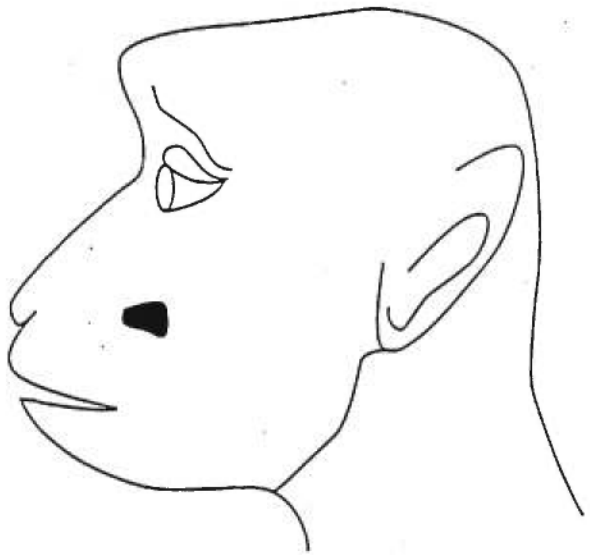
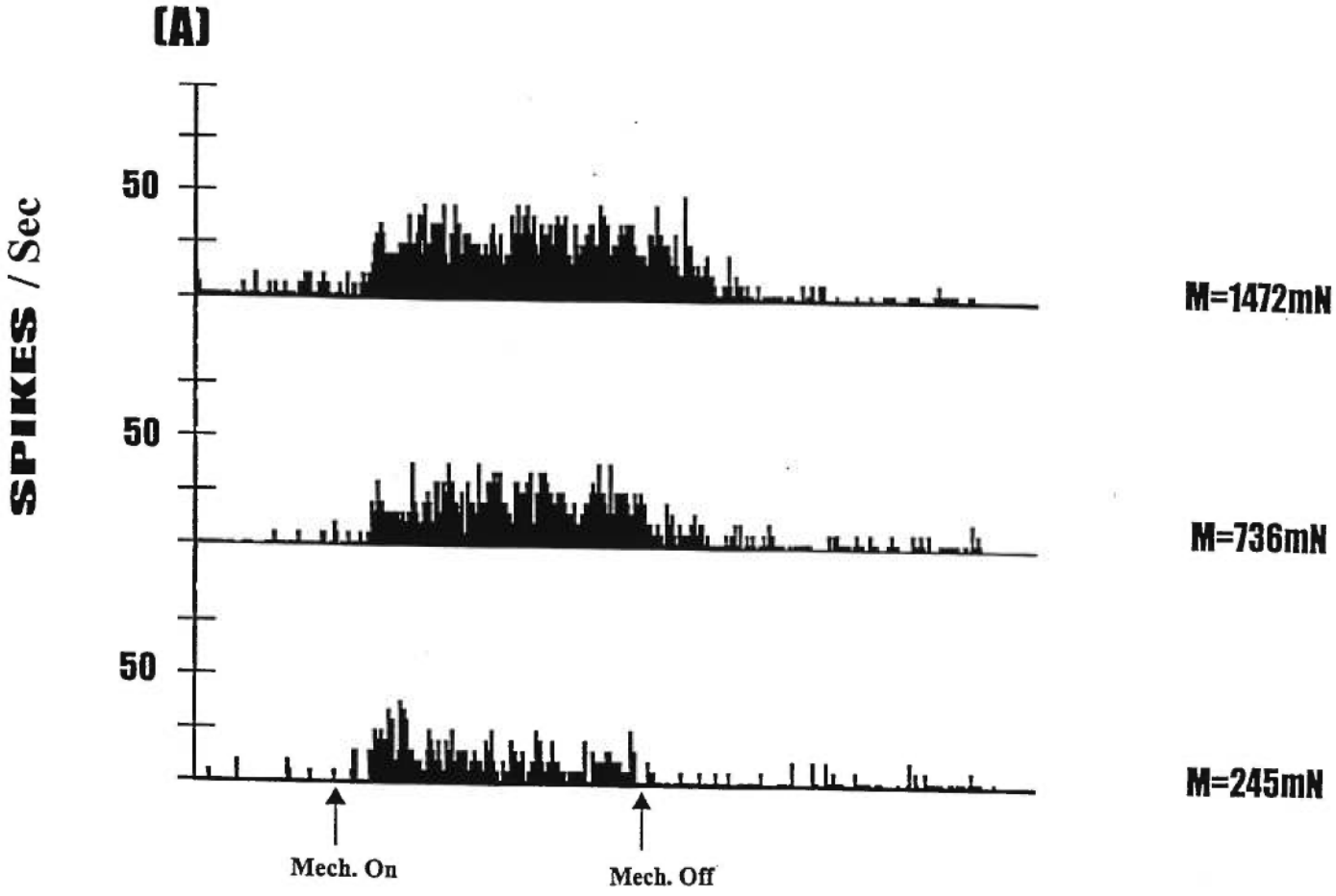
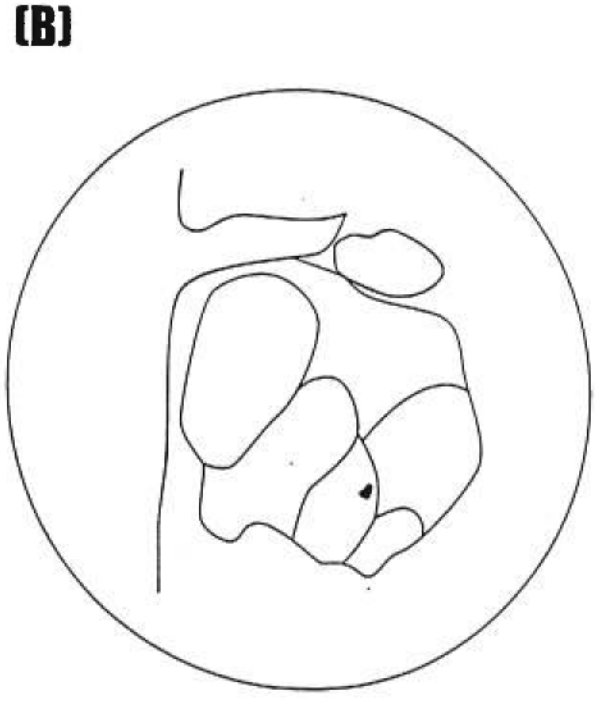


Figure 4. Response of a wide-dynamic-range VPM neuron (M1022) to punctate mechanical stimuli. The figure is organized in the same manner as Figure 3. The WDR neuron had an increasing discharge from innocuous to noxious stimuli in (A). The average discharges were 16.2, 26.2 and 33.6 spikes/s to the forces of 245, 736 and 1472mN, accordingly. This neuron was located at the lateral border of VPM (shown in (B), and had a receptive field of 8x6 mm on the upper face area as shown in (C).


(A)
SPIKES / Sec
50
50
50
M=1472mN
M=736mN
M=245mN
Mech. On
Mech. Off


(B)

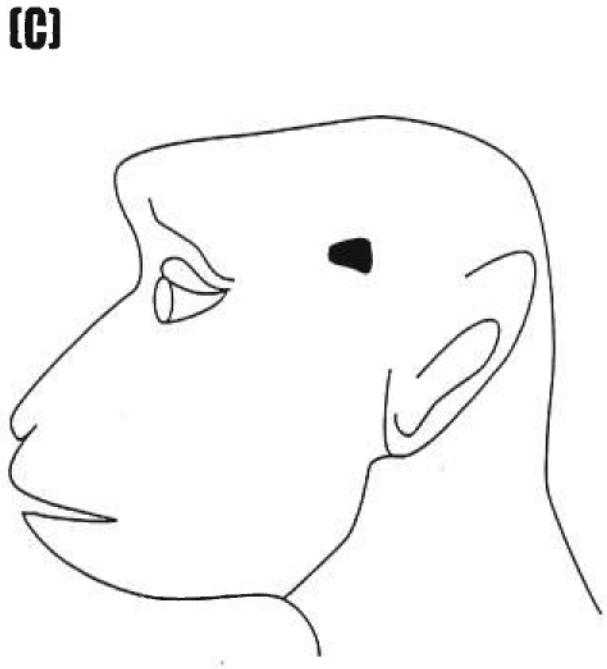

(C)

Figure 5. The stimulus-response functions of individual LT and WDR neurons to punctate mechanical stimulation. Each line represents an individual neuron. The X-axis represents different mechanical intensities; the Y-axis represents the average spikes/sec. (A) shows the spontaneous activity recorded before the application of mechanical stimuli. The entire population showed constant spontaneous activity during the pause between mechanical stimuli. (B) represents neuronal responses evoked by different intensities of mechanical forces. Neuronal responses of all the LT neurons reached a plateau in response to the innocuous stimulus (M=736mN) and did not show further significant increases to noxious stimuli (M=1472mN). On the other hand, the group of WDR neurons showed gradual but statistically significant increasing responses with increases in the intensity of the stimulation from the innocuous to the noxious range. Similar characteristics were also observed for the difference between evoked and spontaneous activity (MEv-S) as shown in (C).

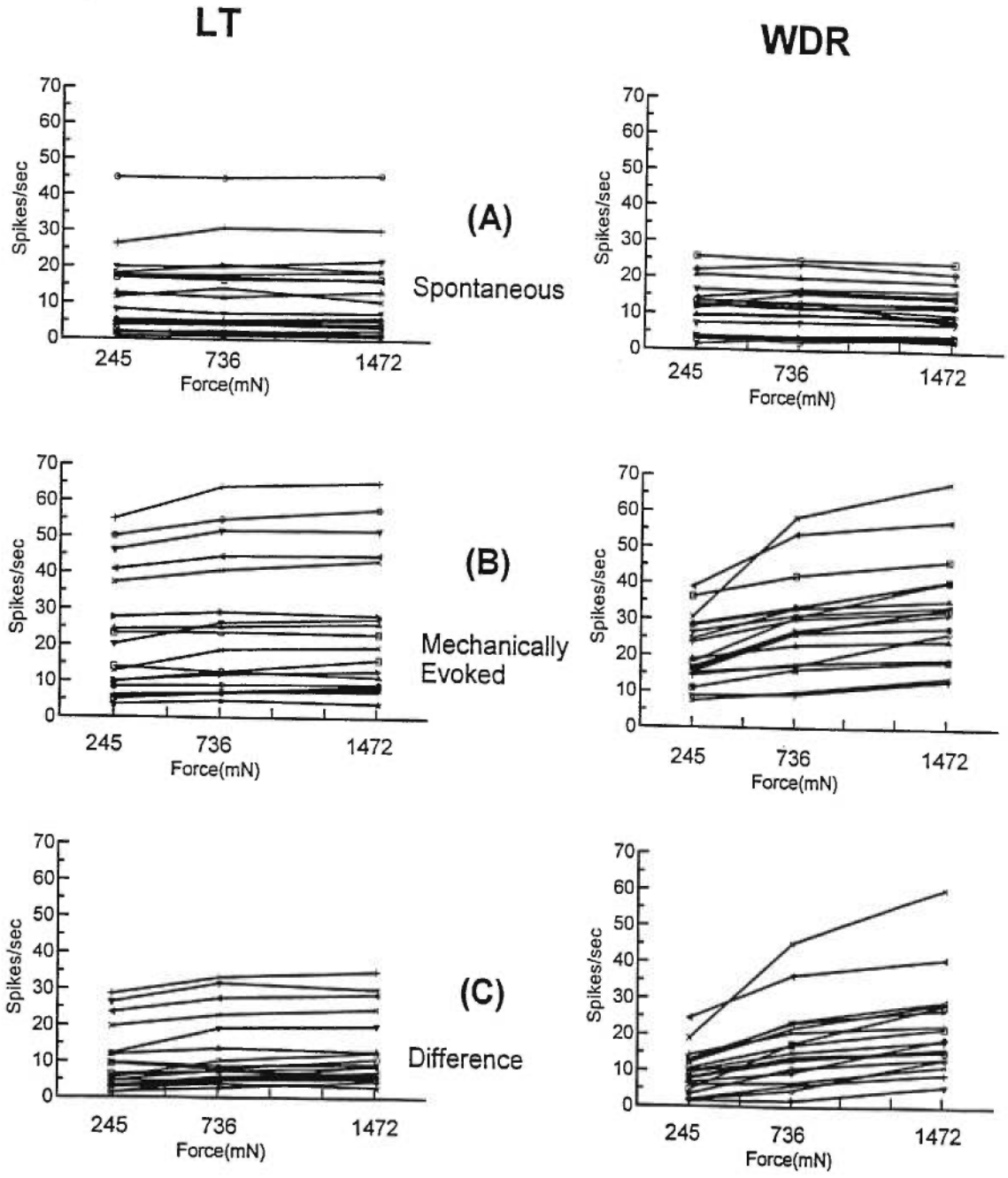

LT
WDR
(A)
Spontaneous
(B)
Mechanically
Evoked
(C)
Difference
Spikes/sec
Force(mN)
245
736
1472
0
10
20
30
40
50
60
70

Figure 6. Mean activity of two types of neurons. (A) represents the average neuronal activity during different intensities of mechanical stimuli of 19 LT neurons and (B) shows the responses of 18 WDR neurons. Spontaneous activity showed constant discharges all the time. LT neurons responded to low-intensity mechanical stimuli and reached maximum responses to innocuous stimuli. In contrast, WDR neurons showed an obviously higher frequency to moderate-intensity mechanical stimuli and responded with the highest frequency to noxious mechanical stimuli ($p<0.001$).

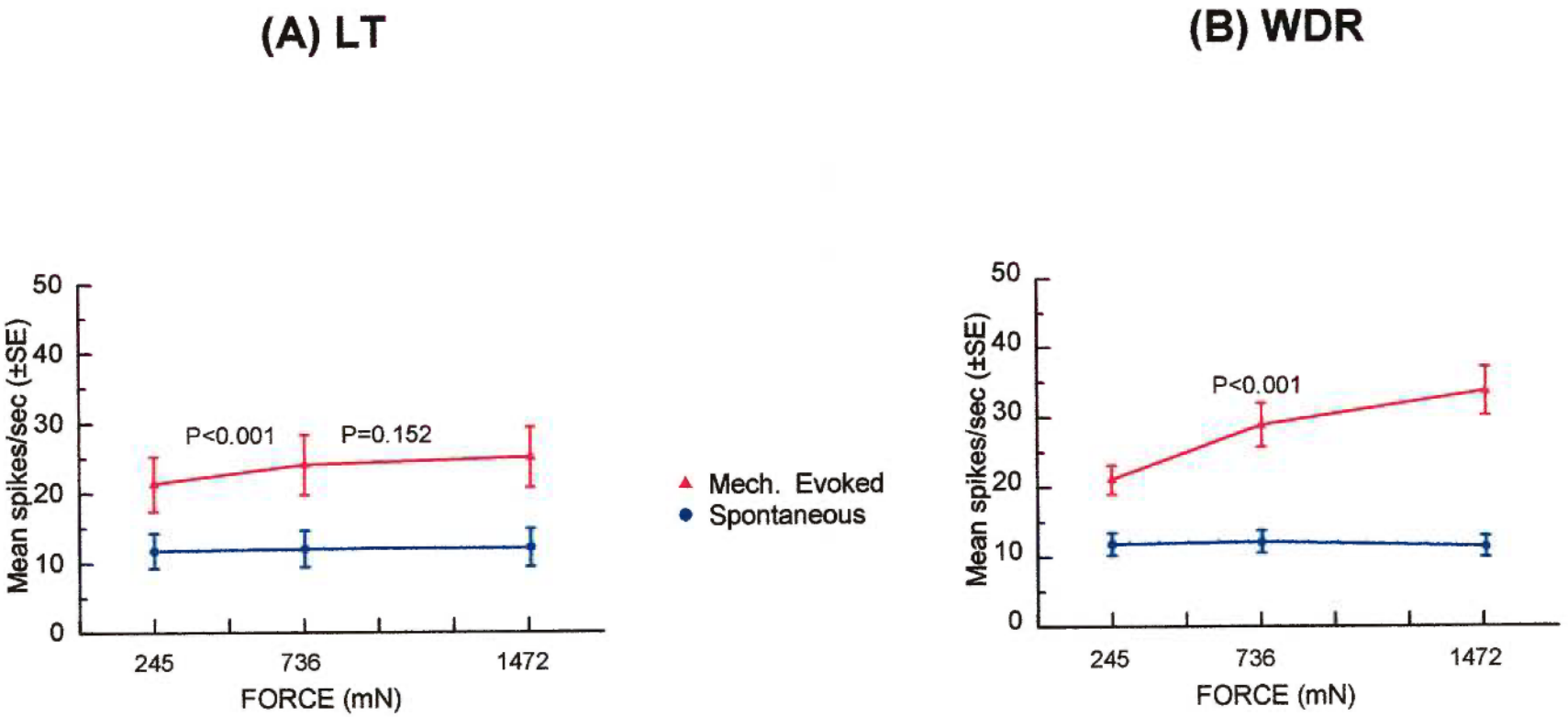
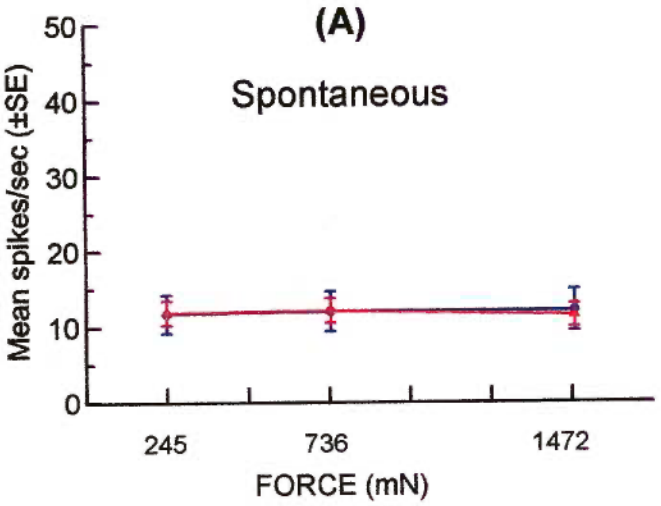
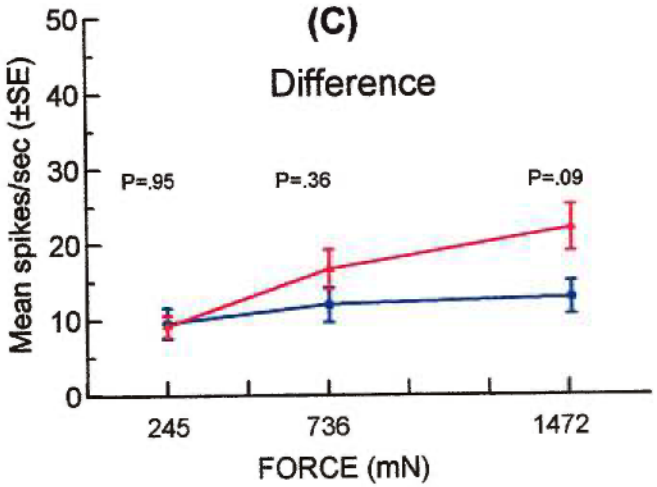
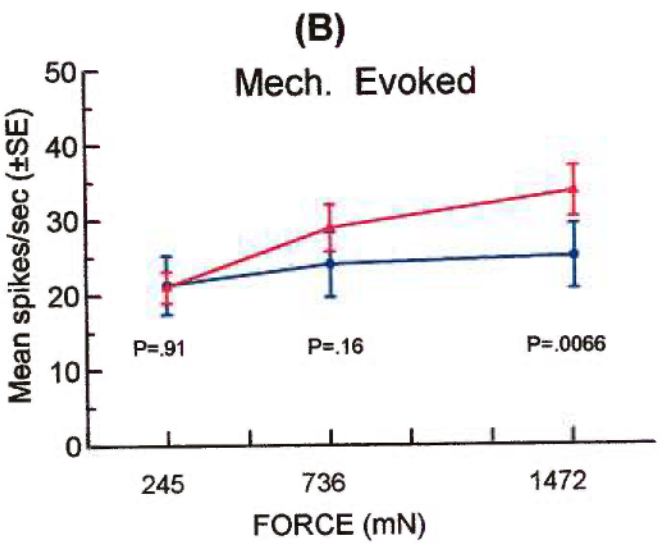

(A) LT
(B) WDR
Mean spikes/sec (±SE)
50
40
30
20
10
0
P<0.001
P=0.152
245
736
1472
FORCE (mN)
Mech. Evoked
Spontaneous
Mean spikes/sec (±SE)
50
40
30
20
10
0
P<0.001
245
736
1472
FORCE (mN)

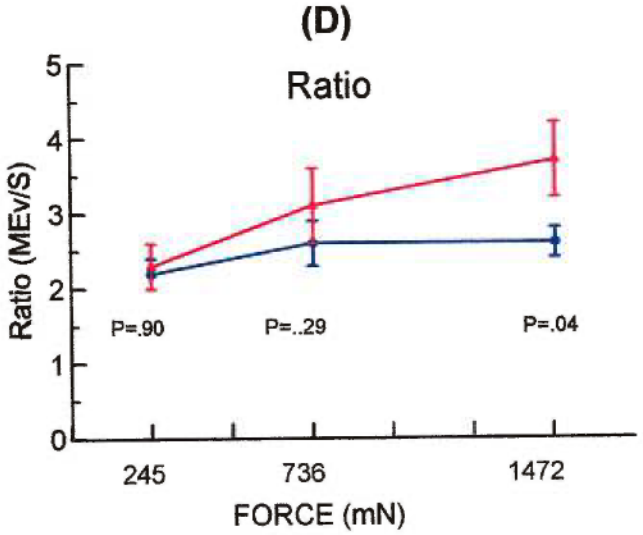
Figure 7. Comparison of LT and WDR neuronal activity. Each line represents the average activity of all neurons from each group. In (A), both groups showed similar spontaneous activity. Neuronal responses to mechanical stimuli became obviously different when the mechanical intensity was increased to the noxious range as shown in (B). Similar findings are demonstrated for the difference between evoked and spontaneous activity (MEv-S) and the ratio of evoked and spontaneous activity (MEv/S), as shown in C and D ($p<.05$).

Comparison of LT and WDR Neurons









Chapter 6 Responses of thalamic VPM neurones to topical application of capsaicin

Note: Part of this work has been published as an abstract in:
Chen, C.-C., Kupers, R.C. and Bushnell, M.C. Neural correlates of static mechanical hyperalgesia in VPM thalamus of the behaving monkey. Society for Neuroscience Abstracts, (1996) p.110, #51.11

6.1 Summary

1. The contribution of the thalamic VPM nucleus to mechanical hyperalgesia was investigated. Activity of thalamic VPM neurons of two awake, behaving monkeys was compared before and after the topical application of capsaicin (0.004M) to the maxillary face area.
2. VPM neurons were classified as LT or WDR according to the responses evoked by graded punctate mechanical stimuli ranging from innocuous to noxious (245, 736 and 1472mN). After the identification of the neuron, a vehicle and capsaicin patch (1x1 cm^2) was applied sequentially to the receptive field for 10 minutes. The neuronal discharges and responses to mechanical stimuli were recorded again after the application of capsaicin.
3. Fifteen neurons (5 LT, 10 WDR) were fully characterized and evaluated for the effects of capsaicin. For WDR cells, both spontaneous and mechanically evoked responses increased after capsaicin was applied, ($p=0.01$ and $p=0.04$, respectively). In contrast, there was a non-significant decrease in both spontaneous and evoked activity of LT neurons. Despite the excitatory effect of capsaicin on WDR neurons, the difference between evoked and spontaneous activity was not altered by capsaicin administered. Furthermore, the ratio between evoked and spontaneous activity of WDR neurons

decreased after capsaicin, while the ratio for the LT cells remained unchanged.

4. These findings suggest that 1) the activity of thalamic VPM WDR but not LT neurons could participate in neural processing underlying capsaicin-induced pain and mechanical hyperalgesia; 2) the WDR discharges may have the maximal discharge rate (ceiling effect) in pain and hyperalgesia states based on the increase of spontaneous activity being greater than the evoked activity after the application of capsaicin in WDR neurons; 3) the trend toward decreased LT neuronal activity during hyperalgesia is consistent with the concept of the touch gate theory, suggesting that low threshold somatosensory transmission can be suppressed in a state of pain.

6.2 Introduction

Peripheral tissue injury can produce hypersensitivity in an area that incorporates the injured site and a larger area beyond the site of injury extending into the undamaged tissue. These phenomena have been known as primary and secondary hyperalgesia. While both heat and mechanical stimuli can evoke pain sensation in the area of primary hyperalgesia, the area of secondary hyperalgesia has been described as responsive only to mechanical stimulus (Hardy et al., 1950; LaMotte et al., 1982b; Lewis, 1936; Meyer et al., 1992; Meyer and Campbell, 1981; Raja et al., 1984).

Sensitization of nociceptors has been well known to account for the peripheral mechanism of hyperalgesia. For instance, the mechanisms of heat hyperalgesia confined to the primary zone have been described as a consequence of sensitization in A-δ or C-mechano-heat sensitive nociceptors (AMH or CMH) by some investigators (Beitel and Dubner, 1976a,b; Bessou and Perl, 1969; Burgess and Perl, 1967; Campbell et al., 1979; LaMotte et al., 1982, 1983; Meyer and Campbell, 1981; Meyer et al., 1992; Raja et al., 1984; Treede et al., 1992). These studies showed that nociceptors increased their discharges during the hyperalgesic state evoked by heat- or chemical-induced cutaneous tissue damage. However, other studies failed to find a contribution of peripheral mechanisms to capsaicin-induced hyperalgesia in monkeys (Baumann et al., 1991; Simone et al., 1986). From recordings of evoked responses from A-δ and C- afferents before and after injections of capsaicin outside and inside the receptive fields (RFs), these authors found that capsaicin-induced pain was related to the excitation of heat-specific or chemospecific nociceptors, but that these nociceptors were not sensitized to heat or mechanical stimuli after the injection of capsaicin. Therefore, it was concluded that neuronal sensitization occurs in the central as opposed to the peripheral nervous system.

Neural mechanisms underlying mechanical hyperalgesia appear more complicated and are less known (Campbell, 1981; Guibaud et

al., 1992a; Hardy et al., 1950; Koltzenburg et al., 1992; LaMotte et al., 1991; Meyer et al., 1992; Simone et al., 1991b,1989a,b; Torebjork et al., 1992; Woolf, 1983). Mechanical hyperalgesia, which has been found in both the primary and secondary areas, comprises two different components: (1) a change of modality from touch to pain and (2) an increase in response of nociceptors evoked by a noxious stimulus. As in the neural mechanism of heat hyperalgesia, the discharges of nociceptors are necessary to initiate mechanical hyperalgesia. However, there is little information about the central neural mechanisms that may be responsible for the development of hyperalgesia.

Abundant evidence suggests that persistent excitability in peripheral nociceptors results in the increasing response of dorsal horn nociceptive neurons at the first synaptic relay (Cervero et al., 1992; Cook et al,, 1987; Dubner, 1992; Hylden et al., 1989b; Laird and Cervero, 1989; Simone et al., 1989b). Moreover, neurons in the spinothalamic tract (STT) respond not only to noxious stimulation but also can develop enhanced responses to heat and mechanical stimuli after a cutaneous injury within the RFs (Ferrington et al., 1987; Kenshalo et al., 1982, 1979). A series of studies concerning the role of sensitization of STT neurons in secondary hyperalgesia revealed that the majority of WDR neurons and some NS neurons showed evidence of sensitization after a tissue injury. This effect was marked

by a sustained increase in spontaneous activity and an increase in response to mechanical stimuli (Dougherty et al., 1991, 1994; Dougherty and Willis, 1992; Guibaud et al., 1992a; Koltzenburg et al., 1992; LaMotte et al., 1991; Simone et al., 1991b, 1989b; Torebjork et al., 1992; Woolf,1983). An expansion of the receptive field was also reported in some studies (Sherman et al., 1997a). This information supports the participation of STT in both acute pain and hyperalgesia following cutaneous tissue damage.

In higher levels of the central nervous system, the thalamic ventrobasal complex receives spinal inputs which convey somatosensory information from the body and facial areas. Thus, we should expect that during the hyperalgesic state, neuronal alternations that occurr in the STT should be conveyed to the thalamus sequentially. However, except for one study showing that craniovascular application of capsaicin activates thalamic nocioceptive neurons in anesthetized cats (Zagami and Lambert, 1991), no other experiments have investigated the responses of thalamic neurons during mechanical hyperalgesia produced by capsaicin.

The present study examines to what extent a topical application of capsaicin can affect the responses of thalamic VPM neurons in alert monkeys. This capsaicin model produces a transient pain and hyperalgesia without permanent damage to the skin. These

neurophysiological data could be compared with behavioral and psychophysical results in monkeys and humans. Therefore, it is possible to learn more about the correlated responses of thalamic VPM neurons under mechanical hyperalgesia.

6.3 Materials and Methods

6.3.1 Animal training and surgical preparation

All the experimental procedures were approved by the ethics committee of McGill University and the Université de Montréal, and were in accordance with the guidelines of the Canadian Council on Animal Care and the International Association for the Study of Pain. These guidelines ensure that all efforts are made to minimize the suffering and the number of animals used in this experiment.

This study was conducted on two adult monkeys (Macaca Mulattas) weighing about 7.5 kg each. Animal training and surgical procedures were described in detail in Chapters 4 and 5. Briefly, the monkeys were first trained to perform visual tasks in order to get rewards and/or receive punctate mechanical forces on the maxillary area with a plastic probe (tip=0.2mm^2). After the monkeys had learned the tasks, a recording chamber was placed stereotaxically on the right side of the skull under standard surgical procedures and general anesthesia. After the animals had completely recovered from the surgery and re-acquainted themselves with the behavioral tasks, recording of right thalamic VPM

neuronal activity was made with a low-impedance (0.5MΩ, at 1KHz) gold and platinum-plated Tungsten electrode.

6.3.2 Recording procedures

Vehicle and capsaicin patch

A four-layer gauze pad measuring 1x1 cm^2 and a transparent dressing (3M, Tegaderm) were used for the topical application of vehicle or capsaicin. The vehicle patch, saturated with 0.3ml of 70% ethanol, was applied to the center of the receptive field (RF) for 5 minutes. This control patch was then replaced by the capsaicin patch (capsaicin, dissolved in 70% ethanol; 0.004M, 0.3ml; Sigma) for 10 minutes. During the application of vehicle or capsaicin, the monkeys performed only the visual tasks for liquid rewards. The RFs and the patch-applied area were marked in each application to avoid applying capsaicin on the same area of skin within one week. Hair was clipped on a regular basis three days before the experiments to ensure the intimate contact of patch with the skin.

Mechanical stimulation

A punctate mechanical stimulus was applied with the intensity of innocuous (245mN), near-noxious (736mN), and noxious forces (1472 mN) via a computer-controlled stimulator that had a circular probe tip (0.2 mm^2) made of thermally neutral material (see Chapters 4,5). The mechanical probe was positioned orthogonal to the skin at a distance of about 5 mm from the skin surface. There were two recording sessions of mechanical

stimulation, before and after the application of capsaicin, respectively. Each intensity was applied 10 times pseudo-randomly in every recording session; the interval between each stimulus was at least 30 seconds.

Experimental protocol (shown in Table 1)

While the experimenter was searching and isolating VPM neurons, the monkeys continued to perform the visual tasks for reward. After isolating a neuron, the receptive field was carefully marked. Three different intensities of punctate mechanical stimuli (245, 736, 1472 mN) were then applied to the receptive field and the neuronal responses were observed and recorded. Then, a vehicle patch was applied to the receptive field for 5 minutes and was replaced by the capsaicin patch for 10-15 minutes. The neuronal activity was recorded during this patch-applied period. After removal of the capsaicin patch, the neuronal responses to mechanical stimuli were tested again. Only those neurons recorded during the complete experimental protocol were selected for further analysis to compare the capsaicin effects on the mechanical responses. In some neurons, we also tested the effect of cold suppression on capsaicin-induced hyperalgesia; 5-10°C stimulation was presented for 5 minutes by a contact thermode or ice, following which the mechanical response properties of the single unit were again evaluated.

6.3.3 Data Analysis.

All neuronal discharges were retrieved and analyzed off-line. Accumulated histograms were generated for the neuronal responses, mean spikes per second of neuronal discharges were calculated for further comparison. Each neuron's response was compared before and after the application of capsaicin. Data are represented as means ± SE. Analysis of variance (ANOVA) for repeated measurements or a paired Student's *t*-test was used for statistical analysis. *P* values of <0.05 were considered statistically significant.

Definition and Classification of neuronal activity

The responses to mechanical stimuli were used to categorize the recorded neurons. The definition and classification of neurons were described in previous Chapters. In brief, VPM neurons were classified as LT or WDR according to their responses to graded intensities of the mechanical stimulation ranging from innocuous to noxious. LT neurons responded maximally to innocuous stimulation (245, 736mN), whereas the WDR neurons responded in a graded fashion and significantly increased to noxious stimulation (1472mN).

Spontaneous activity

Spontaneous activity was defined as the number of neuronal discharges that occurred without the presence of either tactile or thermal stimuli in the receptive field. The spontaneous activity was collected for

three seconds before each application of the mechanical stimulus. Mean spikes per second were calculated for further analysis.

Mechanically evoked activity

Neuronal responses recorded during presentation of mechanical stimuli to the receptive fields were defined as mechanically evoked (MEv) activity. These response discharges were used to categorize the neurons. LT neurons did not differentiate between forces higher than 736mN (rated as non-painful by human subjects), while WDR neurons responded differentially up to 1472mN (rated as painful by human subjects). All the neurons presented here showed significant responses when the mechanical stimuli were applied (ANOVA, repeated measure, $p<0.05$).

The difference of the activity

The spontaneous activity subtracted from mechanically evoked activity was defined as the difference of the activity. This absolute value was used to characterize neuronal responses to mechanical stimuli before the application of capsaicin and to compare the extent of change after capsaicin, in which both the spontaneous and mechanically evoked activities were influenced by this chemical.

Ratio of the activity

The mechanically evoked activity divided by the spontaneous activity was defined as the ratio of the activity. This value was especially important to characterizing the change of neuronal discharges after the application of capsaicin.

Gain of the activity

The difference of the neuronal response before and after the capsaicin was defined as gain of the activity. This value could be positive (higher than zero) as an indication of an increasing response, or negative (lower than zero), as an indication of a decreasing response.

6.3.4 Localization of recording sites

After the end of the experiment, the region of one monkey (M-10) was marked by a micro-lesion produced by passing current (10-15 μA for 10s) through the Tungsten microelectrode (See chapter 5). The specimen was obtained through the standard procedures of perfusion. Serial sections of 50 μm were stained with cresyl-violet. Recording sites were identified by camera Lucida and reconstructed afterwards.

6.4 Results

Of the thirty-seven VPM mechanosensitive neurons recorded (see Chapter 5), fifteen (5 LT, 10 WDR) were tested for the effects of capsaicin on the neuronal responses evoked by mechanical stimuli. The receptive fields and thalamic recording locations of seven neurons tested for capsaicin effects are shown in Figure 1 (monkey M-10). The receptive fields were confined to one trigeminal division and were not found to differ in size.

In general, WDR and LT neurons showed characteristic differences in their response to capsaicin. WDR neurons increased their discharge following capsaicin treatment in a manner consistent with the development of hyperalgesia (see Chapter 4). On the other hand, some LT neurons decreased their activity after the application of capsaicin. The neuronal activity recorded during the patch-applied period is presented in Figure 2. For the LT neuron, the discharge gradually decreased through the duration of the capsaicin patch while the WDR showed a marked increase. Representative LT and WDR neurons, showing the typical change of discharge to mechanical stimuli before and after the application of capsaicin, are presented in Figure 3.

6.4.1 Effect of capsaicin on LT neurons

A total of five LT neurons was examined in this study. Figure 4 (A) demonstrates the spontaneous activity of individual LT neurons before and after the application of capsaicin. Three of LT neurons showed

decreasing spontaneous discharges, and two showed slight increases following capsaicin. The constant spontaneous activity recorded between application of mechanical stimuli reveals that there is no interference caused by the different intensities of mechanical stimuli. The mechanically evoked activity shown in Figure 4 (B) demonstrates that four neurons decreased their responses to mechanical stimulation after the capsaicin.

Comparison of LT neuronal average activity before and after the application of capsaicin is shown in Figure 5. Mean spontaneous and mechanically evoked activity (N=5) were lower after than before the capsaicin application. However, these effects were not statistically significant effect ($p=0.44$, $p=0.26$, respectively). That the ratio value of mechanically evoked and spontaneous activity appears similar before and after the capsaicin implies that the magnitude of change in the spontaneous and mechanically evoked activity is similar.

6.4.2 Sensitization of WDR neurons

As shown in Figure 6(A), spontaneous activity of all WDR neurons showed slight or marked responses to capsaicin. Topical application of capsaicin to the receptive fields of these WDR neurons resulted in elevated spontaneous activity for a duration of 30 minutes. The responses to mechanical stimuli were also elevated after the application of capsaicin in eight out of ten neurons. The responses to 245 and 736

mN showed the most marked increases while the increase in responses to the noxious force (1472mN) was less, and in fact three WDR neurons showed decreased responses (Figure 6B).

The comparison of average neuronal activity is presented as mean±SE (N=10) in Figure 7. Both the spontaneous and mechanically evoked activities showed significant increase after the application of capsaicin (ANOVA, repeated measure, p=0.01, p=0.04, respectively). However, the difference and ratio of mechanically evoked and spontaneous activities showed a decrease or no change after capsaicin. This implies that the magnitude of increasing activity caused by capsaicin is similar or larger for spontaneous activity than that for the mechanically evoked activity.

6.4.3 Cold suppression on the effect of capsaicin

Cold suppression on the capsaicin-evoked response was tested in four WDR neurons (Table 2). In Figure 8, a representative neuron shows the suppression of spontaneous activity after the cold was applied to the receptive field. For the mechanical responses, the effect of cold suppression was found in all the mechanical stimuli but more predominant in the noxious range.

6.5 Discussion

This study presents two patterns of responses of thalamic VPM neurons to topical application of capsaicin. First, the majority of WDR

neurons showed an elevated spontaneous discharge and excitatory response to mechanical stimuli after topical application of capsaicin. The second pattern of response was characterized by the decreasing activity of LT neurons. These neurons showed lower spontaneous discharges and mechanically evoked activity following capsaicin application.

Experimental considerations

Capsaicin has been used as an inflammatory substance to produce experimental hyperalgesia or allodynia by cutaneous or subcutaneous application. This substance can have both excitatory and toxic effects on cutaneous primary sensory afferents (Russel and Burchiel, 1984). Lower concentrations of capsaicin, such as a 1% (33mM) solution applied topically to the skin could excite C-mechanoheat (CMH) nociceptors in rats (Kenins, 1982), in monkeys (Baumann et al., 1991) and in humans (Konietzny and Hensel, 1983, Liu et al., 1998). However, repetitive topical application of capsaicin can suppress the discharge of the primary afferents (Carpenter and Lynn, 1981). Intra-arterial injections of a higher dosage of capsaicin (200ug) in rats were found to desensitize CMHs to heat, mechanical, and chemical stimuli (Szolcsányi, 1988). A model of intradermal injection of capsaicin, extensively used by LaMotte and his colleagues, demonstrates that the injection site itself is analgesic (Lamotte et al., 1991; Simone et al., 1989a, 1987). Therefore, extreme caution should be taken when using capsaicin in the chronically electrophysiological study of pain. In this study,

we used low concentrations of capsaicin solution (0.004M) to induce hyperalgesia. In the preceding studies, we have proven that this concentration can induce transient hyperalgesia without permanent tissue damage, and does not distress the primates (i.e. does not disrupt performance of operant tasks). Moreover, in order to minimize the possible desensitizing effects of capsaicin, we also avoided applying the capsaicin patch on the surrounding RFs within one week of application.

Contribution of VPM WDR neurons to hyperalgesia and allodynia induced by capsaicin

Most studies regarding the central components of hyperalgesia have focused on STT neurons or other spinal cord neurons. Several models have been proposed to elucidate possible central neural mechanisms of hyperalgesia in response to peripheral injury. One of these is the "intensity-based" hypothesis proposed by Dougherty and his colleagues (Dougherty et al., 1994a; Dougherty and Willis, 1991a, b). This hypothesis is based on the notion of central sensitization, in which discharges in primary nociceptors cause dorsal horn neurons to enhance their excitability to acquire new or to increase their responsiveness to other peripheral stimuli (Cervero et al., 1992; Coderre et al., 1993; Cook et al., 1987; Dubner and Ruda, 1992; Hylden et al., 1989b; Laird and Cervero, 1989; Woolf and Thompson, 1991; Woolf, 1983). They pointed

out that the WDR neurons played the role of the "convergent" neurons of this central sensitization.

Other findings of Simone et al. (1991b, 1989b) show that intradermal injection of capsaicin causes excitation of STT neurons followed by a sensitization of these neurons to mechanical stimuli. They found that both types of WDR and High-threshold (HT) neurons discharged vigorously immediately after the injection of capsaicin, but only the WDR neurons continued to discharge for an extended period of time. Furthermore, there was a significantly increased response to "brush" and "press" of the WDR but not of HT neurons. These results suggest that WDR neurons are more likely to play an important role in maintaining pain sensation and developing hyperalgesia and allodynia following skin damage.

Our results reveal similar characteristics of WDR neurons in thalamic VPM following the topical application of capsaicin. Capsaicin significantly increased both the spontaneous and mechanically evoked activity of WDR neurons for at least 30 min. following capsaicin application. The observed increases in spontaneous activity were comparable to the increased incidence of spontaneous pain reported by human subjects (LaMotte et al., 1991, Morris et al., 1997; Simone and Ochoa, 1991a; Simone et al., 1989a; Chapter 4 and following Chapter 7). Our findings of increased mechanically evoked activity suggest that WDR neurons could at least partially subserve hyperalgesia. Further analysis

shows that this increase is not uniform but in preference to the responses to non-noxious mechanical stimuli. We suggest that VPM WDR neurons show enhanced excitability to low-threshold inputs after sensitization, and might play a role in mechanical allodynia. Alternatively, the lower increased response to noxious stimuli may simply be due to a ceiling effect, in which the maximal rate of neuronal discharge has already been reached under the hyperalgesic state, and therefore, cannot, to show any further significant increase.

However, the difference and ratio values of both LT and WDR neurons were not significantly altered by the effects of capsaicin. We predict that these unchanged magnitudes before and after the hyperalgesia might result in there being no difference in the ability of human subjects for mechanical discrimination. We will continue this psychophysical study in the next chapter.

LaMotte et al. (1991) reported that cooling the injection site of capsaicin to 1°C for several minutes could reduce the stroking hyperalgesia area in human subjects. A similar observation was also reported by Cervero and Laird (1996a), who found that cooling the primary hyperalgesia site (induced either by capsaicin or mustard oil) abolished both the allodynia and the blood flow response. In our study, we tested this suppressive effect of cold on the neuronal activity in some WDR neurons. All neurons showed general suppression of the

spontaneous discharge and mechanical responses after the cold application to the RFs (Figure 8).

Moreover, LaMotte and his colleagues (1991) proposed a model of neurogenic hyperalgesia, which states that the projection neurons in the dorsal horn can be sensitized by the chemospecific afferents. Cervero and Laird (1996a,b) proposed a new model of allodynia with the idea that the A-β mechanoreceptors can gain access to nociceptive neurons by a presynaptic link at the spinal level. Whatsoever, the similar findings of cold suppression in the spinal cord observed by these two investigating groups, as well as ours in the thalamus, suggest that the neural mechanisms of hyperalgesia or allodynia might preserve the same character throughout different level of CNS.

Contribution of LT neurons

The gate control theory of pain (Melzack and Wall, 1965) suggests that tactile stimuli can modulate the perception of pain. Recently, an opposing theory named "touch gate" has been proposed by Apkarian and his colleagues (1994). They demonstrated that heat-induced pain could substantially suppress tactile sensitivity, independent of a shift in attention or arousal in human subjects. Our results showed that most of LT VPM neurons (N=4) have a tendency toward decreased spontaneous discharge and decreased responses to mechanical stimuli during the hyperalgesic state. However, we also found one LT neuron showing

excitability following the capsaicin. This is perhaps due to a phenotypic switch of larger myelinated afferents under the state of tissue injury (Neumann et al., 1996). Due to the small sample size, we would need further investigation to confirm these findings in other LT VPM neurons.

Conclusion

These findings suggest that 1) thalamic VPM WDR, but not LT, neurons can participate in neural processing underlying capsaicin-induced mechanical hyperalgesia ; 2) WDR neurons may have maximal discharge rate in pain or hyperalgesic states; 3) low threshold somatosensory transmission can be suppressed by the state of pain or hyperalgesia, supporting the touch gate theroy that predicts decreased LT neuronal activity.

Table 1. The experimental protocol for testing the capsaicin effect on the neuronal responses

The experimental protocol

Parameter	Duration	Comments
Visual task	Variable	Isolating the neurons, monkeys receive the liquid rewards
Pre-capsaicin mechanical stimuli	10 –15 min	10 times for each intensity of 245, 736, 1472mN
Vehicle patch and visual task	5-10 min	Receive the rewards
Capsaicin patch and visual task	10-15 min	Observe the monkey's behavior
Post-capsaicin mechanical stimuli	10 – 15 min	10 times for each intensity
Cold temperature	5 min	Contact thermode at 5°C on the RFs
Post-cold mechanical stimuli	10 –15 min	10 times for each intensity

Figure 1. Thalamic schema and monkey face diagrams showing the receptive fields of seven thalamic VPM neurons in one monkey (M-10). The diagrams and the receptive fields were drawn proportional to the original size. Unfilled areas represents the receptive fields of LT neurons (N=3) ; filled shadow represents the WDR neurons (N=4). For all neurons, the receptive fields are confined to one trigeminal division and did not differ between the two types of neurons. Thalamic schema showing four WDR (filled circle) and three LT (open circle) neurons is presented at A+11.0~12.0 with mediolateral arrays between L7.5~9.0.

1. 2. 3. 4.

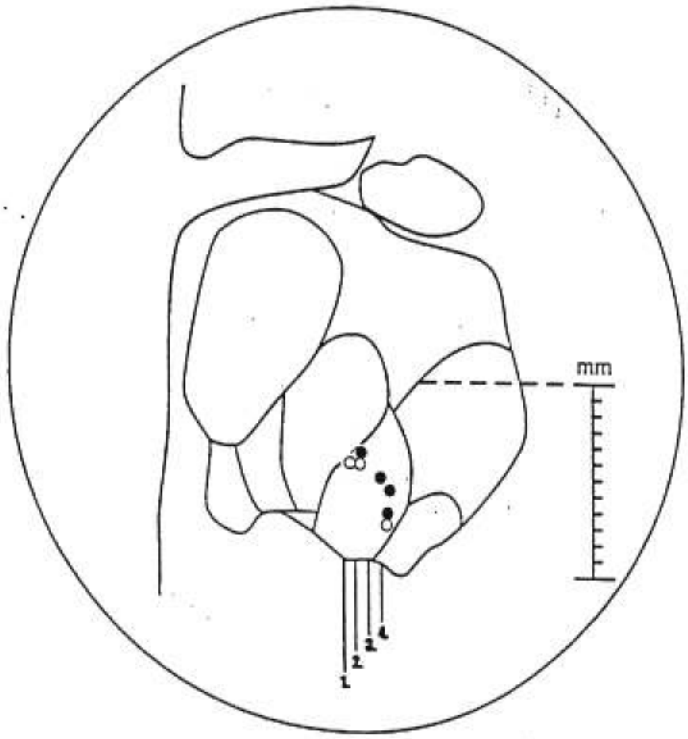


Figure 2. Typical change of neuronal discharge and time course during the patch-applied period of one LT and one WDR neuron (Veh: vehicle, Cap: capsaicin). The intervals shown in the X-axis are about 5 minutes. Total spikes were recorded while the patch was placed on the receptive field. The value is presented as Mean±SE (spikes/s). For the LT unit, there is a gradual decrease in discharge, whereas the WDR neuron showed a marked increase and reached a peak in 10 minutes. This increasing discharge then decreased, but was still significantly higher than the discharge during the vehicle patch application.

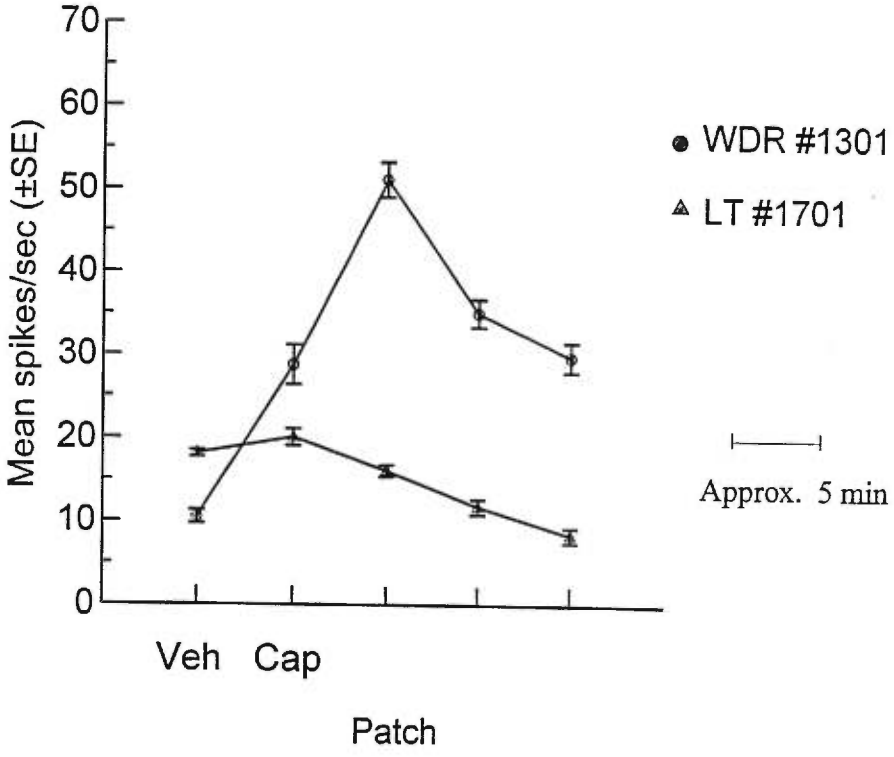

70
60
50
40
30
20
10
0
Mean spikes/sec (±SE)
Veh
Cap
Patch
WDR #1301
LT #1701
Approx. 5 min

Figure 2A. One WDR neuron shows continuous firing during the application of vehicle and capsaicin patches. Neuronal discharge is significantly increased during the capsaicin treatment in comparison with the vehicle patch ($p<0.05$).

Fig. 2-A WDR neuron

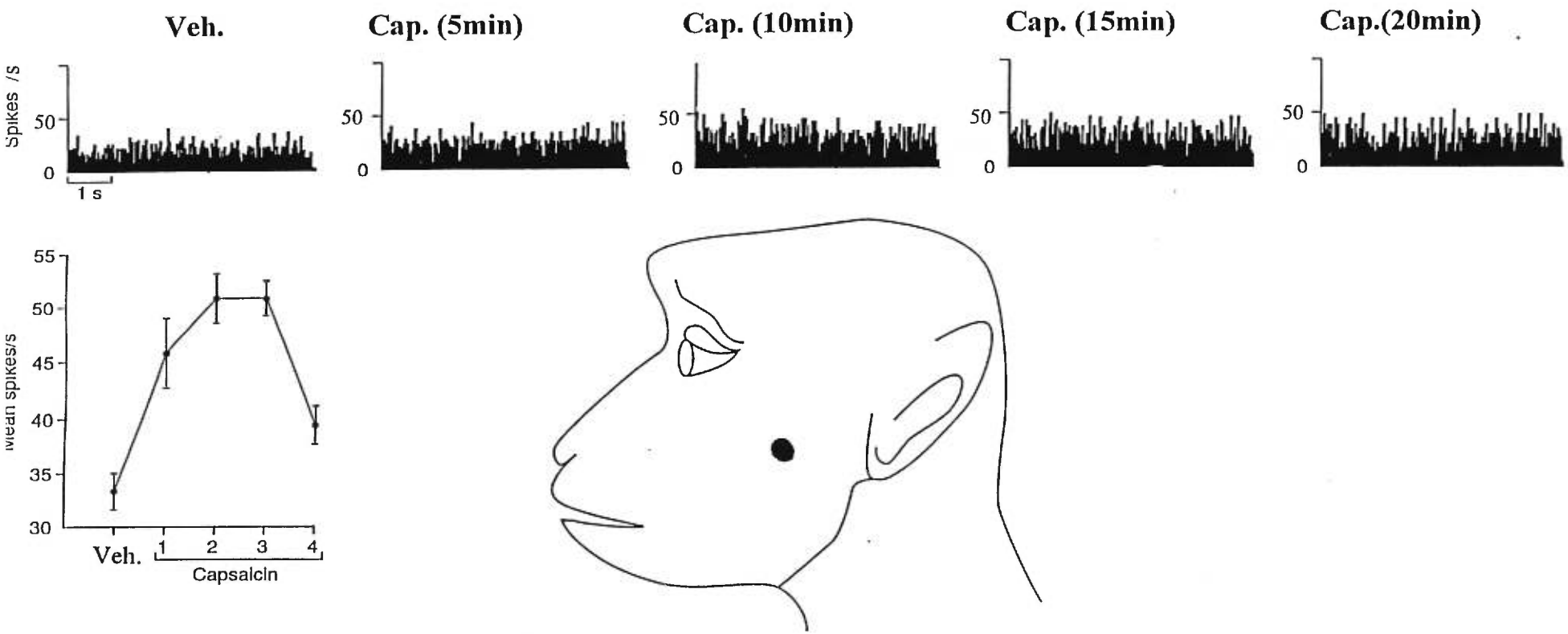
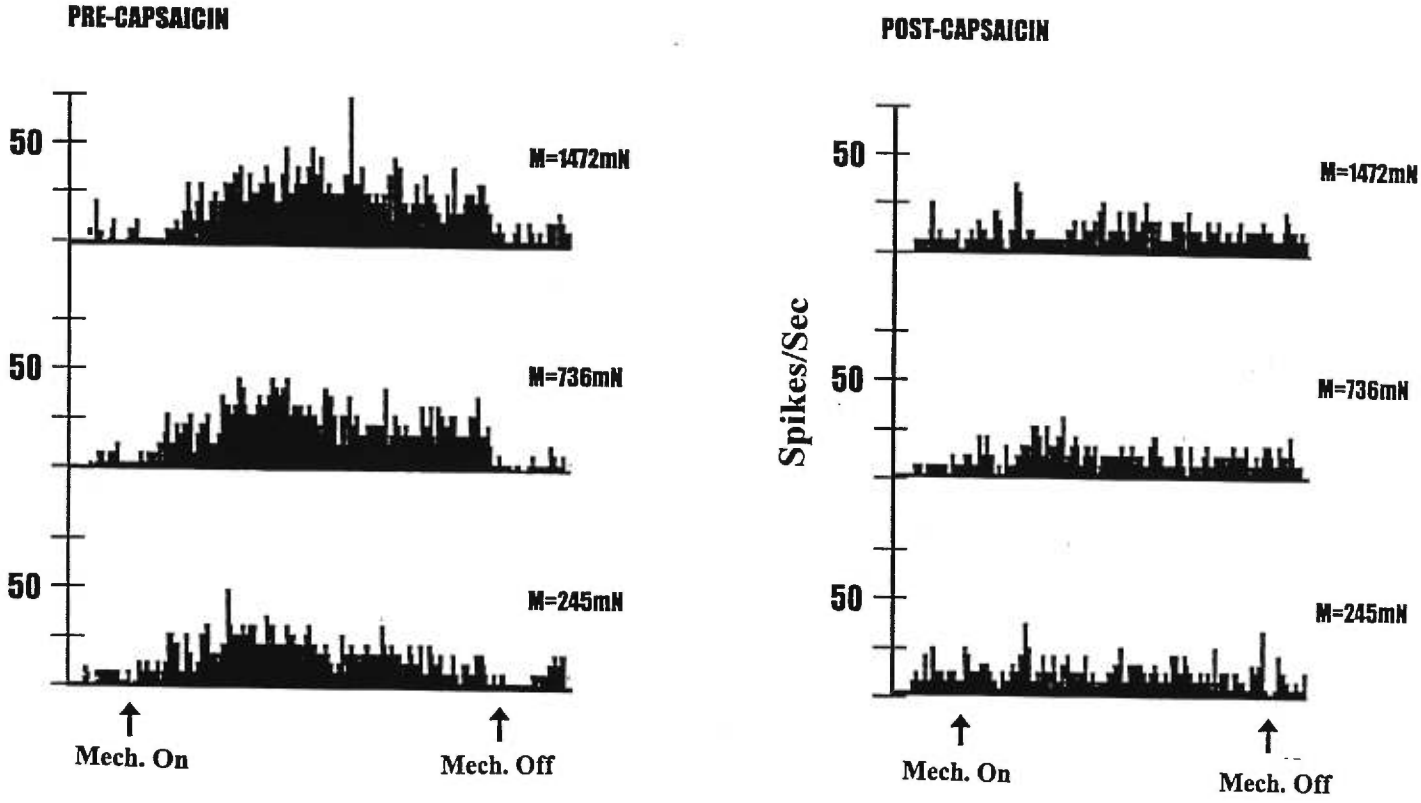
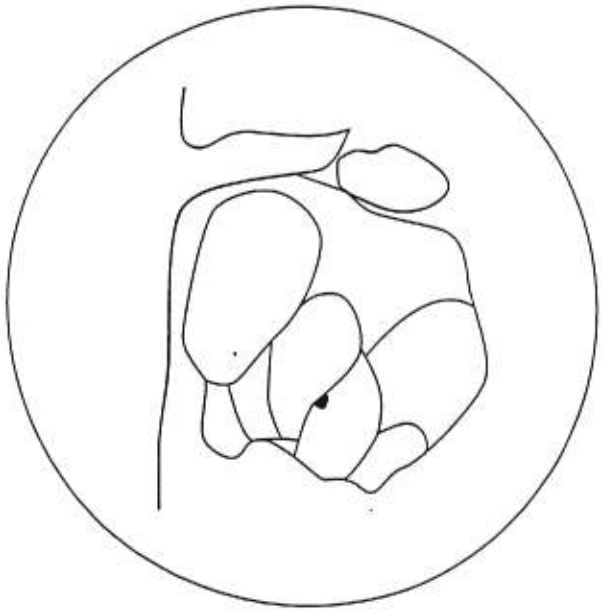
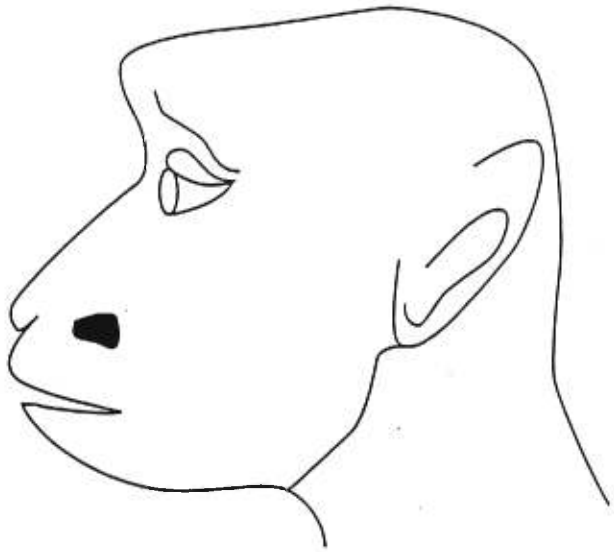


Figure 3. Representative responses of LT (A) and WDR (B) neurons to mechanical stimulation before and after the application of capsaicin. *Bottom (right):* capsaicin was applied to the RF and mechanically evoked activity was recorded. *Bottom (left):* The thalamic location of the recorded neuron. *Top:* peristimulus histograms showing the neuronal responses to mechanical stimuli before and after the application of capsaicin. Note decreased responsiveness in LT neuron and increased discharge in WDR unit.

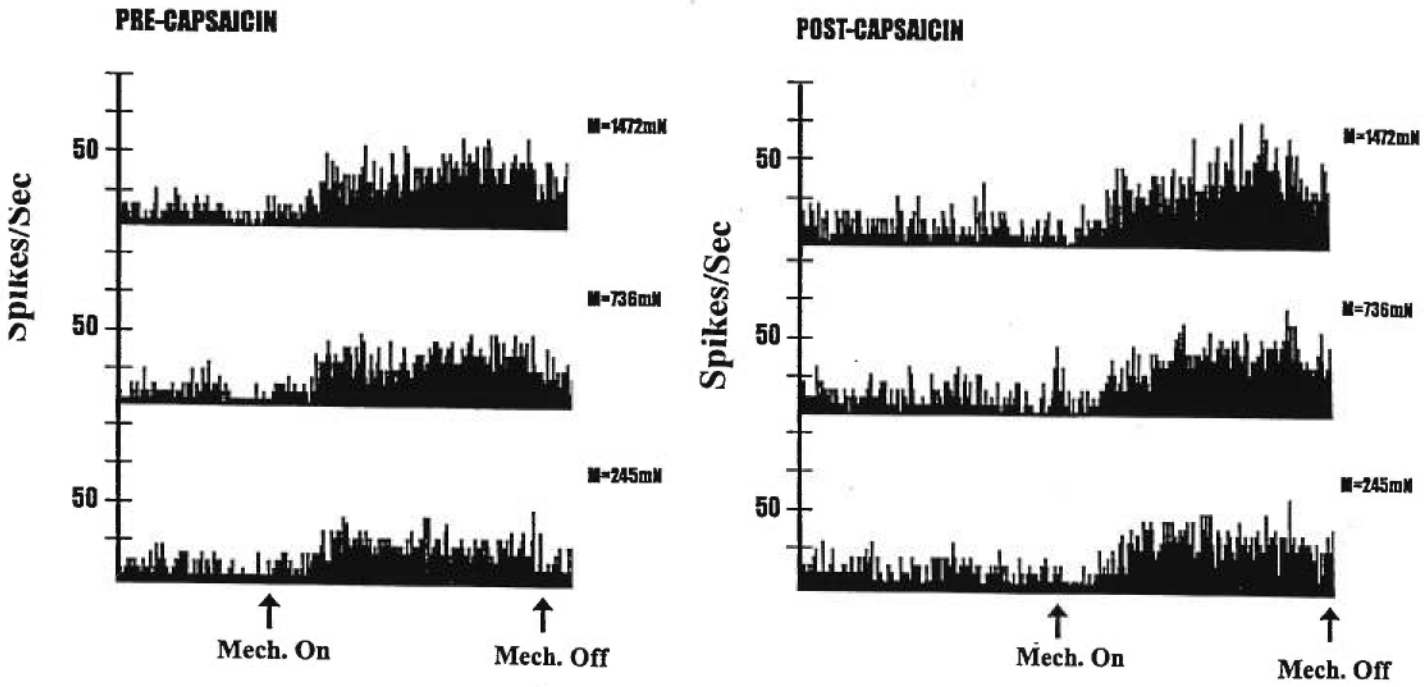
(A) LT NEURON


PRE-CAPSAICIN
POST-CAPSAICIN
Spikes/Sec
50
50
50
50
50
50
M=1472mN
M=736mN
M=245mN
M=1472mN
M=736mN
M=245mN
Mech. On
Mech. Off
Mech. On
Mech. Off





(B) WDR NEURON


PRE-CAPSAICIN
POST-CAPSAICIN
Spikes/Sec
50
50
50
M=1472mN
M=736mN
M=245mN
Mech. On
Mech. Off

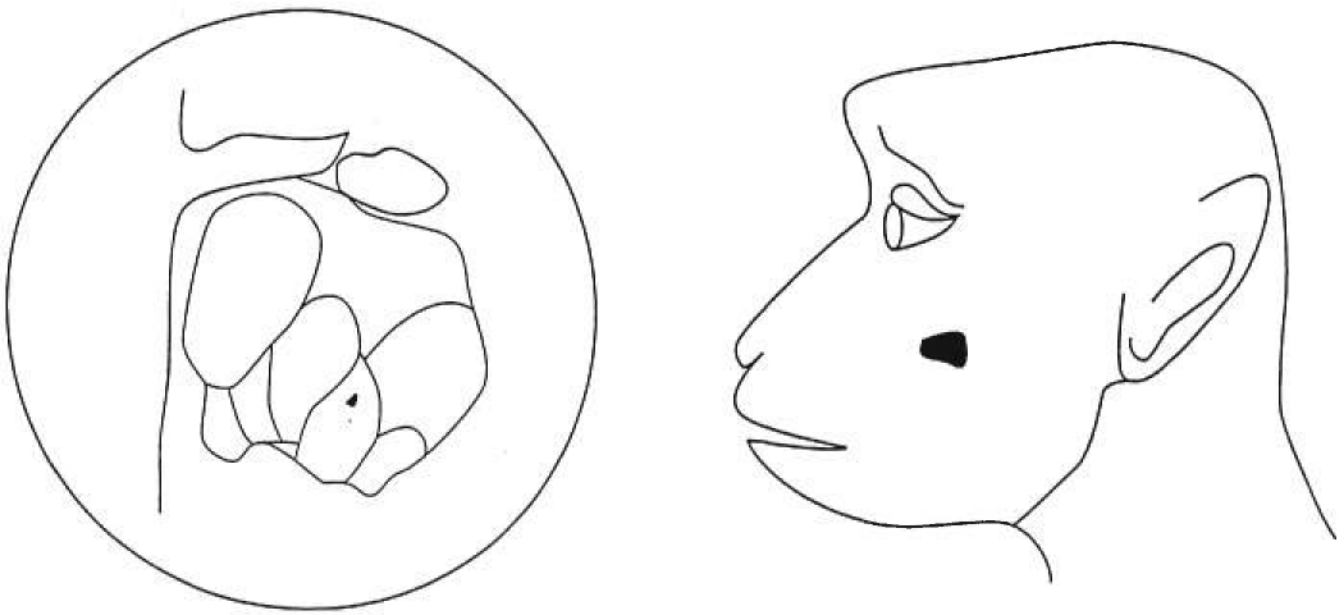


Figure 4. Discharge profiles of individual LT neurons. A: spontaneous activity, B: mechanically evoked activity. *Left:* neuronal activity before the application of capsaicin, *Middle:* neuronal activity after the capsaicin and *Right:* gain of the activity. Positive values (higher than zero) indicate increased responses, whereas, negative values (lower than zero) indicate decreasing responses after the capsaicin patch was applied on the receptive field. Three LT neurons showed decreases in spontaneous activity and four units showed decreasing discharge to mechanical stimuli.

Fig. 4.(A) **Spontaneous activity (LT neurons)**

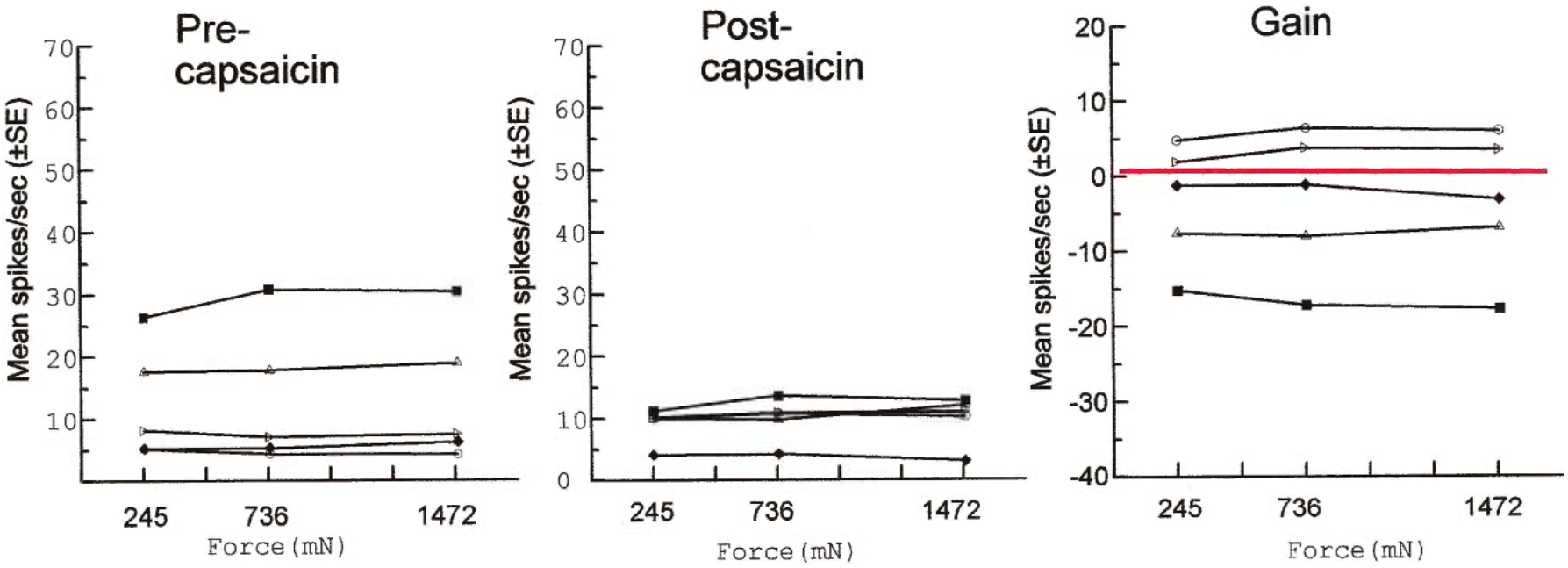


Fig.4.(B) **Mechanically evoked activity (LT neurons)**

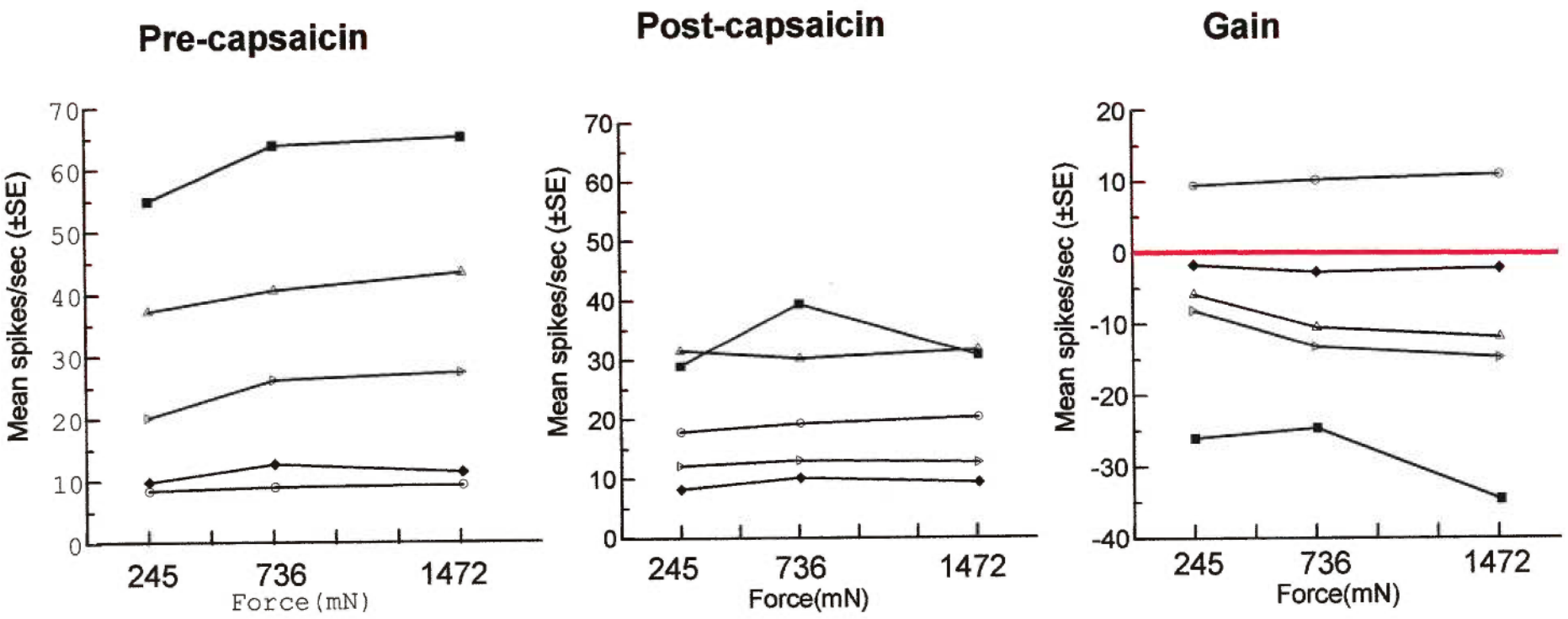


Figure 5. The comparison of mean discharge of LT neurons (N=5) before and after the application of capsaicin. A: spontaneous , B: mechanically evoked , C: difference activity, D: ratio. The data are presented as Mean± SE. A non-significant decrease of neuronal activity was noted in spontaneous and mechanically evoked activity ($p=0.44$; 0.26, respectively). The ratio of mechanically and spontaneous activity remained the same before and after the capsaicin.

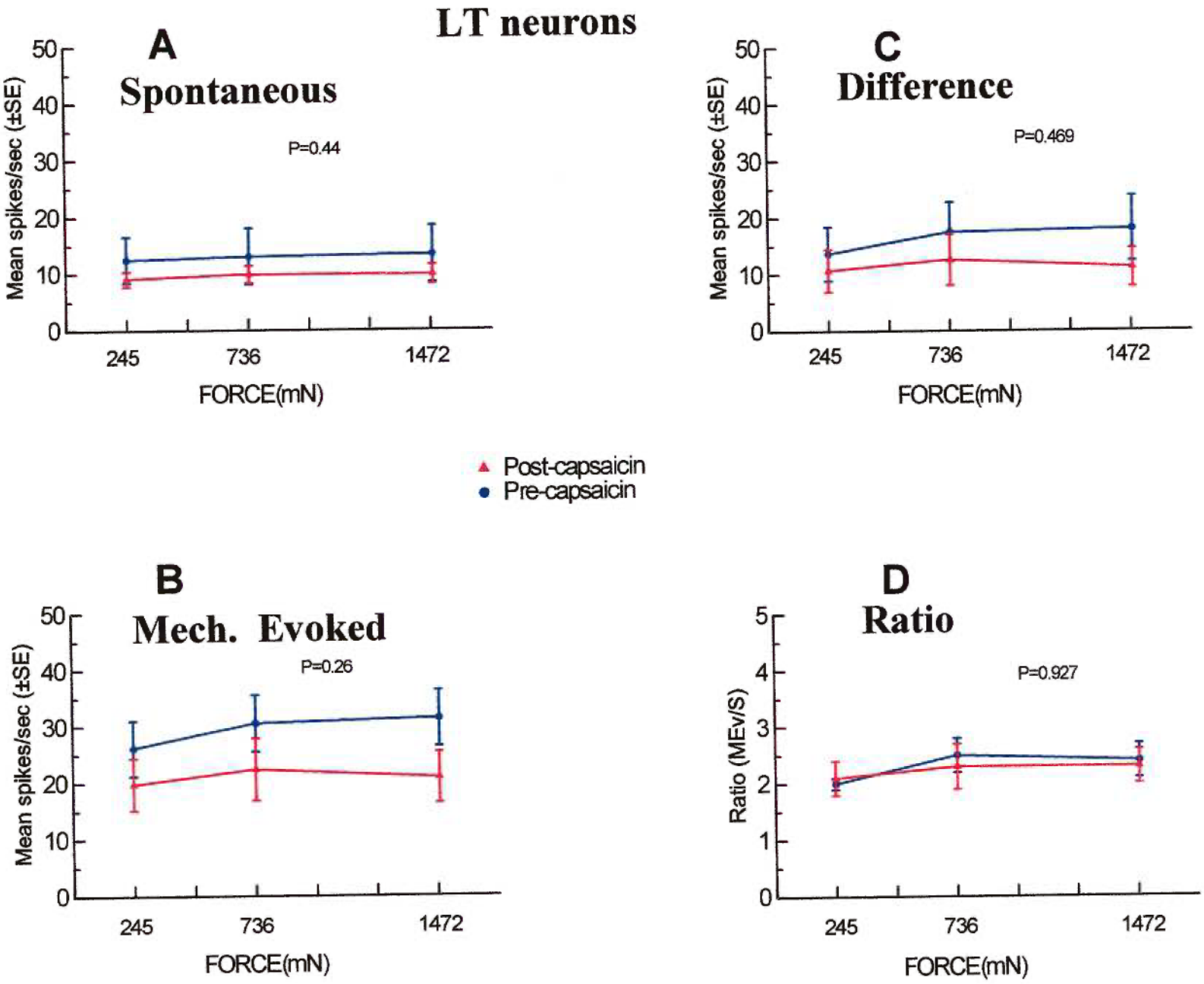
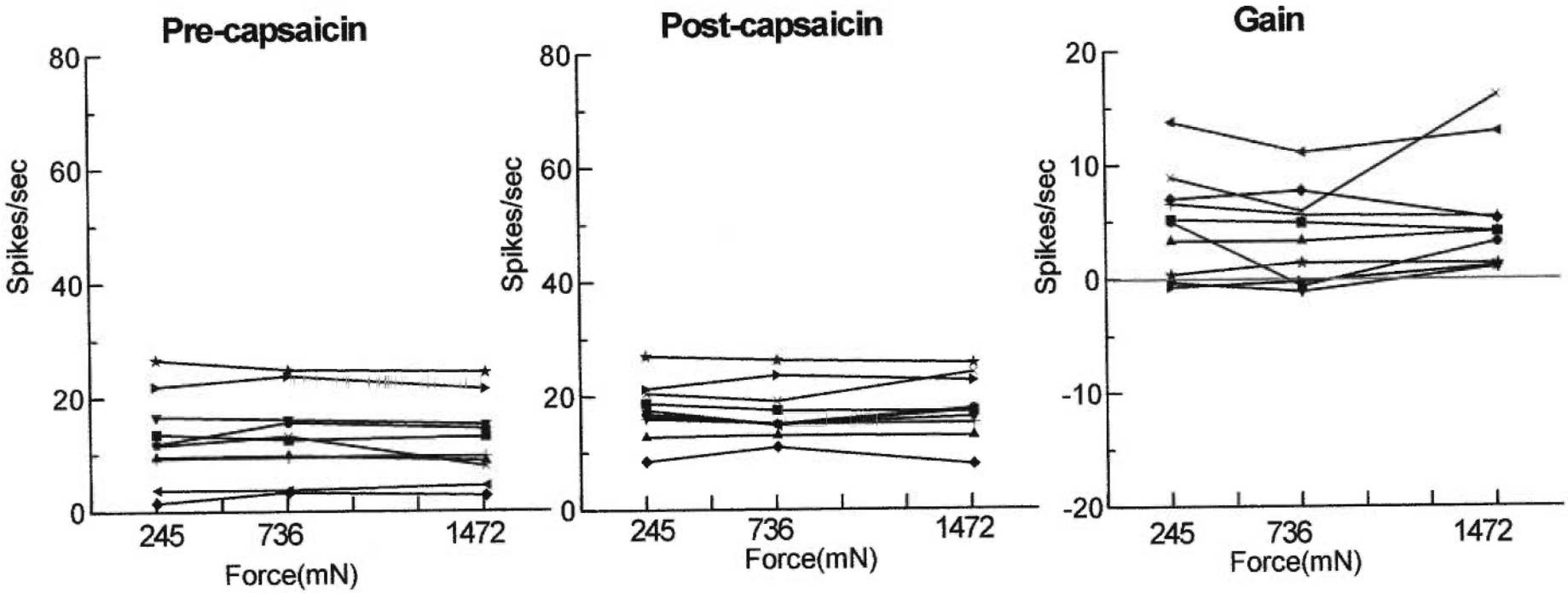

LT neurons
A
Spontaneous
P=0.44
Mean spikes/sec (±SE)
50
40
30
20
10
0
245
736
1472
FORCE(mN)
C
Difference
P=0.469
Mean spikes/sec (±SE)
50
40
30
20
10
0
245
736
1472
FORCE(mN)
Post-capsaicin
Pre-capsaicin
B
Mech. Evoked
P=0.26
Mean spikes/sec (±SE)
50
40
30
20
10
0
245
736
1472
FORCE(mN)
D
Ratio
P=0.927
Ratio (MEv/S)
5
4
3
2
1
0
245
736
1472
FORCE(mN)

Figure 6. Similarly organized as Figure 4, discharge profiles of individual WDR neurons are presented. A: spontaneous activity, B: mechanically evoked activity. *Left:* neuronal activity before the application of capsaicin. *Middle:* neuronal activity after the capsaicin, *Right:* gain of the activity; positive values (higher than zero) indicate increased responses, whereas, negative values (lower than zero) indicate decreasing responses after the capsaicin patch was applied on the receptive field. Most of the WDR neurons showed increases of spontaneous activity and responses to mechanical stimuli after the hyperalgesia produced by capsaicin.

(A) Spontaneous activity (WDR neurons)



(B) Mechanically Evoked Activity (WDR neurons)

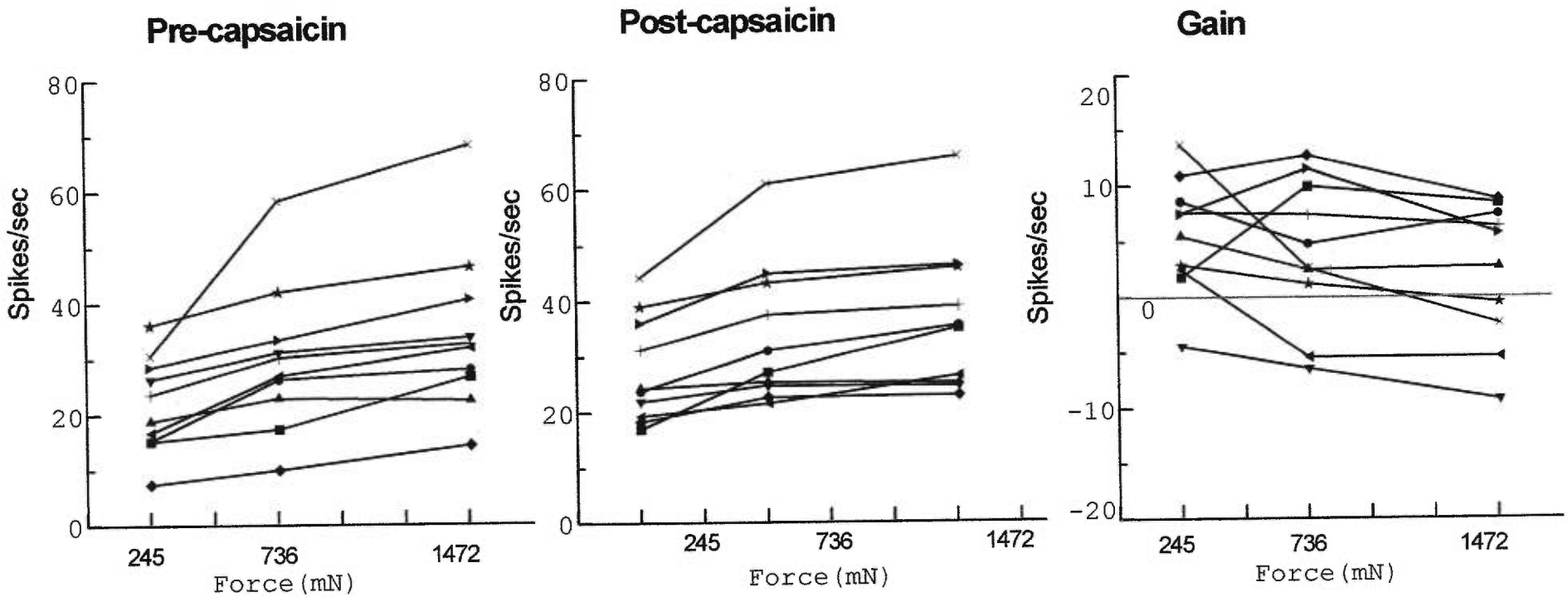


Figure 7. Comparison of mean discharge of WDR neurons (N=10) before and after the application of capsaicin. A: spontaneous , B: mechanically evoked , C: difference activity, D: ratio. The data are presented as Mean spikes/s ±SE. Significant increases of spontaneous and mechanically evoked activity were noted (p=0.01; 0.04, respectively) while the difference of mechanically evoked and spontaneous activity remained the same and the ratio of these activities became reversed after applying capsaicin.

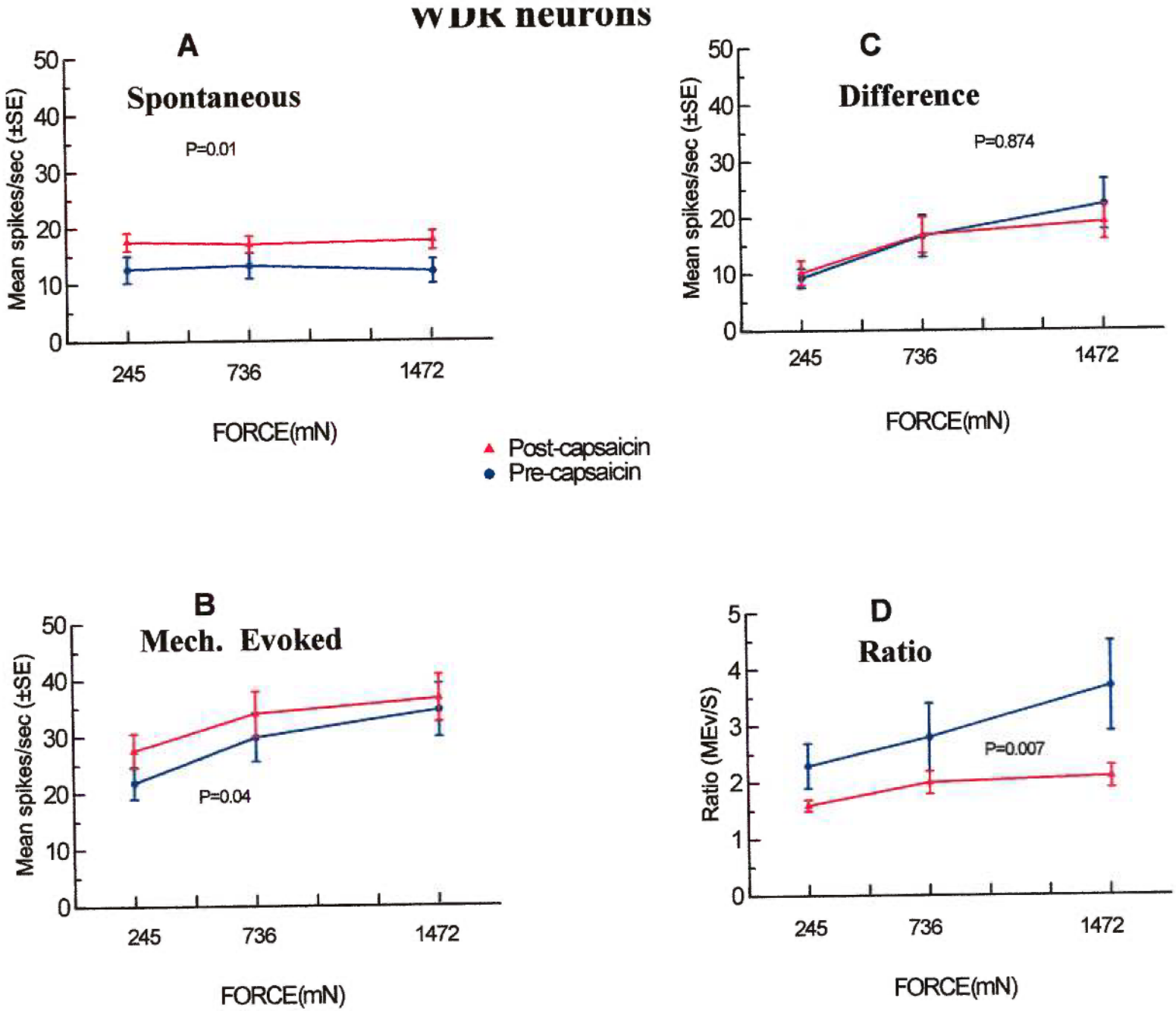
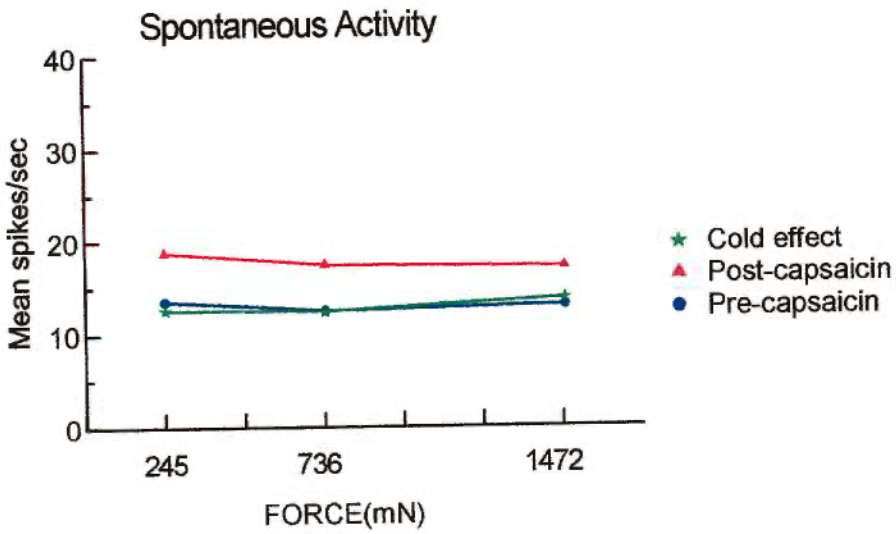

WDR neurons
A
Spontaneous
P=0.01
Mean spikes/sec (±SE)
FORCE(mN)
C
Difference
P=0.874
Mean spikes/sec (±SE)
FORCE(mN)
Post-capsaicin
Pre-capsaicin
B
Mech. Evoked
P=0.04
Mean spikes/sec (±SE)
FORCE(mN)
D
Ratio
P=0.007
Ratio (MEv/S)
FORCE(mN)

Figure 8. Example of a WDR neuron tested for the effect of cold temperature. The increased spontaneous activity caused by capsaicin was suppressed by the cold temperature applied to the testing area. In the mechanically evoked activity, this suppression was not clear except in the noxious range.

Cell #3312


Spontaneous Activity
Mean spikes/sec
40
30
20
10
0
245
736
1472
FORCE(mN)
Cold effect
Post-capsaicin
Pre-capsaicin

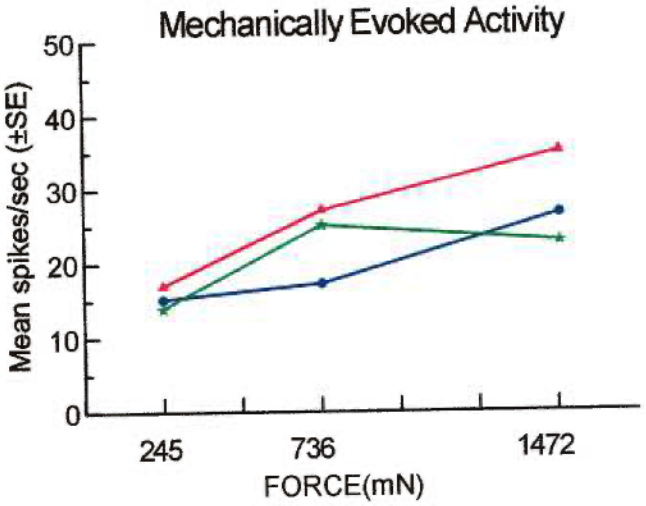

Mechanically Evoked Activity
Mean spikes/sec (±SE)
50
40
30
20
10
0
245
736
1472
FORCE(mN)

Table 2. Cold suppression on the capsaicin effect in four WDR neurons

Four WDR neurons tested with cold (5-10°C)

Cell ID	Spontaneous Activity (mean spikes/s)			Mech. Evoked Activity (245/736/1472 mN) (mean spikes/s)		
	Pre-cap	Post-cap	Post-cold	Pre-cap	Post-cap	Post-cold
#3304	14.0	17.5	15.0	15.3/26.3/28.1	23.9/31.1/35.5	17.2/19.8/23.7
#3305	22.4	22.5	22.2	28.6/33.5/40.7	36.1/45/46.4	25.4/40.3/46.7
#3308	12.1	21.2	17.1	30.6/58.4/68.3	44.3/61.0/65.9	40.1/52.0/55.2
#3312	13.1	17.8	13.0	15.2/17.3/26.7	17.0/27.2/35.1	14.0/25.2/22.9

Chapter 7 The effects of capsaicin on mechanical perception and discrimination in humans

Note: Part of this work has been published as an abstract:

Chen,C.-C. and Bushnell, M.C. Discrminative properties of human capsaicin-induced hyperalgesia correspond to activity of thalamic wide-dynamic-range neurons. *Society for Neuroscience Abstracts,* (1998) p.1128, #445.4

7.1 Summary

1. The effects of capsaicin on mechanical perception and discrimination were tested on the facial skin in eight human volunteers (3 females, 5 males; age range: 23 – 45 years).

2. The flare area was about 14 times the size of the capsaicin patch, and area of the mechanical hyperalgesia was consistent with but slightly larger than the flare area. Spontaneous pain started within one minute, and reached the maximum during 5-10 minutes following application of the capsaicin.

3. Capsaicin-increased mechanical sensation was found for both baseline forces ($p=0.001$). For the stronger baseline force (near-noxious), the VAS rating increased from 46.8 (not painful) to 65.2 (painful), indicating the presence of mechanical hyperalgesia.

4. In the discrimination test, two mechanical baseline forces (245 & 882mN) were each paired with two incremental forces (ΔF) and forced-choice discrimination was tested. The results showed that mechanical discrimination was not altered by capsaicin. Although the performance improved in the smaller ΔF and worsened for larger ΔF in both groups, the differences were non-significant ($p=0.66$).

5. These results, combined with the electrophysiological findings in monkeys, suggest that 1) thalamic WDR neurons participate in neural processes underlying capsaicin-induced pain sensation and mechanical hyperalgesia, 2) the unchanged discriminative ability in humans is

consistent with lack of alteration of difference and ratio between mechanically evoked and spontaneous activities after capsaicin treatment in monkeys.

7.2 Introduction

Intradermal injection of capsaicin is known to cause excitation of spinothalamic tract (STT) neurons followed by a sensitization to mechanical stimuli in anesthetized monkeys (Dougherty and Willis, 1992; Simone et al., 1991b). These studies demonstrated that both NS and WDR neurons discharged vigorously immediately after the injection of capsaicin, but only WDR neurons continued to discharge for an extended period of time. Therefore, it was postulated that STT WDR neurons not only contributed to the acute pain immediately after injection of capsaicin (LaMotte et al., 1991), but were also most likely to be responsible for maintained pain and hyperalgesia.

In addition, capsaicin also has been known to increase the size of receptive fields of WDR neurons in monkey and cat (Dougherty and Willis, 1992; Simone et al., 1989b), or to induce reorganization in receptive fields in cat cuneate neurons (Petit and Schwark, 1996, 1993). WDR neurons in the spinal cord have the capacity to code tactile, as well as noxious mechanical and thermal, signals (Willis and Coggeshall, 1991). The alteration in receptive fields and the responsiveness of WDR neurons to mechanical stimuli after capsaicin-induced inflammation could

impair two-point discrimination and could reduce the ability to detect differences in the roughness of various stimulation surfaces (Kauppila et al., 1998).

In the previous chapter, we presented evidence showing capsaicin increases spontaneous and mechanically evoked activity of WDR neurons of thalamic VPM nucleus in alert monkeys. However, neither the difference nor the ratio of mechanically evoked and spontaneous activity were altered by capsaicin. Based on these findings, we predict that capsaicin should not alter the ability of human subjects in mechanical intensity discrimination.

7.3 Materials and methods

The effects of capsaicin on perception and discrimination of mechanical intensities were tested in eight human volunteers (3 females, 5 males, ages 22 to 44).

The experimental protocol was approved by the Ethics Committee of McGill University. All participants were given clear instructions and information regarding the procedures, and they understood that they were free to withdraw from the experiment at any time without any objection.

7.3.1 Preliminary tests

Preliminary studies were done by the author and his colleagues in order to decide on the experimental parameters. These pilot tests

included: 1) observing the effects of a capsaicin patch on facial skin and measuring the areas of visible flare and mechanical hyperalgesia; 2) temporal courses of spontaneous pain sensation during and after the application of capsaicin; 3) adequate increment of forces (ΔF) for the mechanical discrimination under normal situation.

7.3.2 Experimental protocol

Testing site and application of capsaicin: The left facial area near the lower border of the zygomatic arch was chosen for the experiment. Capsaicin was prepared following the procedures used in monkey behavioral and electrophysiological studies and those used in human psychophysical studies (see Chapters 4,5 and 6). In brief, one 1x1-cm gauze pad saturated with capsaicin (0.004M, 0.3ml) was applied to the testing area and sealed with a 3M transparent adhesive film for five minutes. Prior to the study, the testing site was cleaned with 70% ethanol and drawn with a marker pen. Subjects were asked to rate the intensity of spontaneous pain sensation caused by the capsaicin patch with a visual analogue scale (VAS) in which "0" represented no sensation and "100" meaning intolerable pain. Subjects were instructed to use "50" as a pain threshold if the stimulus was felt as painful.

Mechanical discrimination: Results obtained from the preliminary tests were used to choose the mechanical baseline forces. Two baseline forces, one innocuous (245 mN) and one near-noxious (882 mN) were selected for two sessions of experiments. The ΔFs were also determined

in the preliminary studies to ensure that the correct percentage of discrimination was always higher than the guessing rate under the normal condition (i.e. baseline 245mN paired with 29.4, 98mN; baseline 882mN paired with 98 & 343mN, respectively). In each session, the baseline force was paired with one smaller and one larger incremental force (ΔF). The sequence of the paired stimuli was pseudo-randomized.

All experiments were conducted in an isolated room in which the subject was seated in a comfortable position with his head supported by a chin-rest to help immobilize the head. The mechanical stimulator was positioned orthogonally about 5 mm from the skin, and the probe remained within the testing area throughout the experiment. A 3-cm-diameter light and a response lever were attached to the chair and located 50 cm in front of the subject. The subject initiated the trial by pressing the lever when the cue light was on. When the lever was pressed, the mechanical probe would deliver the first stimulus intensity (baseline plus ΔF) onto the face for one-second. Then the probe would retract and advance again to deliver the second stimulus intensity. The subject was then asked to indicate the stronger force between the two by pointing to a letter A (the first one) or B (the second one). Feedback was provided to the subjects verbally by the experimenter as to whether the response was correct.

In the beginning, all subjects practiced 30 trials to familiarize themselves with the task. Immediately following the practice, two test

sessions with different baselines (245, 882 mN) were conducted before the administration of capsaicin. After the application of capsaicin, the same parameters were tested again.

Subjects were also asked to rate the intensity of the mechanical stimuli using the same VAS that was used for capsaicin pain sensation. This rating of a baseline force was made in the middle and at the end of each discrimination session.

7.4 Results

During the preliminary tests, the spatial extent of mechanical hyperalgesia to punctate stimuli was evaluated after application of capsaicin, and a representative example is illustrated in Fig. 1. The average area of the visible flare was 14 ± 2 cm^2 including the application site. Hyperalgesia, tested with a punctate stimulus (a sharpened pencil) was consistent with but slightly larger than the area of the flare.

Topical application of capsaicin elicited a sensation of burning pain in all subjects. Ratings of pain intensity increased rapidly within the first 2 minutes and reached a elevated level after 5 min (ANOVA, repeated measure, $p<0.001$)(Fig. 2). Previous experiments have demonstrated that the level of pain evoked by capsaicin remains at a high level for about 25 min (see Chapter. 4). As shown in Fig. 3, after capsaicin application, the average rating of sensation evoked by the 245mN stimulus increased from 13.7± 4.0 to 34.7± 5.9, while ratings of the 882mN stimulus increased from 46.8 ± 2.0 to 65.2 ± 2.4 (ANOVA,

repeated measure, $p=0.02$, $p<0.001$; respectively), indicating that capsaicin increases mechanical perception and pain sensation.

Fig. 4 shows the percentage of correct responses in the discrimination task. During all tests, subjects always performed better for the larger ΔF than for the smaller one (ANOVA, $p=0.003$). After the application of capsaicin, subjects showed a tendency toward an increased ability to discriminate the smaller ΔF and a decreased discrimination of the larger ΔF, in both baseline forces (Fig. 4). However, these differences are non-significant (ANOVA, repeated measure, $p=0.103$, $p=0.170$ for the baseline of 245mN with smaller and larger ΔF; $p=0.072$, $p=0.265$ for the baseline of 882mN with smaller and larger ΔF; accordingly).

7.5 Discussion

In this study, an increase in the perceived intensity of mechanical stimuli accompanied by an unaltered ability to discriminate mechanical stimuli were found within the area of primary hyperalgesia on humans' facial skin, following the application of capsaicin. These findings, combined with monkey neurophysiological results (see Chapter 6), indicate that thalamic WDR neurons could subserve tactile sensation as well as pain and mechanical hyperalgesia in thalamus.

Areas of flare and hyperalgesia

We found that the spatial extent of flare coincided closely with the area of mechanical hyperalgesia in the present study. This finding is consistent with findings reported by other investigators using intradermal injection (LaMotte et al., 1991; Serra et al., 1998; Simone et al., 1989a) or topical application of capsaicin in humans (Forster et al., 1995; Kilo et al., 1994; Koltzenburg et al., 1992). The area of flare and mechanical hyperalgesia presented in this study are larger than those reported by Kotzenburg et al., (1992) , in which 1% dyed capsaicin was used, and an area of mechanical hyperalgesia measuring approximately 9 times that of the patch size in forearm was reported. A possible hypothesis for this difference is that the spread of flare caused by topical capsaicin could be influenced by local microvasculature of the skin (Szolcsányi et al., 1992) or by a multifocal dilatation of arterioles around the injury site (Serra et al., 1998). Therefore, a capsaicin model of studying hyperalgesia could be associated with the inter- or intra-subjects variability at different testing sites (Liu et al., 1998).

On-going pain and mechanical perception

Capsaicin is known to be a selective excitant of unmyelinated or thin myelinated nociceptive primary afferents in animals (Baumann et al., 1991; Kenins, 1982; Lynn, 1990; Reeh et al., 1986; Szolcsányi et al., 1988) and humans (Konietzny and Hensel, 1983; Handwerker et al., 1991; LaMotte et al., 1992). Capsaicin-induced sensitization was also

observed in monkeys' NS and WDR neurons of the STT (Dougherty and Willis, 1992; Simone et al., 1991b), implying processing of sensitization at a higher central level (i.e., the thalamus and somatosensory cortex). In our study, topical application of capsaicin to the face produces a burning pain sensation and an increase in mechanical sensitivity in all subjects. These findings, combined with the enhanced responses of WDR neurons after capsaicin treatment (see Chapter 6), suggest that thalamic WDR neurons may participate a role in the processing of pain and hyperalgesia and may code tactile information as well under normal and hyperalgesic status.

Mechanical discrimination

WDR neurons in the spinal cord have the capacity to code tactile as well as noxious mechanical and thermal information. However, the interaction between the noxious and innocuous mechanical stimuli as described in the gate control theory of pain (Melzack and Wall, 1965) and touch gate theory (Apkarian et al., 1994) imply that thalamic WDR neurons might have more complicated integration between noxious and tactile stimuli. For example, capsaicin-induced inflammation could increase receptive fields of spinal WDR neurons in animals (Dougherty and Willis, 1992; Simone et al., 1991b;1989b) and result in the impairment of tactile spatial discrimination ability in humans (Kauppila et al., 1998).

In the previous chapter, we presented the enhanced spontaneous and mechanically evoked activity of thalamic WDR neurons after the treatment of capsaicin. However, the difference and ratio of these activities were not found to be significantly altered; therefore we can predict that humans' mechanical discrimination abilities would not be altered by hyperalgesia. This observation again implies a complexity of integration among the noxious and tactile information in thalamus. This finding is especially important and interesting when studying the mechanisms of central pain syndrome (CPS), in which both the hypoaesthesia and hyperaesthesia are observed (Boivie, 1992; Boivie et al., 1991, 1989,1982).

Figure 1. An example of schematic distribution of capsaicin patch, areas of flare and mechanical hyperalgesia. The flare area is 14 times the patch size. The area of mechanical hyperalgesia is consistent with the flare area. The mechanical forces for the following study of mechanical discrimination and perception were applied within the patch area.

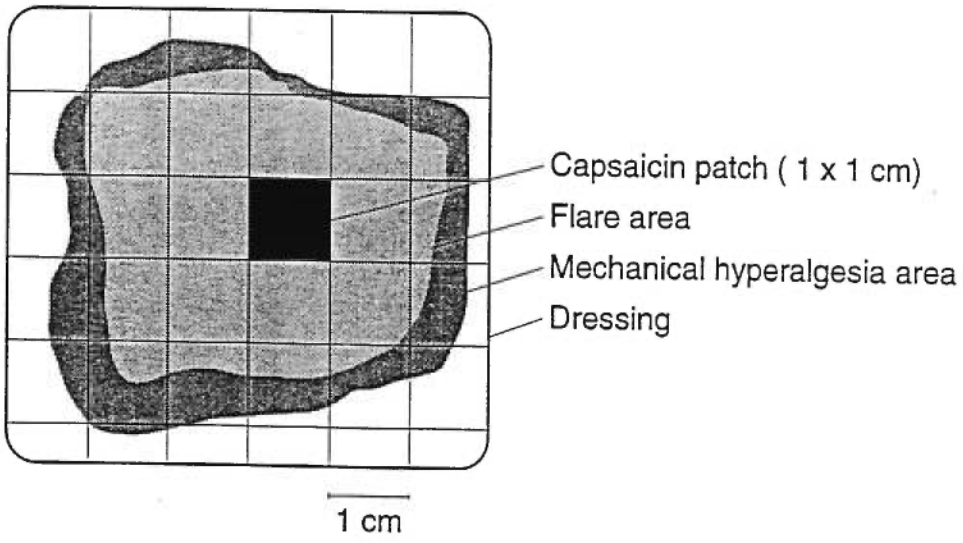

Capsaicin patch (1 x 1 cm)
Flare area
Mechanical hyperalgesia area
Dressing
1 cm

Figure 2. The spontaneous pain rating in eight human subjects during the capsaicin application. The data (Mean ± SEM) are based on a visual analogue scale of 0 to 100; "50" represented the pain threshold and "100" represented intolerable pain. Marked increases in pain sensation occur within 2 minutes.

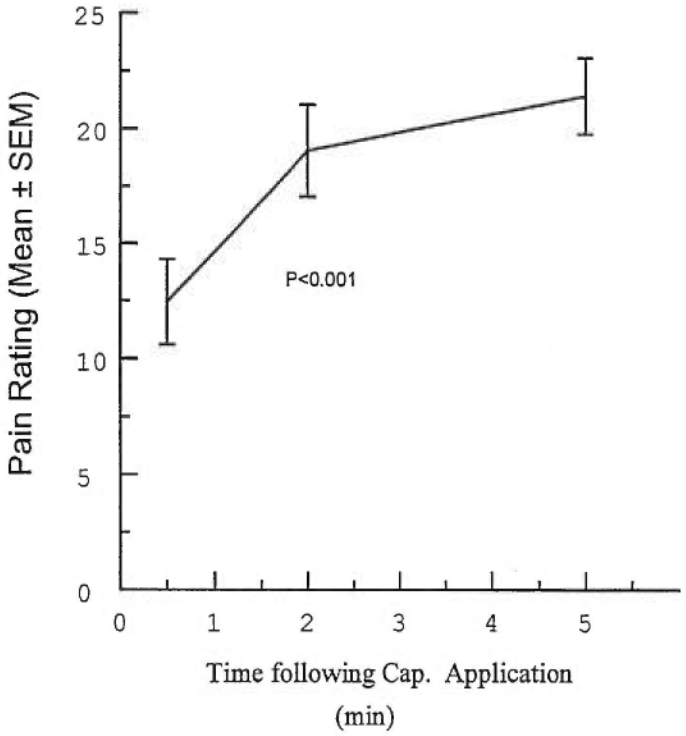

25
20
15
10
5
0
Pain Rating (Mean ± SEM)
P<0.001
0
1
2
3
4
5
Time following Cap. Application
(min)

Figure 3. Capsaicin effects on mechanical perception. Both baseline intensities showed significant increases after capsaicin. The average rating of sensation of 245mN increases from 13.7 to 34.7 and from 46.8 to 65.2 for 882mN, indicating that capsaicin increases the mechanical perception and pain sensation. (Blue: before, Red: after)

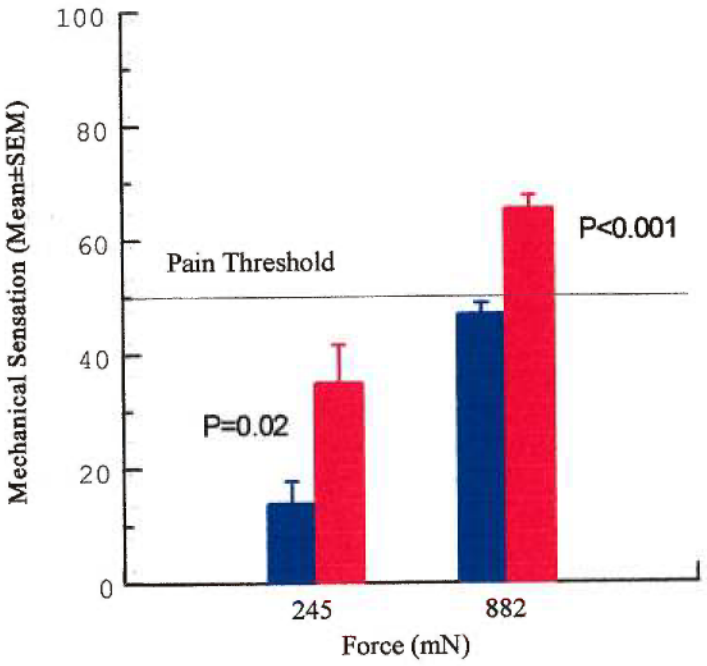
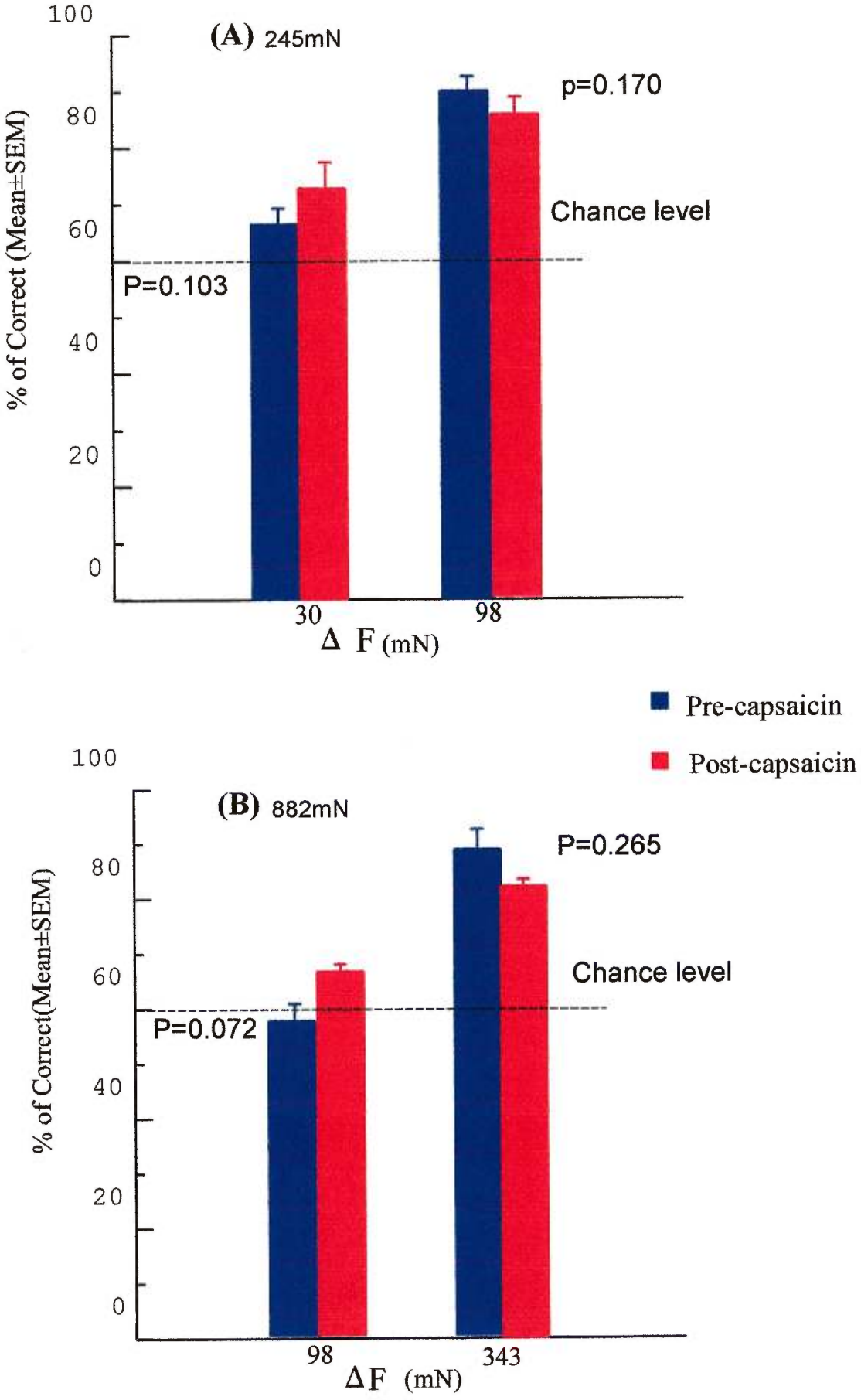

100
80
60
40
20
0
Mechanical Sensation (Mean±SEM)
Pain Threshold
P=0.02
P<0.001
245
882
Force (mN)

Fig. 4 shows the percent of correct performance in the discriminating task. During all tests, subjects always had higher correct percentage with larger ΔF. After the application of capsaicin, subjects had the tendency to improve their performance with the smaller ΔF and showed decreased ability of discrimination with the larger ΔF for both baseline forces (A: innocuous baseline force (245mN), B: near-noxious baseline force (882mN).).


(A) 245mN
p=0.170
Chance level
P=0.103
% of Correct (Mean±SEM)
100
80
60
40
20
0
30
98
Δ F (mN)
Pre-capsaicin
Post-capsaicin
(B) 882mN
P=0.265
Chance level
P=0.072
% of Correct(Mean±SEM)
100
80
60
40
20
0
98
343
ΔF (mN)

Part III. DISCUSSION AND CONCLUSION

CHAPTER 8 General discussion and conclusion

Application a variety of extreme stimuli (mechanical, heat or chemical agents) to peripheral receptors can evoke verbal reports of pain in humans and escape behavior in animals. It has been suggested that the circuitry which subserves the transduction and the encoding of noxious information has two components. First, at the peripheral level, noxious stimuli evoke activity in both small myelinated and unmyelinated primary afferents that synapse in the dorsal horn of the spinal cord. By way of ascending systems, this information gains access to supraspinal centres located in the brain stem and thalamus. Based on the anatomical association with spinal tracts and the neuronal response to peripheral stimuli, these supraspinal regions participate in the processing of pain-related information. A number of thalamic nuclei are thought to be associated with the transmission of somatic information evoked by noxious stimuli, and they are located in the lateral, medial and posterior part of thalamus. In lateral thalamus, the ventrobasal complex is particularly important for somatosensory processing, and the majority of neurons recorded here are responsive to innocuous tactile or thermal stimuli. A relatively smaller number of neurons are responsive to noxious stimuli. These nociceptive neurons include a population of "high-threshold" neurons which can only be activated by extreme stimuli, and a group of wide-dynamic-range (WDR) neurons which respond in a graded fashion to stimuli from innocuous to noxious range.

In addition, damage to the peripheral tissue could also result in stronger pain sensations to noxious stimuli or change the modality of sensation to innocuous stimuli from non-painful to painful. These phenomena are called hyperalgesia and allodynia, and both peripheral and central neural mechanisms are involved. The activation of primary nociceptive afferents is necessary to initiate hyperalgesia but the central sensitization is essential to maintain hyperalgesia and allodynia. This central sensitization occurs at spinal and supraspinal levels in neurons that receive convergent input from mechanoreceptors and nociceptors.

In this thesis, a series of experiments were designed in order to investigate the mechanical responses of thalamic VPM neurons under normal and hyperalgesic states. These data provide important information for better understanding central neuronal mechanisms of pain and hyperalgesia.

8.1 Summary of results

8.1.1 The transient hyperalgesia model in awake primates (Chapter4)

The aim of this study was to develop a transient hyperalgesia model in the awake monkey. One monkey and seven human subjects participated in this experiment. Capsaicin (0.3 ml; 0.004M) was topically applied on the maxillo-facial area. Innocuous and noxious thermal (43, 47 and 51°C) or mechanical (245, 736 and 1472mN) stimuli were used.

At the site of capsaicin application, the monkeys escaped more thermal and mechanical stimuli, suggestive of thermal and mechanical hyperalgesia. Human subjects reported higher pain intensity for similar stimuli after capsaicin application, in accordance with the monkey's escape behavior.

The procedure is repeatable and produces no overt sign of distress in animals. Therefore, this model of acute, transient hyperalgesia could be suitable for the following neurophysiological studies.

8.1.2 Response characteristics of thalamic VPM neurons to quantitative mechanical stimulation in awake monkeys (Chapter 5)

This study investigated the responses of thalamic VPM neurons to punctate mechanical stimuli in two awake behaving monkeys (Macaca Mulatta). The intensity of a mechanical stimulus applied to the monkey's face ranged from innocuous (245mN) to noxious (1472mN), and was controlled by a computer. Thirty-seven neurons with contralateral cutaneous receptive fields on the face were collected.

All neurons showed spontaneous activity in the absence of stimulation. Both spontaneous and mechanically evoked activity were recorded while the monkey performed visual tasks and received the mechanical stimuli. The difference of activity was calculated by subtracting the spontaneous activity from the mechanically evoked

activity. The ratio of spontaneous and mechanically evoked activity was also measured to verify the characteristics of neuronal responses.

Nineteen neurons were classified as low-threshold since the mechanically evoked activity was not significantly different between the innocuous and noxious stimuli. Eighteen neurons were defined as WDR as the neurons showed a significant, graded response to noxious stimuli. LT neurons were further classified as having slowly adapting (SA) or rapidly adapting (RA) properties according to their responses at the onset or offset of the stimuli. Fourteen of them were SA and five were RA.

The size of the receptive fields was not significantly different between LT and WDR neurons (averaged receptive fields were 44.9, 36.2m^2, respectively). The distribution of LT and WDR neurons within the VPM nucleus is somatotopically organized.

8.1.3 Responses of thalamic VPM neurons to topical application of capsaicin (Chapter 6)

In this experiment, the spontaneous neuronal discharge and the stimulus-related responses of thalamic VPM neurons were compared before and after the topical application of capsaicin (0.004M) to the maxillo-facial area in two behaving monkeys.

Fifteen neurons (5 LT, 10 WDR) were fully characterized. The results revealed two response of thalamic VPM neurons to topical application of capsaicin. First, the majority of WDR neurons showed an elevated

spontaneous discharge and increased excitatory response to mechanical stimuli after topical application of capsaicin. The second pattern of response was characterized by decreased activity of LT neurons. These neurons showed lower spontaneous activity and lower mechanically evoked activity following the capsaicin.

Nevertheless, despite the increase in spontaneous and evoked activity, the difference of WDR neuronal activity did not change after the capsaicin. Furthermore, the ratio between evoked and spontaneous activity of WDR decreased after capsaicin, while the ratio for the LT cells remained unchanged, suggesting that the discriminative capabilities of these neurons were not altered during a hyperalgesia state.

8.1.4 The effects of capsaicin on mechanical perception and discrimination in humans (Chapter 7)

Based on the previous findings that neither the difference nor the ratio of mechanically evoked and spontaneous activity were altered by capsaicin, we predict that the ability of human subjects to discriminate these stimuli would, likewise, be unaltered by the capsaicin treatment, in spite of the obvious perceptual consequences of the capsaicin-generated hyperlagesia and allodynia. In this study, we tested this hypothesis in eight human volunteers.

Two mechanical baseline forces (245 & 882mN) were each paired with two incremental forces (ΔF) and forced-choice discrimination was

tested. The flare area was also measured; it was about 14 times the size of capsaicin patch and the mechanical hyperalgesia area was consistent but slightly larger than the flare area. The results showed that subjects' ability to discriminate mechanical intensities was not altered by capsaicin. However, the mechanical sensation was found to have increased following capsaicin application for both baseline forces, indicating hyperalgesia. Spontaneous pain also occurred during capsaicin application, starting within one minute and reaching the maximum within 5-10 minutes. For the stronger baseline force (near-noxious), the mechanical intensity rating increased from 46.8 to 65.2, indicating the presence of mechanical hyperalgesia.

8.2 Aspects of methodology

8.2.1 Animal models

In the study of hyperalgesia and allodynia, several animal models have been developed in which partial lesions were inflicted on a peripheral or spinal nerve, ultimately leading to thermal and mechanical hyperalgesia (Bennett and Xie, 1988; Kim and Chung, 1992; Seltzer et al., 1990). These models have the disadvantage of being invasive and irreversible.

Lamotte and colleagues were the first to use the chemical irritant capsaicin in monkeys to study the neurophysiological mechanisms underlying hyperalgesia. These authors injected capsaicin intradermally

in anesthetized monkeys and recorded from primary afferents and spinothalamic tract neurons (Baumann et al., 1991; Simone et al., 1991b). This approach has the disadvantage that no direct correlation can be made between the behavioral consequences and the induced electrophysiological changes. Furthermore, in neurophysiological studies, anesthesia and the state of arousal of the animal have a potential confounding influence on the interpretation of the experimental results (Dougherty et al., 1997; Duncan et al., 1982b; Guilbaud et al., 1981; Morrow and Casey, 1992, 1988).

The present model, using the awake, behaving primate, can offer several advantages. First, operant responses are used in the present model. These are at a hierarchically higher level than reflex-type responses that are used in many studies. Second, a direct correlation between behavior and neuronal discharge can be studied. Third, the obtained results are not influenced by the administration of anesthetics and allow the comparison with human data. Fourth, the present approach allows the study of dynamic changes in single neurons that cannot be studied with models of peripheral nerve ligation that produce long-duration or permanent hyperalgesia. Finally, the present approach poses fewer ethical problems than models involving permanent lesions. However, this model requires an extensive period of laborious training of the monkey in order to get reliable and stable data.

8.2.2 The application of capsaicin

Many substances have been reported to produce hyperalgesia or allodynia after cutaneous or subcutaneous application. Among these, capsaicin, the pungent ingredient of hot chilli pepper, is the best studied both in human psychophysical and animal electrophysiological studies. This substance can have both excitatory and toxic effects on cutaneous primary sensory afferents (Russel and Burchiel, 1984). Lower concentrations of capsaicin, such as 1% solution applied topically to the skin, can excite C-mechanoheat (CMH) nociceptors in rats (Kenins, 1982), monkeys (Baumann et al., 1991) and humans (Konietzny and Hensel, 1983, Liu et al., 1998). However, repetitive topical application of capsaicin can suppress the discharge of the primary afferents (Carpenter and Lynn, 1981). A model of intradermal injection of capsaicin extensively used by LaMotte and his colleagues demonstrates that the injection site itself is analgesic (Lamotte et al., 1991; Simone et al., 1989a,1987). Therefore, extreme caution should be taken when using capsaicin in the chronic electrophysiological study of pain. In our study, we used low concentrations of capsaicin solution (0.004M) to induce hyperalgesia. We chose topical application of capsaicin over intracutaneous injection, as has been used in some human psychophysical and neurophysiological studies. Human psychophysical studies reveal that intracutaneous injection of capsaicin produces an immediate and extremely severe burning pain, which is inappropriate for awake monkey

experimentation (LaMotte et al., 1992; Simone et al., 1989a). We also took other measures to prevent such desensitization. First, we avoided applying the capsaicin at the same site within 1 week. In Chapter 4, we observed no systematic change in escape behavior across the pre-capsaicin test days, indicating that in fact no nerve damage occurred. However, in future applications of this model that might require shorter interstimulation intervals or repeated stimulation to the same skin region, it might be advantageous to use an algesia agent that has less potential for creating long-term nerve damage, such as mustard oil.

8.2.3 Mechanical stimulator

Mechanical stimulus

Adequate mechanical stimulation is especially important for studies in hyperalgesia and allodynia. The traditional methods use brush, pinch or von Frey hairs to investigate the mechanosensitivity of neurons in electrophysiological and psychophysical studies. These methods have the disadvantage in that the mechanical forces applied to the receptive area might not be constant throughout the experiment, thus making the comparison of neuronal activity among different experiments difficult. In addition, the size of the mechanical probe and the angle of stimulus could affect perception as reported by Greenspan and McGillis (1991). Our studies used a 0.02mm probe tip and computer-controlled forces, and the results were consistent with the findings in animal behavioral

observations and human psychological data (see Chapter 4; Kupers et al., 1997,1996). In these studies, a force of 245mN was barely sensed and 746 mN force was reported to evoke a strong sensation but not pain, and 1472mN was almost always reported as painful in human subjects. In the behaving monkey, the animal escaped fewer of the lowest intensity stimuli but did escape most of the 1472mN stimuli. These results indicate that the mechanical stimuli were constant and the evoked neuronal responses were reliable. Special attention was also given to the angle when applying the stimulus. Greenspan and McGillis (1991) described that stimulus angles larger than 15 degrees could result in a change of perception and pain thresholds. In this report, the mechanical probe applied to the receptive fields of all the selected neurons was always positioned perpendicularly to the skin area as much as possible so that the applying angle was within the range of 5 degrees. Thus, by using this method, the mechanical forces could be consistently applied and were easily quantified in animal electrophysiological studies, allowing them to be reliably related to human psychophysical studies. Using these controlled reproducible stimuli, we were able to perform statistical analyses of response characteristics of VPM neurons. These statistical analyses allowed us to observe such distinctions between neurons whose activity continued to increase into the noxious range and those that did not. Using quantitative analyses, we observed a higher proportion of VPM neurons that could be classified as WDR than is usually observed

when less precise methods are employed. The finding that almost all cells we classified as WDR were excited by capsaicin whereas those that were classified as LT were not , further substantiates that our classification has functional significance.

However, one should note that the innocuous stimulus can still cause escape behavior in the animals. This is probably due to the dynamic mechanical stimulator that may have the disadvantage of causing annoyance and distraction in the animal. Therefore, longer training periods are needed to train the animal to reach the reliable psychophysical results.

8.3 Functional mechanisms of thalamic VPM nucleus

The role of the thalamic VPM nucleus in pain and somatosensory processing appears to be more complex than once thought, serving as more than just a simple relay station between the dorsal horn and somatosensory cortex. The altered response characteristics of VPM neurons and the reduced receptive fields indicate that there is reorganization, integration or gating of information occurring under physiological and pathological condition. This study demonstrates that thalamic VPM could encode peripheral mechanical stimuli in both the innocuous and noxious range and showed different response characteristics after the application of capsaicin.

8.3.1 Properties and contribution of VPM LT neurons

Properties of LT neurons

The ventrobasal complex is situated in the ventrolateral part of the lateral thalamus. Neurons in this region project in a somatotopic manner to SI and SII. The majority of neurons in the ventrobasal complex are responsive to innocuous tactile stimuli. The neuronal discharge can be evoked by touch and under normal conditions they never show increasing responses to extreme mechanical stimuli. Therefore, they are defined as low-threshold neurons (LT). According to the properties related to the onset and offset of the stimulus, LT neurons can be further classified as being slowly adapting (SA) or rapidly adapting (RA). Substantial populations of SA neurons in thalamic VPM and VPL have been reported (Baker, 1971; Darian-Smith, 1966; Iwamura and Inubushi, 1974; Loe et al., 1977; Perl and Whitlock, 1961). However, these investigators reported that there is a predominant population of RA neurons in anesthetized cats and monkeys. In present study, approximately 74% (14/19) of low threshold neurons were found to exhibit SA properties. This finding is consistent with the high incidence of SA neurons in awake monkeys described by Bushnell and Duncan (1987), in which they found that about half of the low-threshold neurons demonstrated slowly adapting properties.

Anesthesia and the state of arousal of the animal have a potential confounding influence on neuronal responses. It was reported that anesthetics increase the receptive field sizes and the responses of low-threshold thalamic neurons in rats (Guilbaud et al., 1981) and in monkeys (Dougherty et al., 1997). In addition, it was shown that strong arousal produced increases in thalamic neuronal activity in awake primates (Morrow and Casey, 1992, 1988).

The neuronal distributions of SA and RA neurons were found to be aggregated in clusters in the thalamic VP nucleus of anesthetized (Dykes et al., 1981; Kaas et al., 1984) or alert monkeys (Bushnell and Duncan, 1987; Hayward, 1975). Similar observations in the somatosensory thalamus of humans were also reported (Lenz et al., 1998a,1990a). In the present study, SA neurons showed a tendency to be located in clusters, however, the sample was too small to show a clear clustering of these neurons.

Contribution of LT neurons to hyperalgesia and allodynia

Recently, a touch gate theory has been proposed by Apkarian and his colleagues (1994). They demonstrated that heat-induced pain could substantially suppress tactile sensitivity, independent of a shift in attention or arousal in human subjects. This is consistent with our neurophysiological results which showed that most of VPM LT neurons (N=4) showed a tendency toward decreased spontaneous discharge and

reduced responses to mechanical stimuli during the hyperalgesic state produced by capsaicin. However, one LT neuron was found to show excitability following the application of capsaicin. A phenotypic switch of larger myelinated afferents under the state of tissue injury may partially explain the observed result in this particular neuron (Neumann et al., 1996). Due to the small sampling size, we would need further investigation to confirm the findings on LT neurons.

8.3.2 Properties and contribution of nociceptive VPM neurons

Properties of WDR neurons

The nociceptive neurons found in the present study were classified as WDR neurons. This result is consistent with other reports showing that most of nociceptive thalamic VP neurons were WDR with only a small populations of nociceptive-specific (NS) neurons in primates (Apkarian and Shi, 1994; Chung et al., 1986a,b; Kenshalo et al., 1980; Perl and Whitlock, 1961) or in raccoons (Simone et al., 1993). All the WDR neurons showed increased responses in a graded fashion to the increasing intensities of mechanical stimulation. Therefore, this result demonstrates that VPM WDR neurons could encode peripheral punctate mechanical information in the innocuous to noxious range. The low percentage or absence of NS thalamic neurons in the unanesthetized monkey may have resulted from the difficulty in presenting noxious stimuli to the animal. In addition, species difference may also contribute to the observed result, as reported by Yokota

and his colleagues, in which most of nociceptive neurons in cat sensory thalamus are NS (Yokota and Matsumo, 1983a,b, Yokota et al., 1985). The distribution of nociceptive neurons recorded in this study showed an intermixture with that of LT neurons.

Contribution of WDR neurons to hyperalgesia and allodynia

Simone et al. (1991b) showed that intradermal injection of capsaicin causes excitation of STT neurons followed by a sensitization of these neurons to mechanical stimuli. They found that both types of WDR and High-threshold (HT) neurons discharged vigorously immediately after the injection of capsaicin, but only the WDR neurons continued to discharge for an extended period of time. Furthermore, there was a significant increased response to "brush" and "press" of the WDR neurons but not of HT neurons. These results suggest that WDR neurons are more likely to play an important role in persistent pain sensation and the development of hyperalgesia and allodynia following skin damage.

Our results show a similar response pattern for WDR neurons in thalamic VPM following the topical application of capsaicin. Capsaicin significantly increased the spontaneous and mechanically evoked activity of WDR neurons. This observed increase in spontaneous activity was comparable to the increased incidence of spontaneous pain reported by human subjects (LaMotte et al., 1991, Morris et al., 1997; Simone and Ochoa, 1991a; Chapter 4 and Chapter 7). Our findings of increased

mechanically evoked activity following capsaicin treatment suggest that WDR neurons could at least partially subserve hyperalgesia. Further analysis showed that the increase was not uniform but in preference to the responses to non-noxious mechanical stimuli. We suggest that VPM WDR neurons enhance their excitability to low-threshold inputs after sensitization, and might play a role in mechanical allodynia. Alternatively, the smaller increased response to noxious stimuli may simply be due to a ceiling effect, in which the maximal rate of neuronal discharge has already been achieved in the hyperalgesic state, and therefore the neurons are unable to show any further significant increase in their activity.

Cooling the injection site of capsaicin could reduce the hyperalgesia reported in humans' psychophysical studies (LaMotte et al., 1991). A similar observation was also reported by Cervero and Laird (1996a), who found that cooling the primary hyperalgesia site (induced either by capsaicin or mustard oil) abolished both the allodynia and the blood flow response. In our study, we also showed this cold-evoked suppression of hyperalgesic responses in WDR neurons. All WDR neurons tested showed general suppression of the spontaneous discharge and mechanical responses after cold application to the RFs. Similar findings of cold suppression in the spinal cord by these two investigating groups as well as ours in the thalamus, suggest that the

neural mechanisms of hyperalgesia or allodynia might be preserved throughout different level of CNS.

8.3.3 Discriminative ability and hyperalgesia

It appears that intensity information is transmitted to VPM thalamic nuclei and is necessary for discriminative ability. Duncan et al., (1993) showed that lidocaine injection into VPM reduced the monkey's discrimination of noxious heat and innocuous cool and mechanical intensity. Kauppila et al., (1998) also reported that capsaicin-induced inflammation could impair two-point discrimination and could reduce the ability to detect differences of roughness. In the present study, we show that the ability of human subjects to discriminate mechanical intensity is not altered by the application of capsaicin. This finding is consistent with our neurophysiological results, in that the difference and ratio values of mechanically evoked and spontaneous activity recorded in LT and WDR neurons were not significantly altered by the effects of capsaicin. These results suggest a complexity of integration among the innocuous and noxious mechanical information within the thalamus which is especially relevant to central pain syndromes, in which both the hyper- and hypoaesthesia are observed.

8.4 Other thalamic mechanisms involved in mediating hyperalgesia

In our study capsaicin-induced hyperalgesia increased the activity of VPM WDR neurons in monkey's thalamus and enhanced the mechanical sensitivity in human subjects. Simone et al., (1991) demonstrated that capsaicin significantly increased the responses of STT WDR neurons in anesthetized monkey. Therefore, in addition to VPM, other thalamic regions may receive enhanced inputs from spinal or medullary dorsal horn and may therefore be involved in mediating hyperalgesia. For example, Apkarian and Shi (1994) reported a high percentage of nociceptive neurons (50% of all recorded neurons) in monkey VPI in which 43% of nociceptive cells were NS type. These NS neurons could receive enhanced activity from peripheral noxious mechanical and thermal stimuli. Furthermore, another study demonstrated that somatosensory convergence occurs in monkey's VPI with section of spinal dorsal-cord (Pollin and Albe-Fessard, 1979). These findings suggest that VPI may play an important role in discriminative processing and perception of painful stimuli. In addition, a study using horseradish peroxidase injection (HRP) has shown that VPI projects mainly to SII area (Friedman and Murray, 1986a).

Another thalamic region that may receive enhanced neuronal activity from spinal or medullary dorsal horn is the posterior part of ventral medial nucleus (VMpo) (Craig et al., 1994; Craig, 1991). Using

Phaseolus vulgaris leucoagglutinin (PHA-L) tracer, Craig and his colleagues showed a particular termination site of lamina I STT neurons rostral to the medial geniculate, which joined anteriorly with the ventral medial nucleus in cat and monkey; they termed this area as VMpo. Recordings in anesthetized monkeys revealed that VMpo contains nociceptive- and thermoreceptive-specific neurons. These findings in monkey indicate that VMpo is a specific, topographic lamina I STT projection site that serves as a thalamic relay nucleus for nociception and thermoreception. The stereotaxic coordinates of this nucleus have also been identified in human thalamus (Bushnell et al., 1993;Craig et al., 1994). Furthermore, the cortical projection of VMpo to the rostral insular cortex has been demonstrated in a topographic fashion (Craig, 1994).

Possible neuronal alteration in thalamic circuitry between the termination of STT and DCML tracts may occur in patients with thalamic lesions. WDR neurons in lamina V of spinal (medullary) dorsal horn travel in STT and terminate in VPL (VPM) with an overlapped termination of lemniscal inputs. Some neurons in VP area would be expected to receive convergent input from DCML and STT or input from the STT WDR neurons that in turn receive convergent input from noxious and non-noxious primary afferents. Ralston's group investigated this synaptic organization and interaction between the termination sites of STT and DCML in monkey and cat. They demonstrated that thalamic interneurons exhibit gama-aminobutyric acid immunoreactivity (GABA-ir) and that

about 84% of DCML projections are involved in this GABAergic modulation. In contrast, STT terminations lack interaction with GABAergic interneurons and therefore lack thalamic GABAergic modulation before projecting to somatosensory cortex (Ralston and Ralston, 1994; Ralston, 1991). Consequently, they proposed that injury of STT may result in thalamic sprouting of DCML into non-GABAergic modulated area, which alters normal DCML GABAergic circuitry in primate thalamus, leading to the perception of pain following a non-painful input.

8.5 Cortical regions involved in pain and hyperalgesia

To understand which thalamic regions are involved in different aspects of pain, we need to consider the cortical connections of thalamic neurons. Several regions of the primate cerebral cortex have been implicated in processing of the nociceptive inputs from thalamus. These regions are thought to encode various modality, intensity and location of noxious stimuli and therefore contribute to different aspects of pain experience, such as sensory-discriminative and affective-motivational. The regions most consistently involved in processing painful stimuli include SI which receives inputs from VPL and VPM neurons (Jones, 1979; Jones and Friedman; 1982; Jones and Seavitt, 1974; Pons and Kaas, 1985); and the area of SII that receives input mainly from VPI (Friedman and Murray, 1986a). In our study, WDR neurons probably

project primarily to SI. Accumulating evidence also suggests that neurons in MDvc and Pf project to area 24 of anterior cingulate cortex (Musil and Olson, 1988; Vogt et al., 1987) while VMpo neurons project to the rostral insular cortex (Craig,1994; Craig et al., 1994). While SI and SII cortices are likely to contribute to the sensory-discriminative aspect of pain, the cingulate and insular cortices may primarily subserve the affective-emotional component of pain. Recent brain imaging studies with positron emission tomography (PET) and magnetic resonance imaging (MRI) support these findings, ratifying that various types of painful stimulation are associated with increased cerebral blood flow (CBF) or local metabolism in SI, SII, area 24 of the anterior cingulate cortex, and rostral insular cortex (Casey et al., 1994; Coghill et al., 1994; Derbyshire et al., 1994; Jones et al., 1991; Talbot et al., 1991).

8.6 Perspectives

It is well documented that two zones of hyperalgesia develop after capsaicin application. These are primary and secondary hyperalgesic areas. Mechanical hyperalgesia is found both in primary and secondary areas, whereas the heat hyperalgesia occurs only in the primary area. However, some investigators reported that heat hyperalgesia also occurs in the secondary area when induced by capsaicin (Arent-Nielsen et al., 1996; Wallace et al., 1997) or heat burn in human (Pedersen and Kehlet, 1998). They argue that the absence of heat hyperalgesia in the secondary area

observed in earlier studies might be attributed to the small size of the stimulator and insufficient stimulus intensity.

Using topical capsaicin application, secondary hyperalgesia has been reported to occur for light brushing mechanical stimulation but not for heat or tonic pressure (Kilo et al., 1994; Koltzenburg et al., 1992; Schmelz et al., 1996). Thus, in our study we limited our tests to the area where the capsaicin was applied and did not test for secondary hyperalgesia. Further studies could adapt this model for the study of secondary hyperalgesia by using a computer-controlled brush stimulus.

As mentioned above, mechanical hyperalgesia and allodynia are not only found in peripheral neuropathy; it is also a clinical finding in central pain syndrome (CPS) patients. In these CPS patients, a deficiency in thermoreception is accompanied by increasing reactions to tactile or noxious stimuli. Furthermore, clinicians have observed that skin cooling can provide pain relief (Lehmann and deLateur, 1984; Travell and Simons, 1983). Cold temperatures produce anti-nociceptive effect was reported in animals' study. Response latencies of tail flick and tail pinch increase in rats exposed to 4°C air temperature (Osgood et al., 1990), and response times increase when rats are exposed to an temperature of 10°C (Schoenfeld et al., 1985). Cold temperatures modulate pain perception was also reported in human psychophysical studies (Bini et al., 1984; Strigo et al., 1999).

Our results show that skin cooling tends to suppress the capsaicin-enhanced WDR neuronal activity. This suppression might be due to the conduction block in primary fibers produced by cold or by activating the cool ascending pathway to modulate pain in central nervous system. Physiological studies show that skin cooled below 10°C could produce conduction block in myelinated fibers, and that C fibers' conduction is not blocked until skin temperature is lowered to 3°C (Franz and Iggo, 1968; Kunesch et al., 1987). In our study, we used 5-10°C, which is unlikely to block the conduction of C fibers peripherally. Our results support the evidence that cold inhibits pain processing in the central nervous system (Bini et al., 1984; Craig and Bushnell, 1994; Craig et al., 1996). However, due to the small sample size, this central mechanism of cold suppression of capsaicin effects needs further investigation.

In neurophysiological studies, cold temperature stimuli could suppress the capsaicin-induced neuronal discharge of WDR neurons in spinal cord and thalamus. These findings imply that there is an integration of nociceptive and innocuous thermoreceptive information within the central nervous system, supporting a role for the thalamus as a center of integration. This hypothesis is also supported by anatomical, physiological and lesion data showing that these two systems are found within the same regions of the thalamus (Bushnell et al., 1993; Craig and Dostrovsky, 1991; Duncan et al., 1993;). Results from these studies have shown that some neurons within the medial thalamus are excited by noxious heat and

inhibited by innocuous cool, thus directly indicating an integration of pain and temperature information (Bushnell and Duncan, 1989; Craig and Dostrovsky, 1991; Craig, 1991). Furthermore, neurons responding to innocuous skin cooling were found in VPM (Bushnell et al., 1993; Bushnell and Craig, 1993; Poulos and Benjamin, 1968;). These studies reported that some neurons responding to skin cooling have a slowly adapting response to touch and show a phasic response to skin cooling (Bushnell et al., 1993; Poulos and Benjamin, 1968), possibly due to the input from slowly adapting mechanoreceptors that discharge in response to skin cooling (Iggo, 1985). Lesion-related deficits also suggest an integration of pain and temperature in the thalamus. In monkey lidocaine inactivtation of regions near VPM sometimes produce deficits in both noxious heat and innocuous cold discrimination (Duncan et al., 1993).

Recently, a hypothesis of central unmasking (or disinhibition) of cold-activated C-polymodal afferents was proposed by Craig and Bushnell (1994). They investigated the mechanisms of Thunberg's thermal grill illusion in which the innocuous warm and cool stimuli applied simultaneously to the skin elicited a burning sensation. They recorded from lamina I Cold-specific and HPC cells in anesthetized cat while applying a grill thermode interlaced with 20° and 40° C bars. They found that cool temperature activated both cold-specific and HPC cells, whereas warm temperature reduced activity in cold-specific channel shifting it in favor of HPC channel. This kind of shifted HPC activity pattern is

normally evoked by strong heat stimuli. These findings support the evidence that innocuous cold inhibits central pain processing and provide a possible mechanism for central pain syndrome. Therefore, an investigation of thalamic integration of pain and temperature to elucidate the characteristics and mechanisms of pain/temperature integration in the future is important not only for our understanding of afferent pain mechanisms, but also for the improvement of treatment in pain and hyperalgesia.

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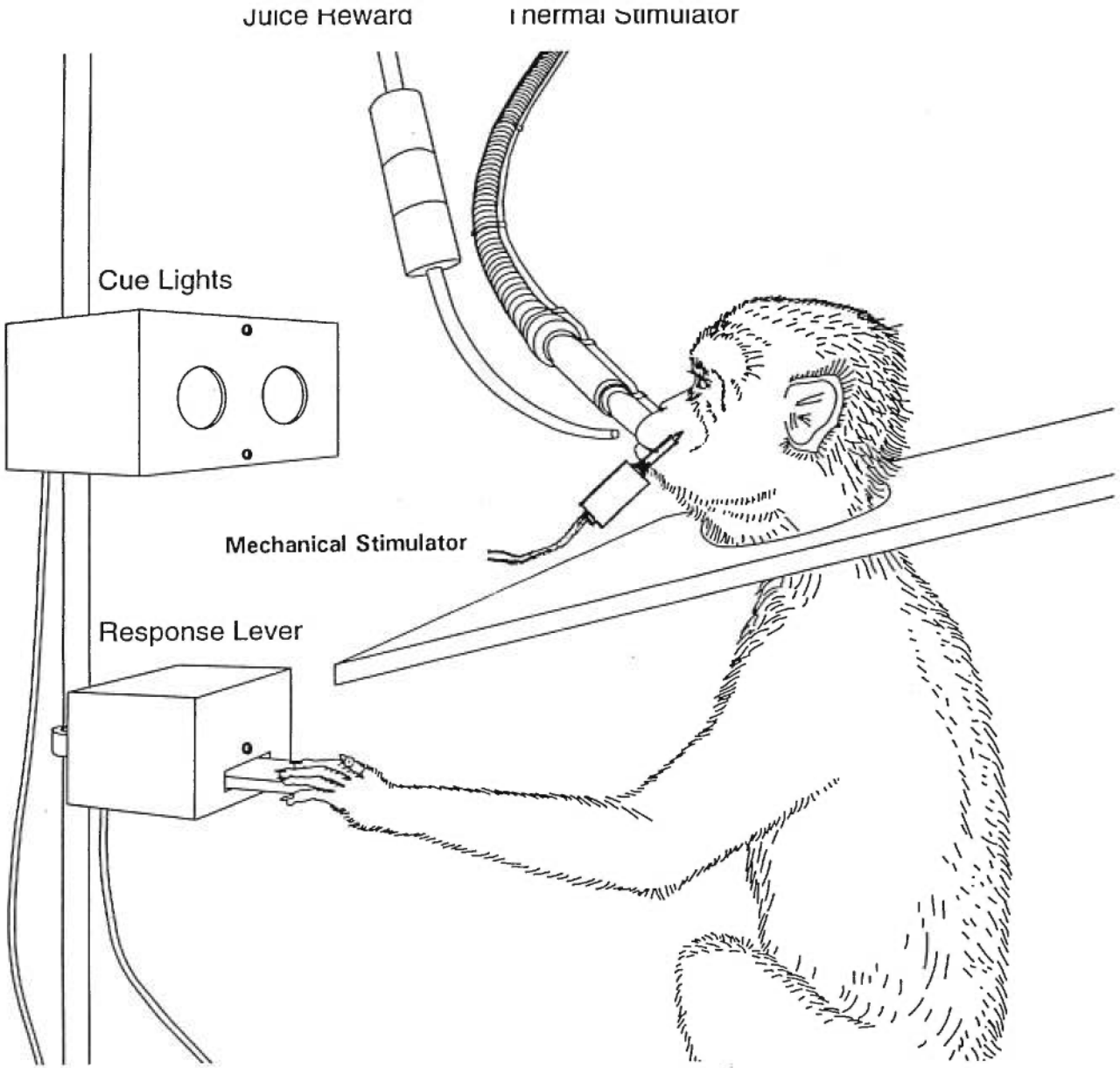
APPENDIX


Juice Reward
Thermal Stimulator
Cue Lights
Mechanical Stimulator
Response Lever

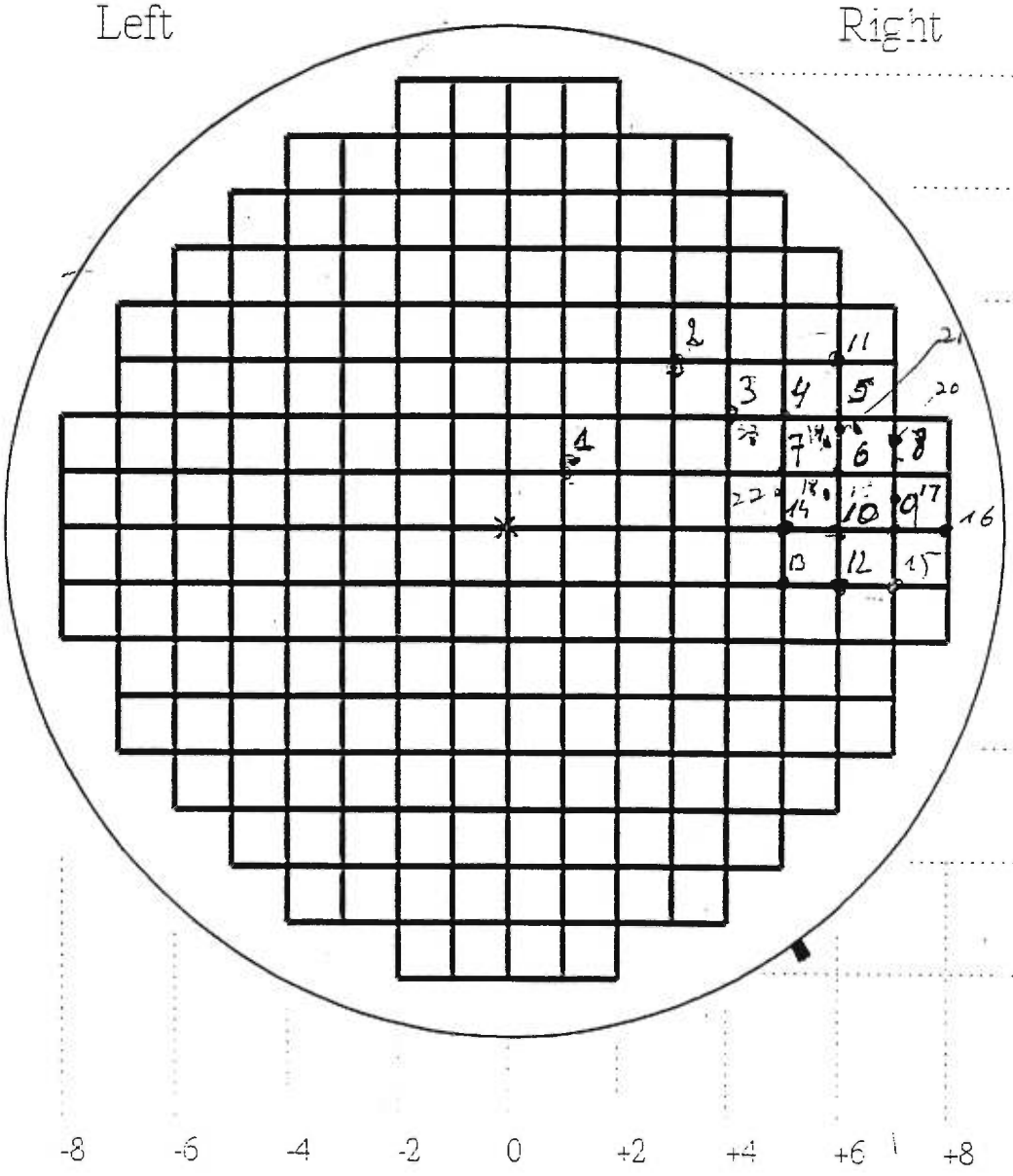
CHAMBRE D'ENREGISTREMENT

Centre de la chambre : Ant. 6.0, Lateral 6.0 (Right UPM)

ANTERIOR

Med Left

Lat Right

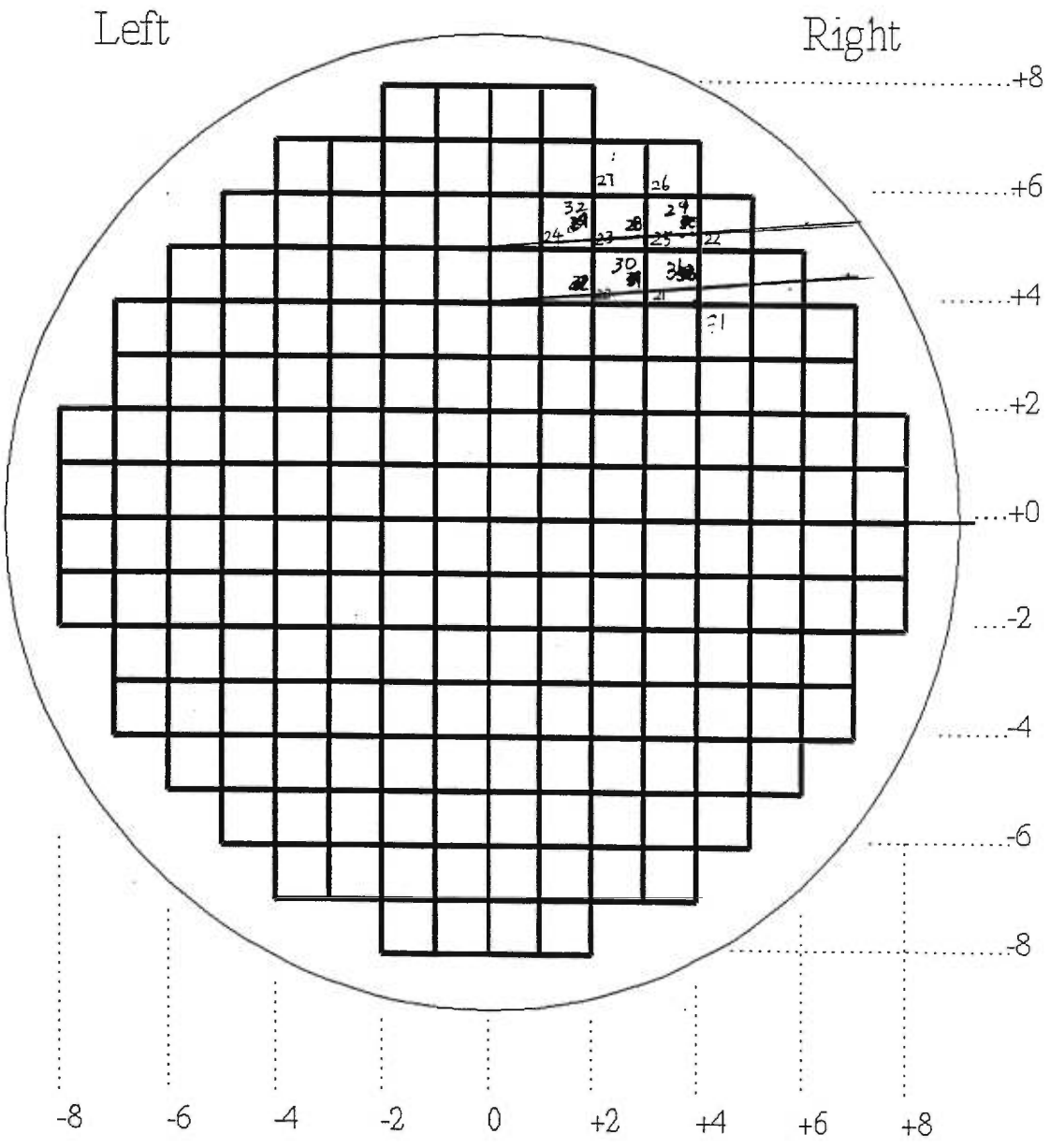


POSTERIOR

CHAMBRE D ENREGISTREMENT

Centre de la chambre : Ant. _____, Lateral _____

ANTERIOR



POSTERIOR

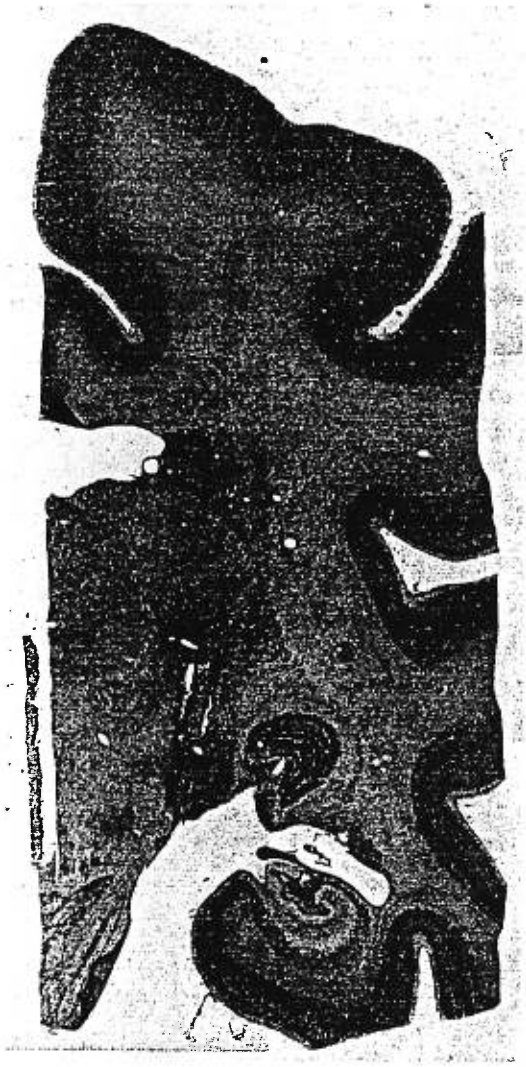


Table 1. Relationship between categories and location of recorded cells (stereotaxic, penetration depth, size of receptive fields)

CELL ID	LOCATION(A-P)	LOCATION (L-M)	TYPE	SIZE OF RECEPTIVE FIELD (MM)
1007(10250202)	A+10	L+9	RA	7x3
3309(33191701)	A+7 (+5)	L+12	SA	----
3311(33060401)	A+7	L+12	SA	----
1003(10230401)	A+11	L+8	SA	8x6
1021(10320401)	A+11(+5)	L+8	SA	6x4
33R1(33190401)	A+7(+5)	L+12	RA	----
33R3(33190701)	A+7(+5)	L+12	SA	---
1002(10230303)	A+11	L+8	RA	6x8
1004(10230501)	A+11	L+8	SA	6x6
1006(10250201)	A+11	L+9	RA	7x3
1010(10280101)	A+11(+5)	L+9	SA	5x4
1012(10280202)	A+11(+5)	L+9	SA	5x5
1013(10280301)	A+11(+5)	L+9	SA	5x4
1014(10280302)	A+11(+5)	L+9	SA	4x3
1017(10231001)	A+11	L+8	SA	10x6
1018(10280401)	A+11(+5)	L+9	SA	15x10
1020(10280501)	A+11(+5)	L+9	SA	3x3
1024(10250701)	A+11	L+9	SA	6x4
1026(10280701)	A+11(+5)	L+9	RA	5x5
3304(33190201)	A+7(+5)	L+12	WDR	----
3308(33191301)	A+7(+5)	L+12	WDR	----
1028(10251001)	A+11	L+9	WDR	7x6
3310(33060301)	A+7	L+12	WDR	---
1001(10230201)	A+11	L+8	WDR	10x8
1025(10250801)	A+11	L+9	WDR	12x8
1015(10250501)	A+11	L+9	WDR	4x6
1011(10280201)	A+11(+5)	L+9	WDR	2x3
3305(33190502)	A+7(+5)	L+12	WDR	----
3312(33061401)	A+7	L+12	WDR	----
1019(10280402)	A+11(+5)	L+9	WDR	12x5
1005(10250101)	A+11	L+9	WDR	8x8
1009(10270101)	A+12	L+8	WDR	8x10
1016(10230901)	A+11	L+8	WDR	3x2
1022(10310401)	A+10(+5)	L+10	WDR	8x6
1027(10280901)	A+9(+5)	L+9	WDR	10x10
3306(33190902)	A+7(+5)	L+12	WDR	----
1008(10230601)	A+11	L+8	WDR	2x3