RESPONSIVENESS OF SPIKING NEURONS IN LATERAL HYPOTHALAMUS TO VISUAL STIMULI AFTER DIPSOGENIC AGENT ADMINISTRATION

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It is known from previous studies that a group of neurons in the lateral hypothalamus of sheep responds to the sight of water by increasing the firing rate. The aim of this research is to study some characteristics of the neuron in sheep which responds to the sight of water.

This research studied the visual response of the water by injecting 200 ng of Angiotensin II into the lateral third ventricle in order to induce the single neuron in the lateral hypothalamus, which responds to the visual of food instead of water, to be highly motivated by water. Then, extracellular recording was performed and analyzed in terms of mean interspike interval (mean ISI) and coefficient of variation (CV).

The study found that the mean ISI can be used, instead of a change in firing rate, to indicate the transition state of sheep due to the sight of water. In addition, the comparison of the mean area under the spikes of a single neuron in the hypothalamus for each trial does not show any significant difference, and the spike distribution, determine from CV, is distributed in Poisson and Gamma distribution.

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อรรถพล เอี่ยมรัตนวงศ์ : การตอบสนองของเซลล์ประสาทในสมองบริเวณ ไฮโปธาลามัส ต่อสิ่งเร้าจากการมองเห็นหลังจากฉีดสารกระตุ้นให้หิวน้ำ (RESPONSIVENESS OF SPIKING NEURONS IN LATERAL HYPOTHALAMUS TO VISUAL STIMULI AFTER DIPSOGENIC AGENT ADMINISTRATION.) คณะกรรมการควบคุมวิทยานิพนธ์: วรากร เจริญสุข, Ph.D., อุดม ทิพยมนตรี, Ph.D. ปร.ด. 85 หน้า ISBN 974-04-1967-4

มีการศึกษาก่อนหน้านี้ พบว่าเซลล์ประสาทจำนวนหนึ่งใน ไฮโปธาลามัสส่วนข้างของแกะที่หิวน้ำจะตอบสนองต่อการเห็นน้ำโดยการเพิ่มอัตราการเกิดสัญญาณกระตุ้น (Firing rate) งานวิจัยนี้จัดทำขึ้นเพื่อศึกษาลักษณะเฉพาะบางประการของเซลล์ประสาทของแกะที่ตอบสนองต่อการหิวน้ำ

ในงานวิจัยนี้ได้ทำการฉีดสารแองจิโอเทนซิน ทู ขนาด 200 นาโนกรัม เข้าไปในโพรงน้ำในสมองแกะเพื่อเหนี่ยวนำให้เซลล์ประสาทเซลล์เดี่ยว ของแกะใน ไฮโปธาลามัสส่วนข้างที่ตอบสนองต่อการมองเห็นอาหารแต่ไม่ตอบสนองต่อการมองเห็นน้ำเปลี่ยนมาเป็นตอบสนองต่อการมองเห็นน้ำแทน ทำให้แกะอยู่ในสภาวะกระหายน้ำอย่างแรง แล้วทำการบันทึกผลและนำไปวิเคราะห์ด้วยพารามิเตอร์ต่างๆอันได้แก่ ค่าเฉลี่ยความห่างของสัญญาณกระตุ้น (Mean interspike interval) และค่าสัมประสิทธิ์ความแปรปรวน (Coefficient of variation)

ผลของการศึกษาชี้ให้เห็นว่า ค่าเฉลี่ยความห่างของสัญญาณกระตุ้นสามารถนำมาใช้แทนค่าของการเปลี่ยนแปลงของอัตราการเกิดสัญญาณกระตุ้น เพื่อใช้ในการบ่งชี้ถึงการเปลี่ยนแปลงสภาวะของแกะอันเนื่องมาจากการมองเห็นน้ำได้ อีกทั้งเมื่อเปรียบเทียบ ค่าเฉลี่ยพื้นที่ใต้กราฟของแต่ละการเกิดสัญญาณกระตุ้นของเซลล์ประสาทเซลล์เดี่ยว จะมีค่าค่อนข้างคงที่หรือเปลี่ยนแปลงอย่างไม่มีนัยสำคัญ ($P>0.05$) นอกจากนี้เมื่อพิจารณาถึงค่าสัมประสิทธิ์ความแปรปรวนยังพบว่าการกระจายตัวของสัญญาณในไฮโปธาลามัสส่วนข้างเป็นการกระจายตัวแบบปัวซองและแกมมา

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LIST OF ABBREAVIATIONS

%	Percent
μg	Microgram
ng	nanogram
ANG	Angiotensin
ANG-II	Angiotensin-II
Bd.wt.	Body weight
CV	Coefficient of variation
CVOs	Circumventricular organs
DLH	Dorsolateral hypothalamus
HPF	High-pass filter
Hz	Hertz
Icv	Intracerebroventricular
ISI	Interspike interval
LH	Lateral hypothalamus
LPF	Low-pass filter
MB	Megabyte
Msec	millisecond
mV	millivolt
n	Number of neurones recorded
N	Number of animals used
OLVT	Organum vasculosum of the lamina terminalis
S	second

LIST OF ABBREVIATIONS (CONT.)

Sc	Subcutaneous
SCSI	Small Computer System Interface
SFO	Subfornical organ
SHE	Spike Histogram Extension
TTL	Transistor-Transistor-Logic
VCR	Video Camera Recorder
ZI	Zona incerta

CHAPTER I

INTRODUCTION

General Background

It was found that hypothalamic regions play vital role in the regulation of feeding and drinking(Grossman 1975). The bilateral lesions of lateral hypothalamus (LH) area produced aphagia and adipsia (Anand and Brobeck 1951; Grossman 1975; Jain et.al. 1999). Many experimental evidences showed that neurons in LH and zona incerta (ZI) are involved in ingestive behavior. Neurons in the hypothalamus in monkey, only in the LH and substantia innominata (SI) have been responsive to the sight of food (Rolls, Burton and Mora 1976). Kendrick and Baldwin (Kendrick and Baldwin 1986) demonstrate that in conscious sheep, small population of neurons in the LH and ZI response to the sight of food. The primate study concluded that LH and ZI also involved in the water intake (Grossman, 1975; Huang and Morgenson, 1974; Evered and Morgenson, 1977).

Recently pieces of evidence suggests that lateral hypothalamus (LH) plays a vital role to the water appetite. It has been shown that sheep's neurons in the LH and zona incerta (ZI) change their firing rate when intracerebroventricular (icv) administration of ANG II 200 ng to that area. Experiments showed that, sheep's neurons which response to sight of food become strongly responsive to the visual presentation of water. Thus, the neuron response, firing rate, could be altered by

changing the animal's dominant motivation state from hungry to thirst (Mungarndee SS et. al. 2002).

Changing of mean ISI is used as a tool to demonstrate the changing of experiment with stimuli. I am interested in analyzing of the change of mean ISI. However biological (neuronal) signal is a nondeterministic signal. The statistics analysis must be used to determine the characteristic of signals.

This chapter, some of the features of food and water intake, angiotensin II and neuronal signals, focusing on the LH and ZI which response to the ANG II and some signal coding were briefly reviewed.

Literature review

UNIT 1 Initiation of Drinking and Thirst

Thirst is regulated by two main factors, which are tissue osmolality (cellular dehydration) and vascular (fluid) volume (Fig 1.1). The cellular dehydration is come from the loss of body water. There is an increase in the osmotic pressure of the extracellular fluids by increasing in exteracellular sodium level. Due to an increase in osmotic pressure of the extracellular fluid, water is drawn from the cells and the blood volume does not decrease.

Osmotic receptors are located in hypothalamus, in the medial preoptic area, the median preoptic nucleus, the organum vasculosum laminae terminalis, the subfornical organ and the supraoptic nucleus. Magnocellular neurosecretory cells may also serve as osmoreceptors (Rolls and Rolls, 1982).

When the osmotic receptors are activated, the suprachiasmatic, supraoptic and paraventricular nuclei of hypothalamus produce vasopressin, which is transported to the posterior pituitary and released from it. The connection of medial preoptic area with mesencephalon (mesencephalic locomotor area) is probably necessary to induce behavioral response to thirst (Fig 1.2).

The volumetric thirst is also called hypovolemic or extracellular thirst. The volumetric thirst is caused by a decrease of the volume of circulating blood or extracellular fluid. It is usually caused by the loss of blood by bleeding, and in the

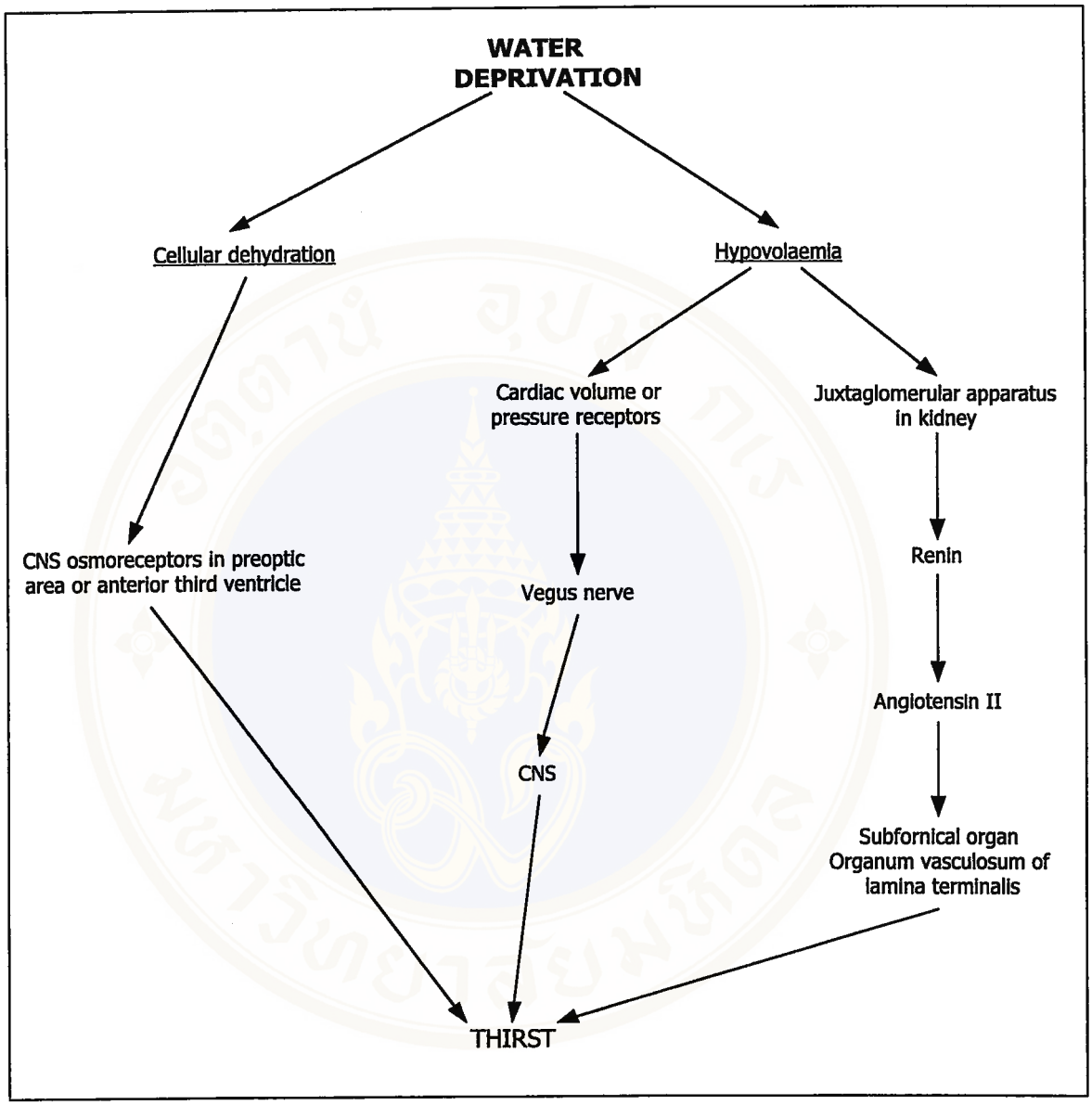

FIGURE 1.1 Summary of the factors that can lead to drinking after water deprivation (Rolls and Rolls, 1982).

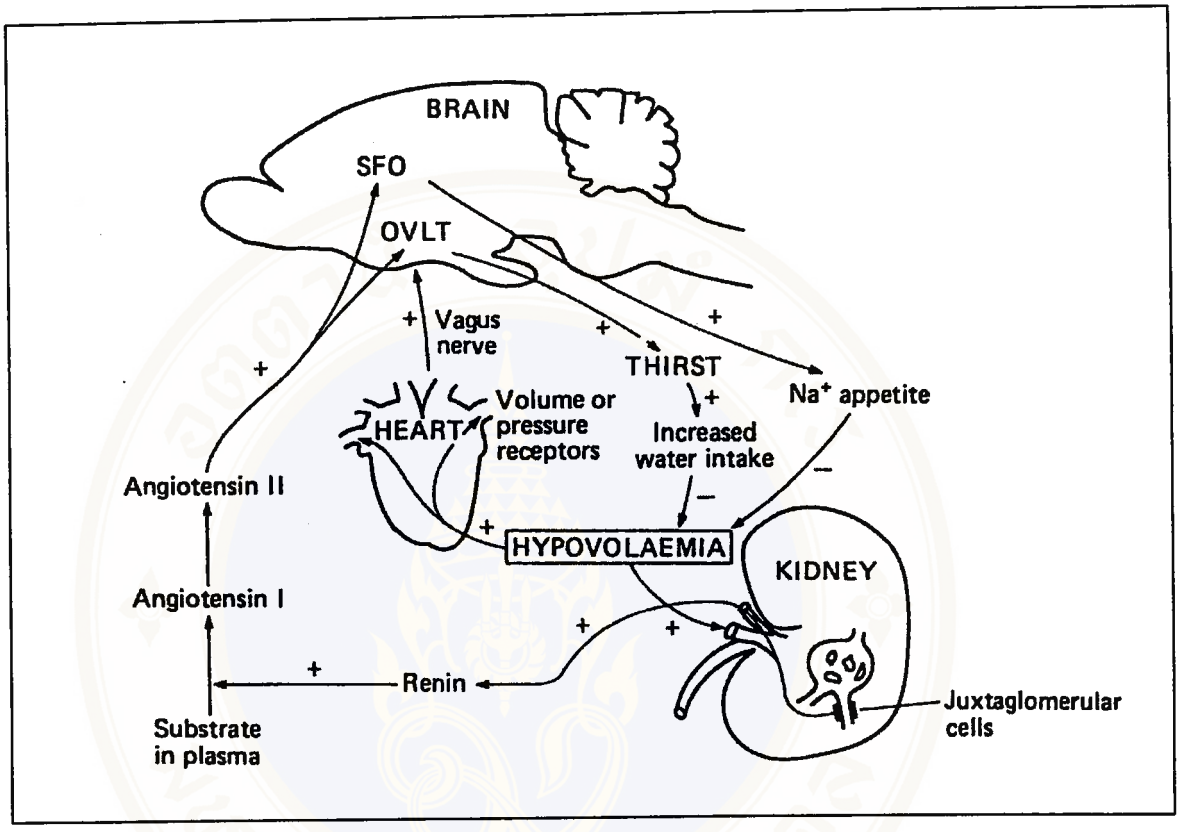

FIGURE 1.2 A summary of the mechanisms involved in extracellular thirst the Subfornical Organ (SFO) and lamina terminalis (OVLT) (Rolls and Rolls, 1982).

experiment. Two mechanisms. firstly, baroceptors in atrium of heart monitor blood pressure, and elicit drinking by direct neural pathway. Second, baroceptors in juxtaglomerular cells of the kidney fall in blood pressure releases enzyme renin, which in turn releases ANG II, a potent dipsogenic peptide. ANG II is thought to act on specific receptors in the Subfornical Organ (SFO) and lamina terminalis (OVLT) (Fig 1.2).

UNIT 2 Angiotensin and Angiotensin II

Angiotensin

The original works postulated that, the effect of Angiotensin (ANG) is very specific. Drinking is the only behavioral response, administration. The experiments show that, after intracranial ANG, a sleeping rat work and went immediately to water. Also a rat which had been deprived of food, but not of water, stopped feeding to drink (Epstin, et al 1970; McFarland and Roll, 1972; McFarland and Roll, 1973)

Ventricle is the important organ for the dipsogenic response (johnson and Epstein, 1975) which show that intracranail ANG was more effective in stimulating drinking if the cannula for its injection pass through a ventricle. Several circumventricular organs have since been suggested as receptive sites for angiotensin. Lesion of the subfornical organ (SFO) completely blocked drinking induced by intravenous ANG. Fitzsimon and Kucharczyk concluded that there are angiotensin-sensitive tissue in both the SFO and preoptic area (Fitzsimon and Kucharczyk, 1978).

Angiotensin II

Angiotensin II (ANG II) is a central effector peptide of the renin-angiotensin system (RAS), responsible for vascular homeostasis. A marked decline in blood pressure and/or volume activates a cascade of events, starting with the release of renin from the kidney into the bloodstream. Renin cleaves circulating Angiotensinogen, producing ANG I, which is then converted by Angiotensin converting enzyme (ACE) to ANG II (Fig 1.3).

Angiotensin II have been reviewed by Fitzsimons, and pointed out that ANG II is a powerful and phylogenetically widespread stimulus to thirst and sodium appetite. When it is injected directly into sensitive areas of the brain, it causes an immediate increase of water intake followed by a slower increase of NaCl intake. An injection of ANG II into sensitive limbic structures causes an animal to stop whatever it was doing and to start drinking water almost immediately. (Fitzsimons, 1998).

ANG II itself also causes contraction and hypertrophy of vascular smooth muscle, activation of sympathetic nerves and release of adrenomedullary hormones, secretion of aldosterone, release of pituitary hormones, and sodium and water conservation through its effects on renal hemodynamics and tubular reabsorption. It is also an exceptionally powerful stimulus of drinking behavior, causing increases in both thirst and sodium appetite (Fitzsimons, 1998).

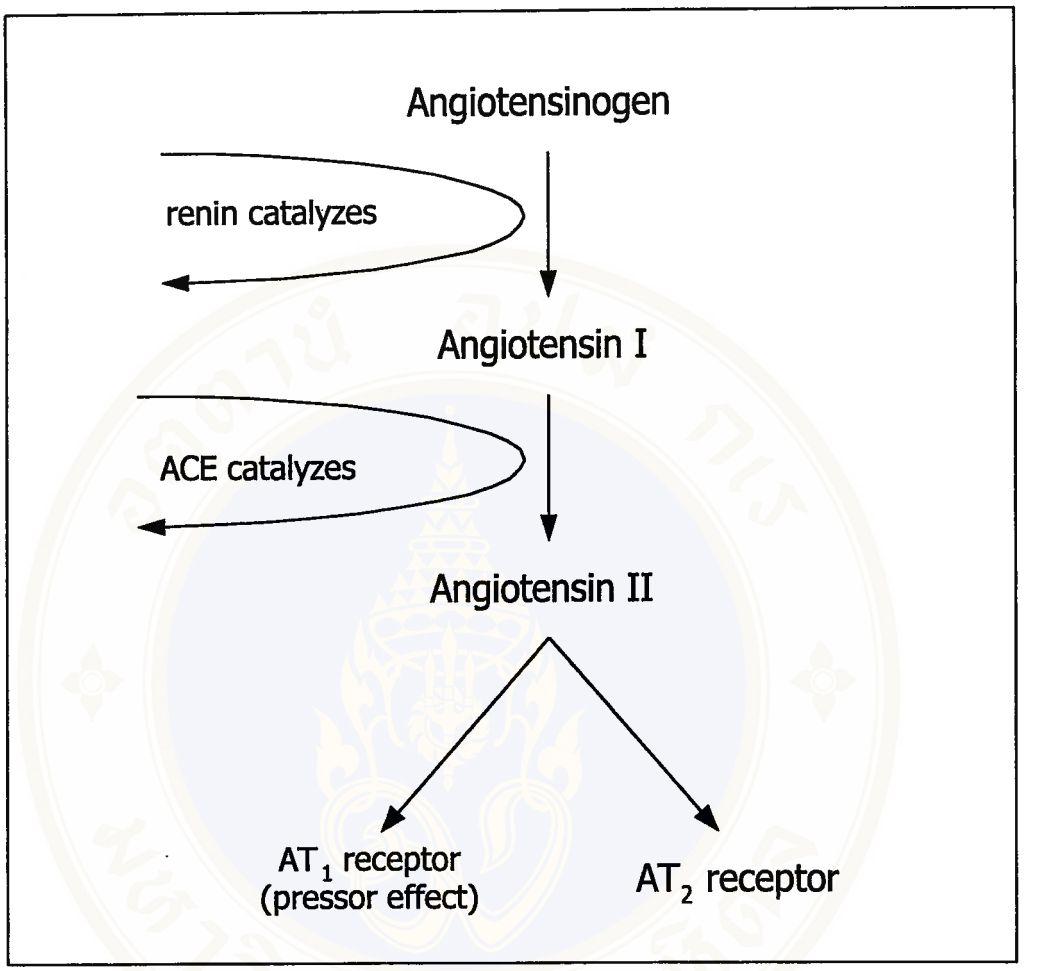

FIGURE 1.3 Summary of Angiotensin II production.

There are numerous reports that the ANG II stimulates drinking in wide variety species such as rat, dog, pigeon, monkey, cow and sheep. Severs and Summy-long (Servers and Summy-Long, 1526) discovered that action of ANG II stimulate in drinking. Administration of the peptide either systemically or directly into the central nervous system elicits drinking in a wide animal species. Experiments show that water intake is increased of a number of situations in which renin secretion and hence circulation ANG II level are elevated; in some experiment the administration of agent which block the formation or action of ANG II can reduced the water intake (server and summy-long, 1975). The lateral hypothalamus (LH) also is an area of closely involve in the regulation of water and sodium. (Ferrai et al., 1990; Ferrai et al., 1991; PereiradaSilva et al., 1995)

Recently researchers suggest that, in hungry but not thirsty sheep, the effect of intracerebroventricular (icv) administration of ANG II 200 ng to the lateral hypothalamus (LH) and zona incerta (ZI) revealed that they become strongly responsive to the visual presentation of water. Thus, the neuron responses could be altered by changing the animal's dominant motivation state from hungry to thirst. The neurons returned to their previous pattern of response when the effects of the thirst-inducing stimuli had dissipated (Mungarndee et. al., 2002).

Action of Angiotensin II in the brain

Angiotensinergic nerve fiber systems are widely distributed in the limbic system and brain stem. Angiotensinergic terminals are particularly densed in the anterior hypothalamus and tissue surrounding the anteroventral third ventricle (AV3V)

region including the organum vasculosum of the lamina terminalis (OVLT) and median preoptic(MnPO) nucleus, in the subfornical organ (SFO), supraoptic nucleus (SON), and paraventricular nucleus (PVN), the central nucleus of the amygdala and brain stem nuclei (Fitzsimons, 1998) (Fig. 1.4).

Several lines of evidence demonstrate that fibers from the SFO converge on nucleus medianus and also project to the supraoptic nucleus, paraventricular nucleus and throughout the LH (Thrasher, 1989). The central part of the SFO, which binds circulating ANG II (Van Houtten, et al 1980); also contain an ANG II – immunoreactive terminal field that appears to arise from cells in LH (Lind et al., 1990).

One recent development has been introduced into the study of receptor subtype selective antagonists in the functional analysis of thirst and sodium appetite. At least two receptor subtypes for Angiotensin have been identified in mammal, subtype 1 or AT1 and subtype 2 or AT2 based on their different affinities for structurally dissimilar angiotensin antagonists. The density of AT1 and AT2 receptors is roughly equal in the brain stem, midbrain and hypothalamus, the AT1 receptors being slightly dominant in the brain stem and hypothalamus, whereas AT2 receptors are mainly expressed in the midbrain (Bottari et al., 1993). The distribution of AT1 receptors in the AV3V region suggests that ANG II induced drinking behavior, like most of the other known functions of ANG II, is mediated through AT1 receptors. Direct support for this view is provided by a number of blocking

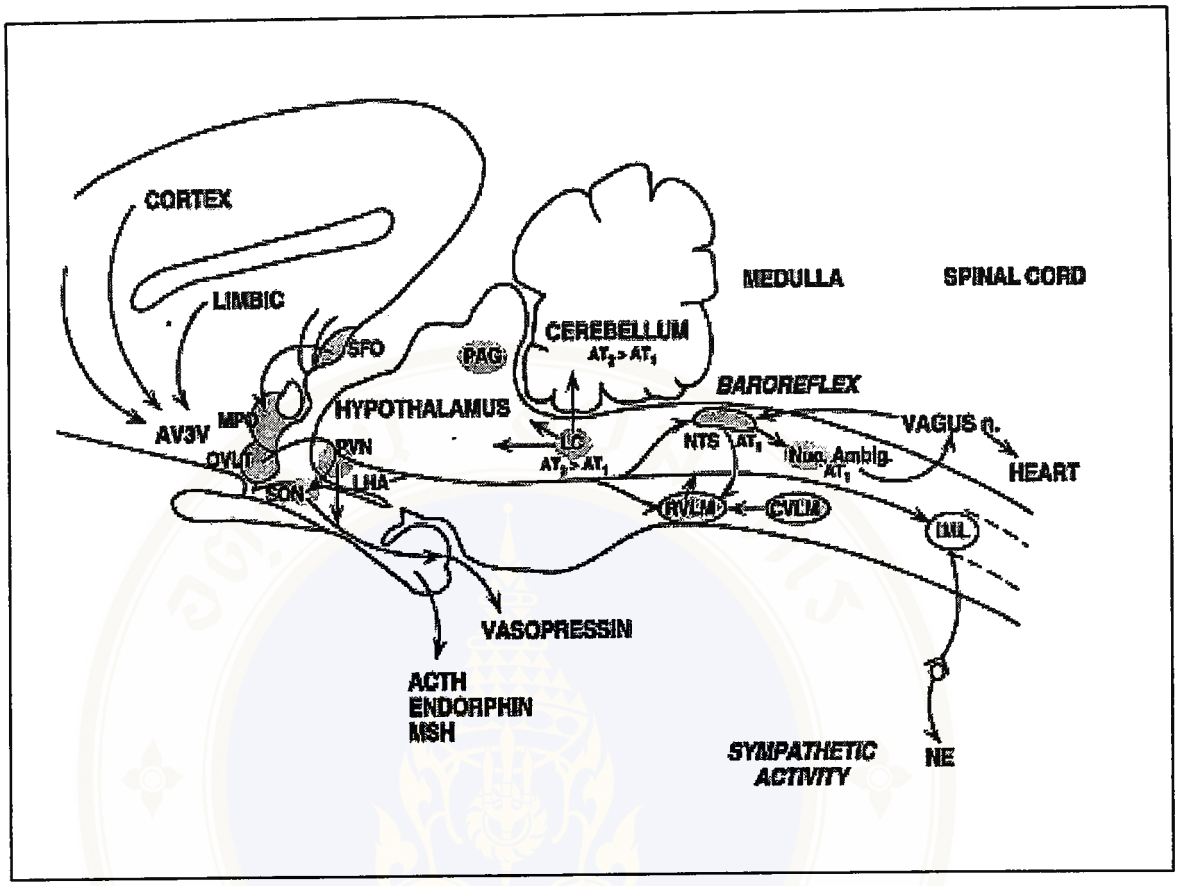

FIGURE 1.4 The distribution of Angiotensin receptor subtypes of central nervous system. AV3V, anteroventral third ventricle; MPO, medial preoptic neuclease; OVLT, organum vasculosum of the lamina terminalis; MnPO, median preoptic nucleus; SFO, subfornical organ; SON, supraoptic nucleus; PVN, and paraventricular nucleus; LHA, lateral hypothalamic area; PAG periaqueductal gray; LC, locus coeruleus; RVLM, rostral ventral lateral medulla; NTS, necleus tractus solitarius; CVLM, caudal ventral lateral lateral medulla; IML, interomedial lateral column; NE, norepinephine.

experiments with the AT1 antagonist losartan, which also has the particular advantage of lacking the partial agonism shown by saralasin (Wong et al., 1990).

Mungarndee (Mungarndee et al., 2002) mentioned that ANG II elicits drinking by acting upon specific ANG II type I receptors which are located in high density in the SFO, OLVT and MnPo nucleus and medial POA which were located near the third ventricle in the lateral hypothalamus. It has been shown that injection of ANG II into the SFO altered the firing rate of neurons in the ZI and LH (Mok and Mogenson, 1987). Recently experiment show that icv injection of ANG II results in the expression of c-fos protein in several brain regions including the medial POA and SFO (Xu and Herbert, 1995). Thus, icv injection of ANG II stimulate c-fos production in regions capable of influencing unit neuronal activity in the ZI and LH.

Unit 3 Extracellular Signal Neuronal Recordings in the Animal.

Noise

There are two ways to record activity of neuronal signal; extracellular recording and intracellular recording. Extracellular recording, records the action potential using a suitable microelectrode inserted in the extracellular compartment, is a simple way of evaluating such activity without significant disruption of system. This method has been developed to study in anaesthetized preparation or in conscious animals. The potential recorded from an electrode is influenced by the electrical signal current setup by the neuronal element (axons, dendrites, and cell body) around it, which is noise in the recording. Recording to isolated the activity of just one neuron is

done by bringing the electrode tip close enough to a neuronal element that the signal to noise ratio from that one is larger than that from other units that can be recorded in the vicinity.

Electrode

The electrodes are either glass micropipettes filled with an electrolyte with a tip diameter 1 micron or metal electrode (usually, tungsten, steel or platinum-iridium) insulate by glass or lacquer with exposed tip of 5 to 10 micron. Tungsten in glass electrode is more commonly used nowadays. The tip size of electrode relates to their resistance, instead capacitance relates to metal/fluid interface and thinness of the glass or lacquer near the tip. The range of impedance of most successful single unit electrodes is 5 to 20 Mohm (measured by an AC current at 50Hz) and 3 to 4 Mohm tend to recorded the multi unit electrode.(Vidyasagar, 1997)

Recording System

Vidyasagar (Vidyasagar, 1997) pointed out that most extracellular studies focused on the frequency of action potential occurred and the shape of the action potential is not necessary. Therefore, the recordings are usually done with an AC amplifier, with which most irrelevant signal such as DC shifts and high frequency noise can be filtered out. These amplifiers usually have filters to cut off frequencies below and above a particular range (band-pass filter), and allow an amplification of at least 10000 times the actual signal. The most convenient range for the filter that optimizes spike recording with out allowing too much noise is 300-6000 Hz. However,

if the study requires attention to spike shape, it is better to widen the filter at the low frequency end or use DC recording as for intracellular recordings. The signal is normally visualized on an oscilloscope. With good ground systems and an appropriate amplifier, the noise level observed in AC recording should be below 100 microvolt. The output of the amplifier as well as the output of window discriminator are also fed into a speaker, so that the experimenter can 'listen' to the electrical activity pickup by the microelectrode.

Unit 4 Neuronal Signal

Neurons are highly specialized for generating electrical signals in response to chemical and other inputs, and transmitting them to other cells. An action potential is a roughly 100 mV fluctuation in the electrical potential across the cell membrane that lasts for about 1 ms. Action potential generation also depends on the recent history of cell firing. For a few milliseconds just after an action potential has been fired, it may be virtually impossible to initiate another spike. This is called the absolute refractory period. For a longer interval known as the relative refractory period, lasting up to tens of milliseconds after a spike, it is more difficult to evoke an action potential. Action potentials are of great importance because they are the only form of membrane potential fluctuation that can propagate over large distances. Subthreshold potential fluctuations are severely attenuated over distances of 1 mm or less. Action potentials, on the other hand, are regenerated actively along axon processes and can travel rapidly over large distances without attenuation. The powerful tool in investigating neuronal function in the intact animal, especially involving behaving animals, is extracellular recording. An electrode is placed near a neuron but it does not penetrate the cell

membrane. Such recordings can reveal the action potentials fired by a neuron, but not its subthreshold membrane potentials, shown in Figure 1.5.

Generally, fiber spikes, such as spike from optic track, and soma spike, like those from the principal cells of the thalamus, differ markedly in their shapes and how these change as the electrode approaches them (Hubel, 1960; Bishop et al.,1962). Fiber spikes generally appear as positive potential, primary because current flowing either in front of, or behind, an action potential is outward current (source). On the other hand, soma electrode is relative to current sinks and sources. When an electrode approaches the soma of neuron which is undergoing synaptic activation the initial wave will be small and positive as current flows into the dendrites. This will be followed by a negative wave due to inward current in the soma as it is invaded by the depolarization of an action potential(Fig 1.6a). A rapid increase in the amplitude of the positive potential occurs as the electrode gets close to the cell surface. Sometimes there are inflections on the ascending potential, indicating that the somatic membrane is acting as a current source for depolarization in dendrites and in the initial segment (Fig.1.6b).

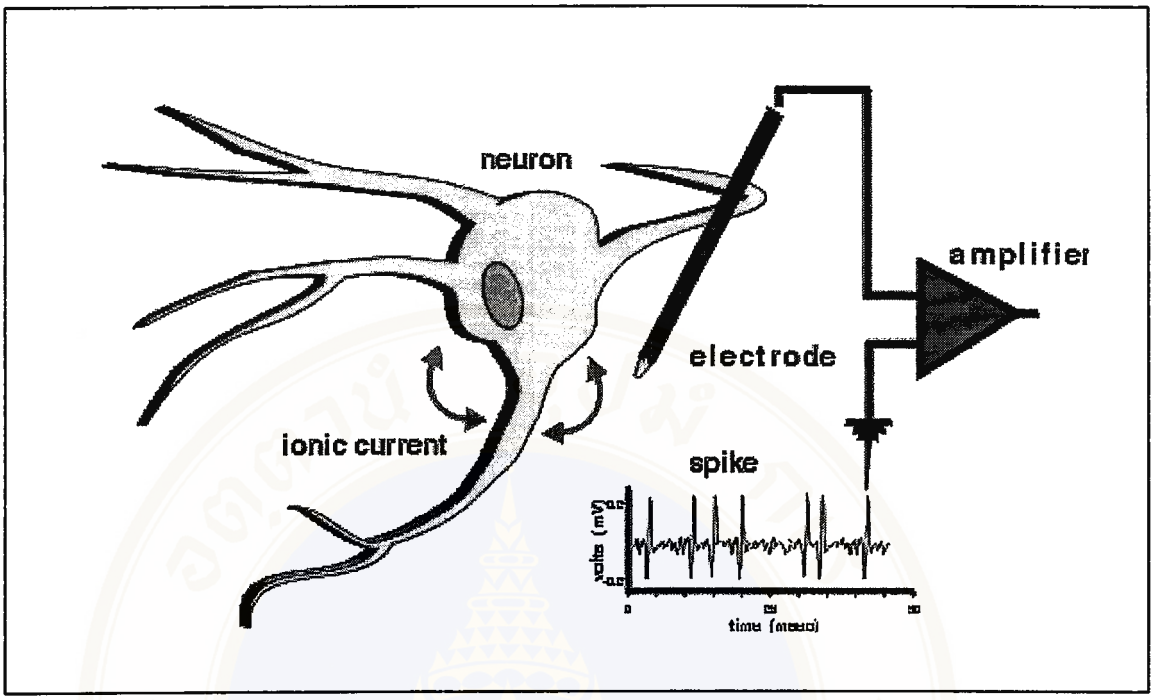

FIGURE 1.5 A diagrammatic summary of the extracellular recording

When the electrode touches the cell body membrane and causes damage locally, there might be deterioration of the recording with the negative wave often dropping out entirely (Fig 1.6c). Another different between fiber and soma recording is the overall duration of spike, which is usually much less than 1 ms for fiber but could be up to 2 ms or more for cell bodies (Vidyasagar, 1997).

When electrode is inserted into the brain tissue, neuron activity is encountered under one of the four conditions;

1) Some neurons are spontaneously active.
2) The electrode tip may injure a neuron and provoke it into firing.
3) The neuron may fire in response to natural stimulation. Such as visual or acoustic stimulus.
4) The neuron may fire due to an artificial stimulus such as electrical stimulus applied.

The brain processes sensory and motor information in multiple stages. At each stage, neural representations of stimulus features or motor commands are manipulated. Neurons represent and transmit information by firing sequences of spikes in various temporal patterns. The study of neural coding, involves measuring and characterizing how stimulus attributes, such as light or sound intensity, or motor actions.

Although researchers tried to find the relation ships between stimulus and response, it is difficult because neuronal signal responses are complex and variable. Neural responses vary from trial to trial even when the stimulus is presented repeatedly. Many theories have been proposed to explain how spike trains encode the

signal. As far back as 1928, Adrian (Adrian, 1928) proposed that information about the sensory environment is conveyed in the time-varying firing rate of spiking sensory (Burns, 1968).

Single signal coding have been reviewed by Christopher de Charms and Anthony Zador (Christopher de Charms and Anthony Zador 2000) and pointed out that, there are two different views of the neuronal coding signal. First, the rate-coding hypothesis posits that the only important characteristic of a spike train is its mean rate. This offers a dramatic simplification because it implies that an entire spike train—a complex time-varying signal comprising a long list of times at which a neuron fired—can be replaced by a single number, the mean rate. The rate coding hypothesis has provided the foundation for our current understanding of the cortical code, but this does not mean that its assumption of simplicity is fully justified. Second, The temporal-coding hypothesis posits that the temporal structure of a spike train carries additional information beyond that signaled by the mean firing rate. There is little debate that the temporal structure of spike trains can carry information about the temporal structure in stimuli, such as modulations in stimulus intensity (Bair and Koch 1996, Buracas et al 1998, Mechler et al 1998).

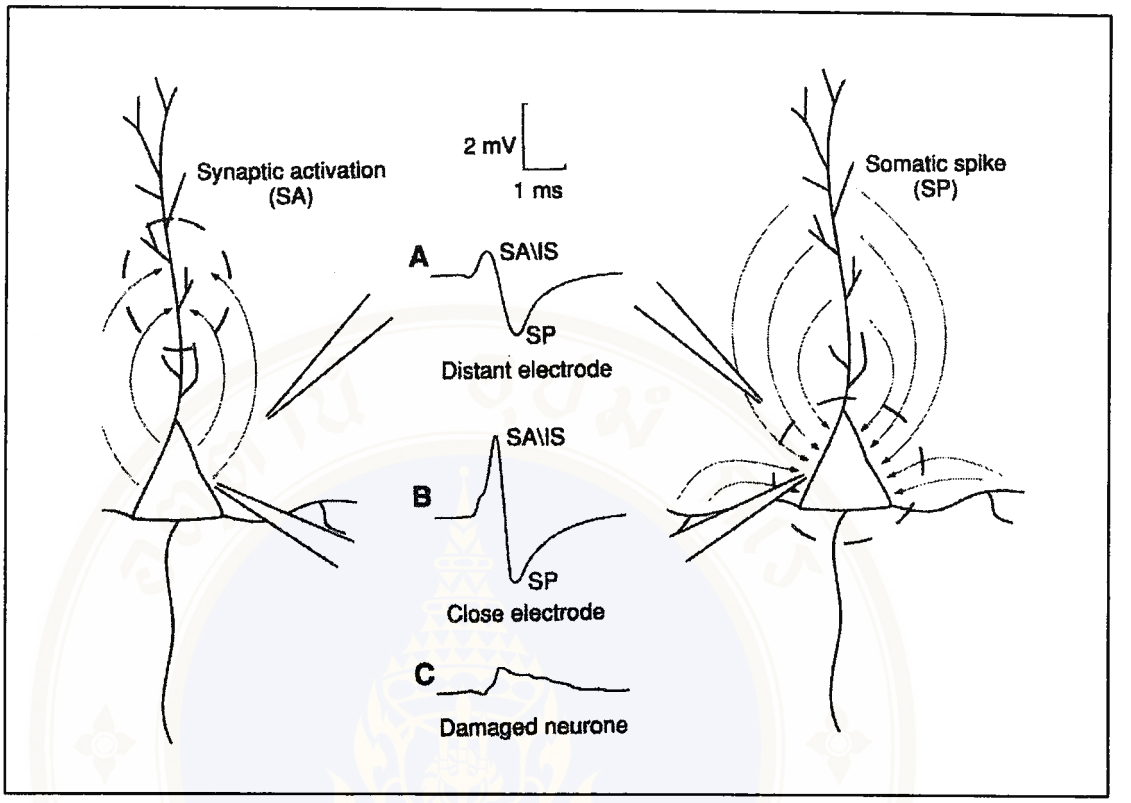

FIGURE 1.6 A simplified schematic diagram of sources and spike sinks that are response for the potential recorded by an extracellular electrode as it approaches the soma of a pyramidal cell. The neuron is shown in two states: depolarization occurs in the apical dendrite (left), soma itself is undergoing depolarization (right). (A) The electrode measure an action potential. (B) electrode being much closer to the cell than in A. (C) waveform when electrode damaged the cell body (Vidyasagar, 1997).

CHAPTER II

PROPOSED STUDIES AND EXPERIMENTAL OBJECTIVES

I analyze the neuronal signal that recorded in the hypothalamic neuron in lateral hypothalamus and zona incerta of sheep which response to sight of water after icv injected of Angiotensin II 200ng . Extracellular single unit was used to record these signals. The details of manipulation of this animals and procedures are presented in ChapterIII. The main spike parameters obtained in this neuronal signal are firing rate, the changing of mean interspike interval (ISI), area under spike, and variation of interspike interval of individual neurons in the lateral hypothalamus (LH).

Study objective

The thesis is concerned with the extracellular single unit recording of action potential in conscious sheep in relation to changes in firing rate and mean interspike interval or patterns associated with defined biological stimuli. From the study of spike distribution in brain cortex, it was found to be Poisson distribution (Softky and Koch, 1993). It is interesting to see how the spike distribution in the lateral hypothalamus, which is done in single neuron, resemble to that in the cortex. In addition, the study is done with the change in the area under spikes in order to see the relationship between the signal coding and the area under spikes.

Objective 1

To compare mean interspike interval (ISI) of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the mean ISI in each period related?

Objective 2

To examine the average area under spike of the neuronal signal of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the power in each period related?

Objective 3

To measure the variability associated with interspike interval distribution by using coefficient of variation of the neuronal signal in each period.

CHAPTER III

MATERIALS AND METHODS

This thesis focuses on analyzing neuronal signal waveform and parameters of unit neuron activity in the conscious sheep in lateral hypothalamus (LH) and zona incerta (ZI) neuron to the sight of water after intracerebroventricular (icv) injection of Angiotensin II. Surgical, raw data recording and experimental preparation in this thesis was recorded by other skillful researchers. The methods used have been adapted from previous experiments using sheep. (Mungarndee et al, 2002; Ngampramuan , 2002)

This chapter clearly describes the surgical procedures used to prepare non-horned sheep with cranial implants for unit neuronal recording. The methods used to provide intracerebroventricular (icv) cannula catheters are described. See detail in Mungarndee et al, 2002 and Ngampramuan, 2002

SECTION I Experimental animals

Experiments were performed on 4 adult ewes, 1-2 years of ages and weight 35-40 kg. They were obtained by purchasing from the Department of Animal Husbandry, Faculty of Veterinary Medicine, Chulalongkorn University, Nakorn Pathom. All newly obtained animals were quarantined in individual cage and fed twice a day. They were surgically prepared for recording by a modified technique of implant a stainless steel cylinder over the recording area.

SECTION II Surgical preparation

Surgical Procedure

Surgeries were performed following the induction of anesthesia by means of an iv 10-mg/kg bd.wt. injection of an ultra-short acting barbiturate (20% w/v preparation of Thiopentone sodium). Then, general anesthesia was maintained by semi-close circuit of halothane (Ohmeda© Model Mark III, UK) induced at 3-5%halothane and maintained under 0.5-2.5% with 95% O_2 / 5%CO_2 during surgical preparation and with full sterile precaution observed throughout surgery. The depth of anesthesia was monitored by frequently testing for the presence of a leg flexion reflex, and if this was present, supplemental anaesthetic was administered. Respiratory condition was clinically monitored (Apalert©, Australia) throughout surgery. The sheep was transferred onto the other surgical table and fixed its head in a stereotaxic frame instrument, which was attached firmly into the top-head of surgical table.

At the surgical stage III plane II, the skin at the frontal bone was incised with the length of approximately 3-4 inches long anterior-posteriorly and was blunt dissected to both sides. The superficial and deep fascia at the surface of the skull were totally removed in order to open a clear skull surface. Location of ZI was achieved by stereotaxic co-ordination referenced at the bregma. (Richard, 1967) and supplemental with the use of X-rays guidance.

Stainless Steel –Well Implant

A 2.0-cm diameter hole was trephined in the skull over the brain region of interest, leaving the dura intact. To aid localization, lateral X-ray were taken and bony landmarks such as the optic recess silhouette used for reference. The most anterior part of the hole will be aligned with the optic chiasma. A stainless steel ring 2.0 cm inner-diameter, 2.0 cm deep was attached to the skull with stainless steel screws and dental acrylic (Dentsply Limited, De Trey Division, Weybridge, Surrey, UK). The dura mater was not be pierced. Then, the well was filled with commercial anti-mastitis suspension (Leo Yellow®, Leo Pharmaceutical Product, Ballerup, Denmark), which it contains in each in each 5 ml: Predinsolone 5 mg to prevent infection and to inhibit granulaton of dura mater. Then, the well was closed and protected from contamination with a removable nylon cap.

Construction of ICV Cannula and its Catheter Indwelling

Permanent indwelling cannula (18-G) acting as guide as guide tubes for access to the lateral cerebral ventricle (M/P* Procedure Needle®, Becton Dickinson, NJ, USA) was used. In brief, by using a stereotaxic apparatus, the cannular implant point was positioned 15-mm posterior to the bregma and 10-mm lateral to the midline. The implanted needle tip was ~ 10 mm ventral to the dural surface of the brain. Dental resin was used to cement the needle firmly to screws, which were affixed to the skull. A radio-opaque agent was then injected into the ventricle (1 ml of Iopromide, Ultravist® 370; Schering, Berlin, FRG) and dorsoventral and lateral X-rays taken starting 30 sec after the end of the injection. A stainless steel well was placed over the

hole in the skull and fixed in position using stainless steel self-tapping screws and dental acrylic. For all animals, the cannular position was visually confirmed by gravity run-in of saline and outflow by a siphon. Cannula can be maintained in most cases for more than 1 yr.

Stainless Steel Cylinder Implantation

To protect the well, a 2.54-cm-high stainless ring will then cemented with dental acrylic in position surrounding the well. The skin was sutured with non-absorbable suture material (Mersilk® No. 2, USP, Ethicon, NJ, USA) so that it healed and seals against the protective ring. A topical aerosol antibiotic (Duphacycline® Spray, Sdvay Duphar B.V.,Weesp, Holland) was applied to the skin around the implant and the animals were given a 4-5-ml subcutaneous injection of long-acting antibiotic penicillin-streptomycin based. Animals were allowed to recover for 2-4 weeks after surgery before the single-unit recording commenced.

SECTION III Neurophysiological Technique

Preparation for electrode

The electrode modification described by Merrill and Ainsworth in 1972, in which tungsten microelectrodes are constructed with glass insular insulation, but without platinum plating was used. Briefly, this technique involves initially etching one end of a 15-cm long of cleaned, straightened tungsten wire (0.13mm diameter, Phillips Element Co.,Lewiston. ME) in Levick's solution (KOH, NaNO2)

(Levick,1972) and inserting it into a 10-cm long glass capillary tube (0.3-mm o.d. x 0.22-mm i.d.; supplied by Plowden and Thompson,Ltd.,. UK). The etched tip of the metal is pull up approximately 2cm into the glass tubing and fixed in position using molton wax applied to the other end. The glass was pull over the tip of the tungsten wire using a microelectrode puller. Then insert the tip into a heated molten bead of sodium borosilicate glass for remove a molten glass on the tip of electrode. The microelectrode impedance was measured at 1 kHz using and impedance meter. The best impedance of electrodes could be 1-3MΩ. (K&B, 1996)

Preparation for ICV Infusion and Single-unit Recording

On the experiment day, 22-G probe is inserted through the guide tube into the lateral ventricle after removed obdurator. When long term infusions were needs, the probe would be connected via a polyethylene canula to a 20-ml injection syringe held in the infusion pump

Each animal was experimented twice every week and 6-8 hours in duration until the conclusion of the sessions. On the recording day, sheep was transferred from cage to the recording chamber by being lifted into a movable canvas hammock sling. Animal's legs hang to holes in the hammock and move occasionally during recording experiment. Sheep's head was designed to fix in position. Two stainless steel bars, fixed with the recording chamber recording by two pairs of plastic clambers, are inserted into the head restraint on the animal's head.

Once the sheep has been readily placed on the sling and being moved into the recording chamber, the nylon cap was removed from the stainless steel well on it head

and commercial anti-mastitis suspension was removed from it using sterile swabs and rinsed with sterile normal saline. The exposed dura was anaesthetized by 0.5 ml of topical application of 0.5% (w/v) solution of tetracaine HCL and Trent-Wells x-y table and hydraulic micro-drive are attached to the top of the well. A deep measurement had taken and a length of the 20-gauge tubing cut so that it penetrated in -10 mm deep down into the brain. This same length of guide length was sterilized and used on all subsequent recording sessions. The sheep was not feel any discomfort when the dura is punctured because the tetracaine in the medium make it insensitive.

The micro-drive was then advanced to the point when it should be level with the end of the guide tube and depth reading was taken. This depth reading was then equivalent to –10.0000 mm below the surface of the dura. Tungsten microelectrode was then hydraulically driven down to the co-ordinate of ZI in lateral hypothalamus (LH) region.

Eventually, sheep in the hammock was moved into the Faraday cage to reduce 50-Hz noise and radio frequency interference. The signal was got from the Tungsten electrode and metal well on the sheep's head, which is a reference electrode. These electrode connected to a pre-amplifier (Digitimer©, NL100;37 Welwyn Garden City, hert, UK).

SECTION IV Data Acquisition

Signals in this work were recorded by MacLab systems with oscilloscope, timing device, and audio amplifier. The MacLab system continuous recorded at 40

kHz. Neuronal signal from the microelectrode were passed through an AC headstage, amplifier with high impedance buffer (Neurolog Digitatimer© Model NL 100 AK, 37 Hydeway, Welwyn Garden City, Hertfordshire, UK) mounted on or nearby the slave microdrive in order to minimize interference. Output from headstage was then 20k amplified (10-20k range form this machine) by analog AC pre-amplifier (Neurolog Digitimer© Model NL 104A). The amplifier signal was then filtered with suitable analog electronic filter (Neurolog Digitatimer© Filter Model NL15), bandpass filter at 500-5kHz. 50 Hz electrical interference is also removed from this bandpass. Then amplified signal were displayed continuously on the oscilloscope (Nikon Kohden, Memory Oscilloscope Model VC-11 Japan) and presented on an audio-monitor at the same time (Grass© Medical Inc., Model AM 4, Quincy, MA, USA). The signal was digitized by the analog to digital (A/D) converter computer interface card at 16 bits (MacLab™). The data signal was also presented on the computer monitor screen, MacLab system.

The raw data of neuronal signal, 35 sec, were recorded on the SCSI internal hard disc of Power PC (Apple© Machintosh™) for offline analysis .In addition, a VHS video camera was placed inside the recording chamber permitting visual observation of the animal via a VCR recorder. These data signal were copy into the CD-ROM.

The affiliated Spike Histogram Extension (SHE) version 1.6.6 was installed in the Chart™ application program version 3.6.1 (ADInstruments Pty Ltd, Australia) for analysis of the recorded files. In this experiment, SHE programming was used as a tool

to convert data to Matlab files format (mat). All neuronal signals were analyze by Matlab program (Figure3-1 show the diagram of data acquisition)

SECTION V Method of Testing

Water is in red plastic bowl, so sheep could easily learn to distinguish the contents of the bowls at a distance, fitted with long handles. During the test, a wooden screen was place at 45° angle between the sheep in the control manner to the left side of animal. The Faraday cage was covered with a cloth so that sheep is not allowed to see the bowl before experimenter offered the stimuli.

Before recording, the neuron was checked to be a single neuron and neuron responding to the sight of food but not response to the sight of water. For this checking, sheep was shown a color plastic bowl containing food for 5 sec, 1 meter far. After 5 sec the stimuli was slowly moved toward the sheep's mouth over 5 sec period. The sheep was allowed to eat for 5sec. This neuron response was detected from the electrode and can be monitored on the oscilloscope screen. Replaced this procedure by water. It could be responsive to the water.

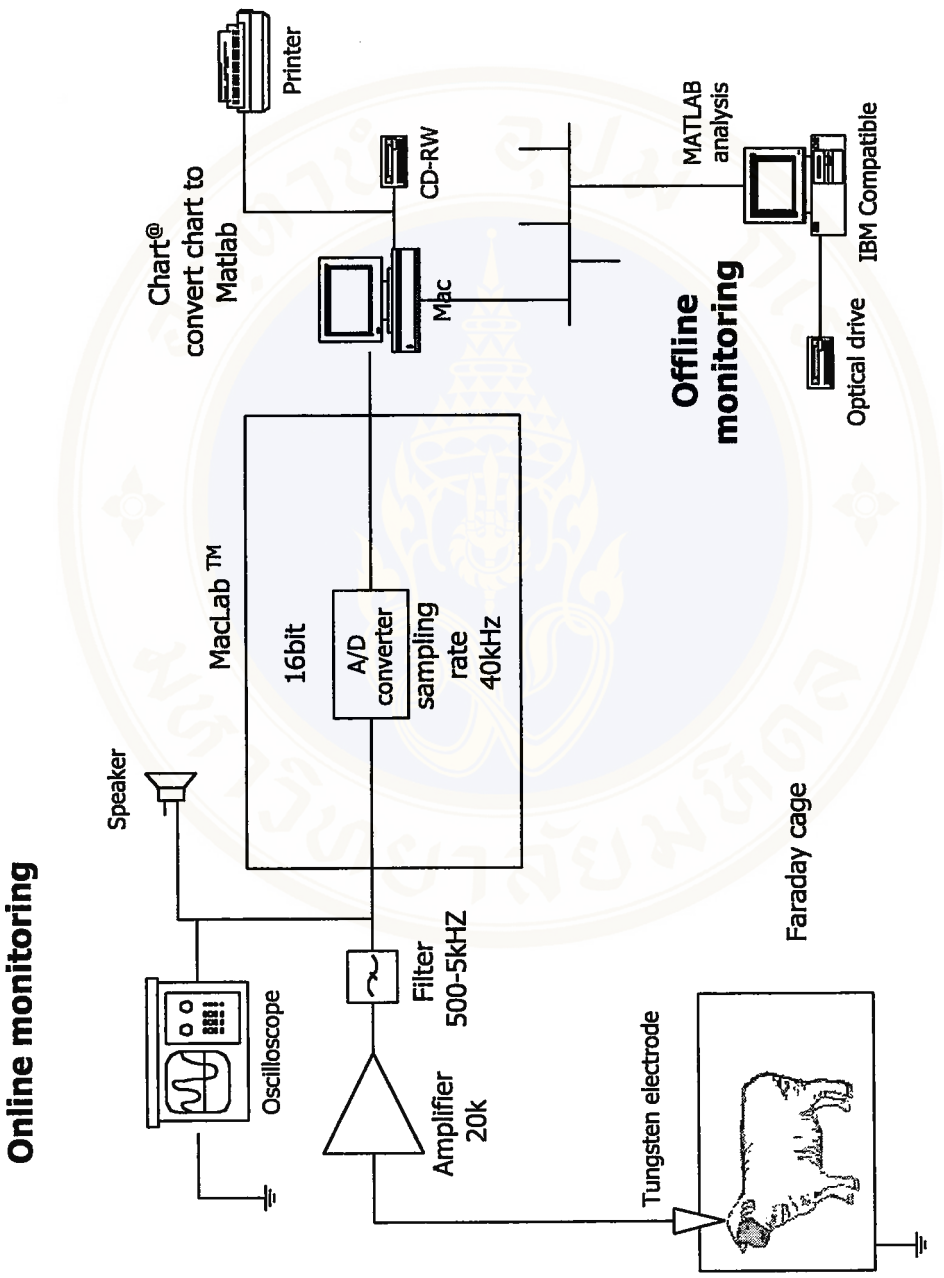

FIGURE 3.1 Diagram of data acquisition.

Before recording, it demonstrated that the single neuron being recorded did not response to general arousal or nonsense visual approach of ingestive stimuli such as VHS video cassette tape, clap hand to make noise, or blow air at sheep face.

The procedure for water presentation is outlined below:

1. First demonstrate icv of 200ng/ANGII dissolved in 1 ml of water and injected into the lateral hypothalamus over 30 sec period, caused neuron response to the sight of water. Then checked to be single neuron and responding to the sight of water.
2. A recording of a clinical test was began with a 5 sec period of baseline control began when it was established that the unit was stable and not response to arousing stimuli.
3. A red color plastic bowl contain water was shown statically to the sheep, at a distance of about 1 m for a period of 5 sec. This procedure establishes wether the neuron being recorded from those responded to static visual presentation of water. A response could readily be detected from the activity on the screens of the oscilloscope or the computer and particular easily by listening to the unit discharges on an audio-monitor.
4. After 5 sec of static presentation the red bowl containing water was slowly moved towards the sheep's mouth over a 5sec period and the unit neuronal response was recorded.
5. When the bowl reached the sheep's mouth, it was allowed to drink if wished to do so and feeding was allowed for 10sec.

6. The red bowl was then slowly withdrawn from the sheep's mouth taking 5sec to do so and again the unit's activity was recorded.
7. The red bowl was then removed from the sheep's vision, and again the unit's activity was recorded for a further 5sec.

These procedures were repeated for ten times (10 trials). Each individual neuron was studied for 2-3 hours if the response was interested and the unit was stable for these long period. A record of the depth XYZ co-ordinate position of each neuron, as well as it response properties was made. (Fig 3.2 shows the procedure of the experimentation)

SECTION VI Signal Analysis

All function in this thesis are wrote on Matlab program because Matlab is a powerful program for mathematics and engineering analysis, It can run on many platforms such as PC, Macintosh and UNIX (in this thesis it run base on PC platform). For offline analysis, SHE program with Chart installed was used as a tool to convert data to Matlab files format (mat). This signal was sent to the PC platform via LAN so this signal, which is a Macintosh platform, was converted to PC platform. Signal was analyzed by Matlab 5.3.1 (Mathwork Inc.). The raw neuronal signal data were recorded along 35 s, but this thesis is focused on the first 3 periods of each trial (control or spontaneous, static visual approach)

The recording signal in this work is spikes (action potential) in the lateral hypothalamus. The electrode often records spike from the several cells. The difference

of spike amplitude will vary upon the distance of electrode and electrode tip. It is difficult to locate the distance between electrode and neuron so there are not equal in the amplitude and width of each neuron.

Schmidt (Schmidt, 1984) suggested that, amplitude and width at half height can separate the neuronal signal into group. This selection technique is done by mean of a "window discriminator", and in this thesis the cluster, which was wrote by Matlab programming, was used. This program allowed the resercher to select the spikes, which have closely amplitude and width at half height with one point selected. (Fig 3.4) The program will find the spikes that close to the selected point by increase the criteria distance from that point until all neuron spikes selected. The noise in raw neuronal signal data was eliminated, then showed on the window. This program also converts data selected to the TTL (Transistor-To-Transistor logic) signal. (See Fig 3.5) TTL were saved as a new data for analysis.

There are 10 steps for cluster spikes, see flowchart on Fig 3.3.

1. Input the signal variable number of trial and name.
2. Function will automatically find the pulse by collect the initial point and terminal point of each spike which is the graph which across zero line.
3. Save basic spike properties, height (zero line to maximum value of spike), width (distance between terminal point and initial point), and position (metric index which is spike position).
4. Calculate the width at half height by finding distance between 2 points that equal to the half height value.

5. Display graph of height and width at half height (Fig3.4).
6. Waiting for position of group selected input.
7. The function will automatically find the appropriate region for grouping spike. Region will magnify until it can group the spike, which assumed same neuron.
8. Function will display the grouping region.
9. Calculate spike data (total spike and selected spike), noise reducing signal, and signal in TTL form.
10. Display spike data (total spike and selected spike), noise reducing signal, and signal in TTL form (Fig 3.4 and 3.5).

- Peri-stimulus time histogram (PSTH) is used as a rate meter to show the firing rate frequency in each period, eg., control, static visual presentation, approach.
- Interspike interval histogram (ISIH), first order, was showed the Poisson distribution of the signal in each period and calculating mean of ISI distribution.
- Measure short time variability by using the coefficient of variation of the interspike interval (CV of ISI).
- The calculation of average mean spike area of spikes in each trial is used for proving objective 2 that mention in Chapter II.
- Pair t-test (2 tails) is used for compare average mean spike area and also mean ISI between control, static visual presentation, and approach.

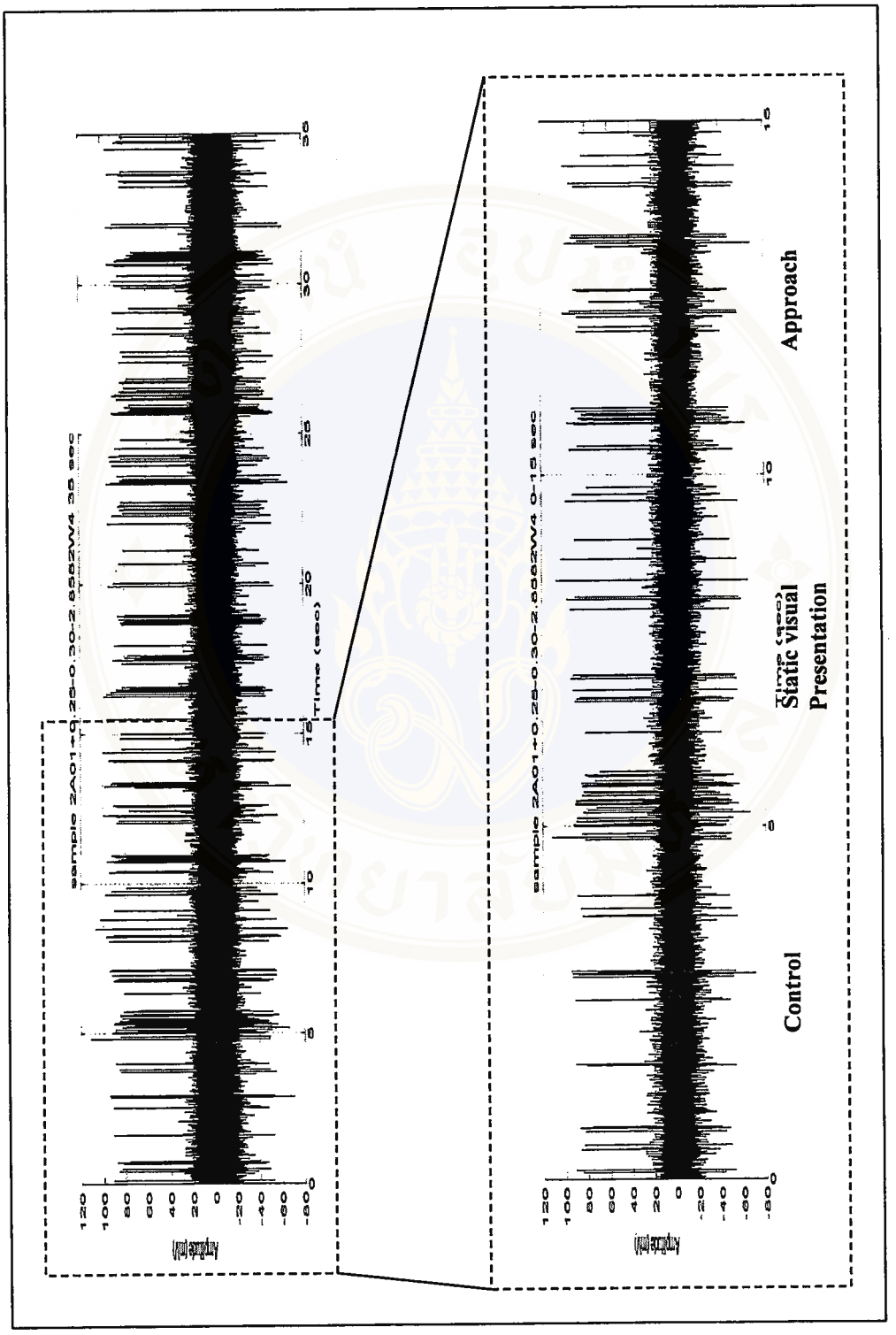

FIGURE 3.2 Procedure of the experimentation

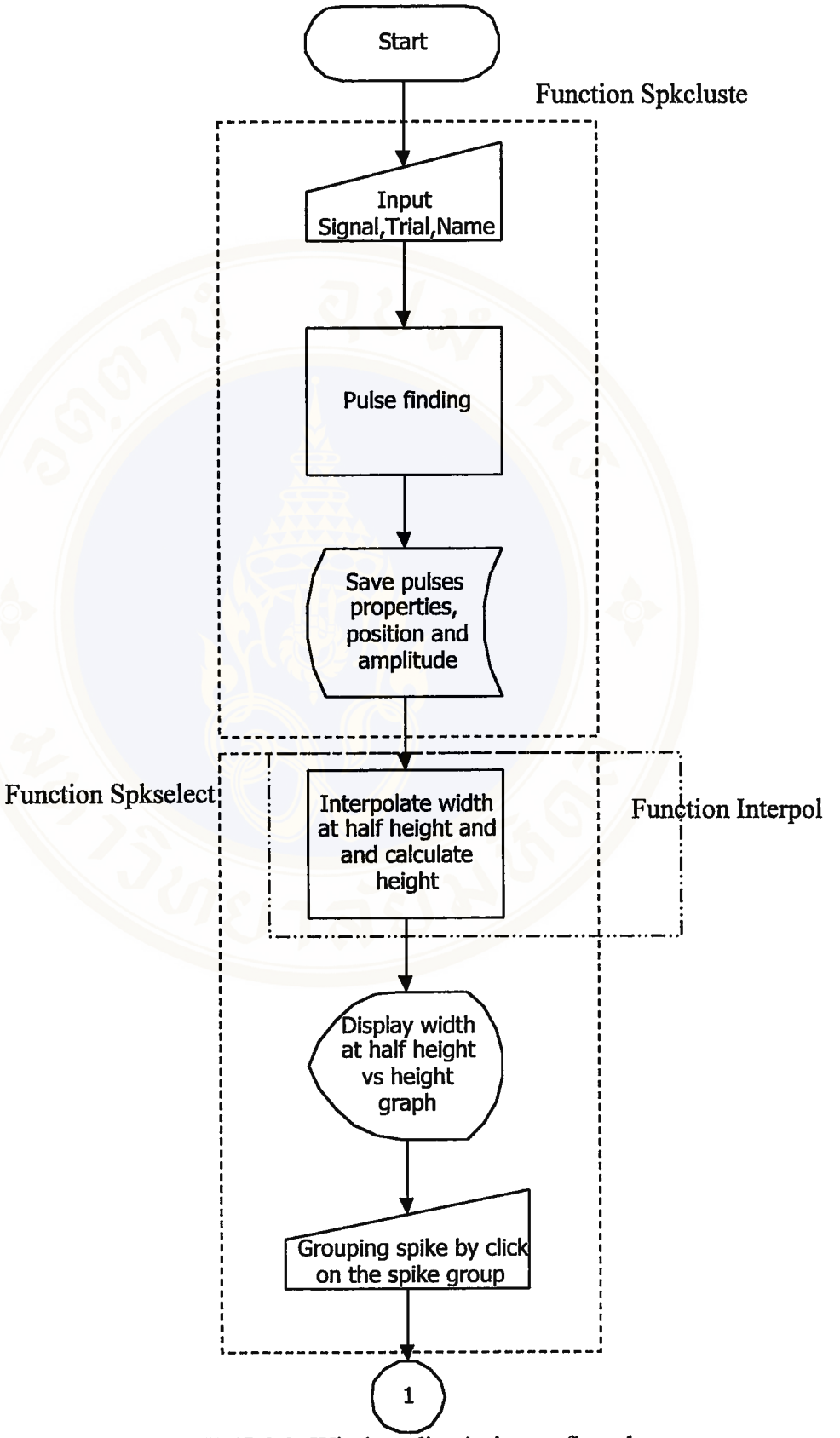

FIGURE 3.3 Window discriminator flowchart

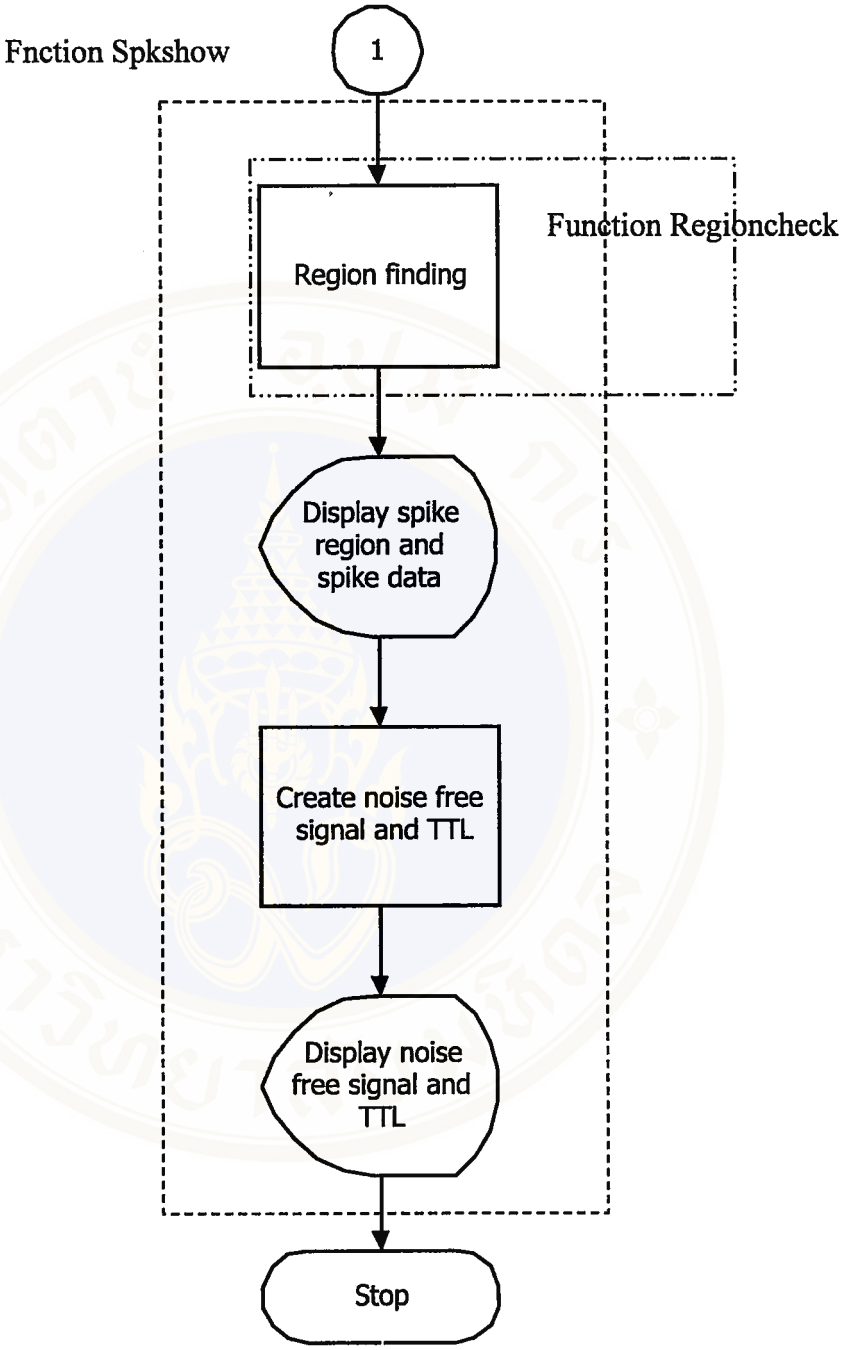

FIGURE 3.3 Window discriminator flowchart (cont.)

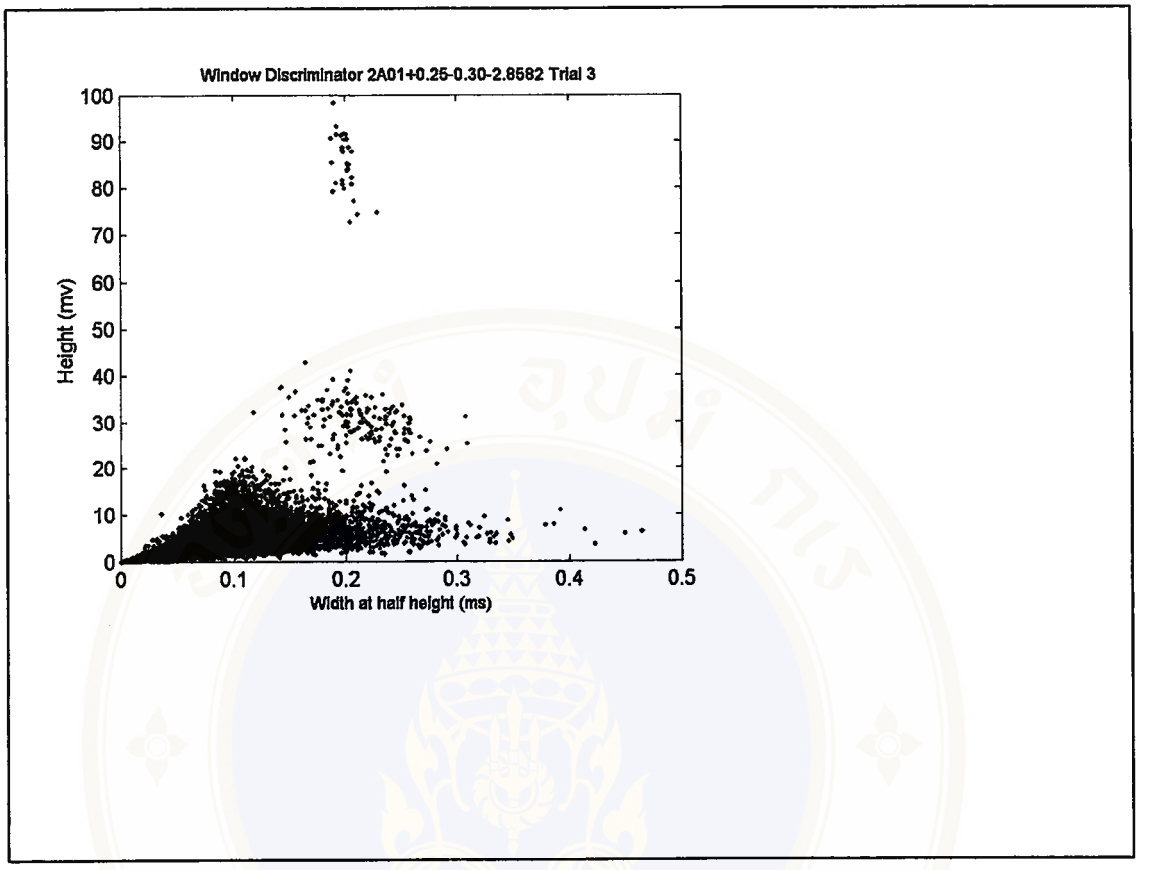

FIGURE 3.4 Window discriminator.

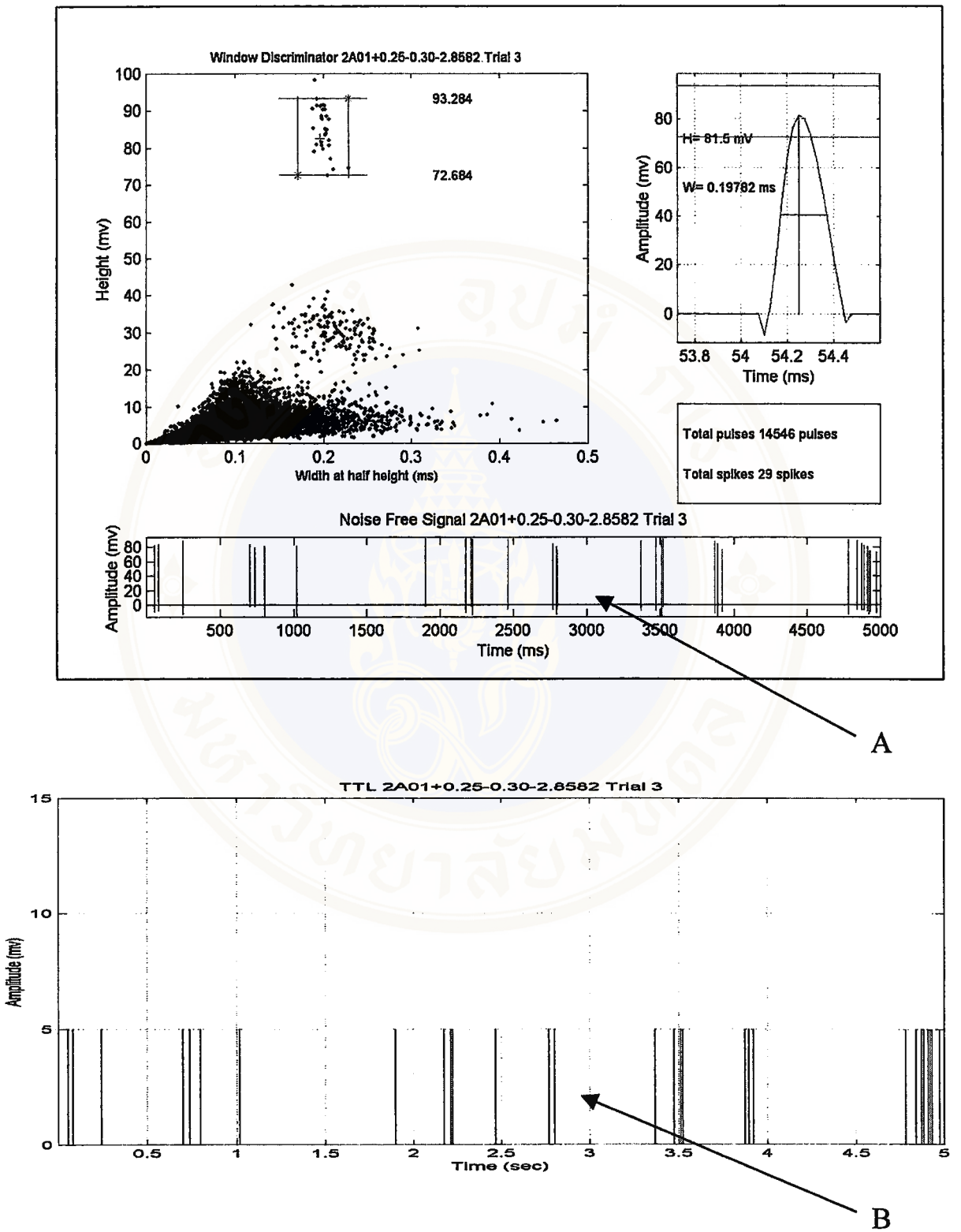

FIGURE 3.5 Window discriminator. **(A)** Noise free signal, this each signal shows their position, amplitude and width that recorded instead of TTL show only their position. **(B)** TTL.

CHAPTER IV

RESULTS

This chapter divided into 2 sections, computer programming (section 4.1) and spike neuron result (section 4.2). Computer programming section describes the Matlab m-files algorithm and also instruction for use. The neuronal activity signal section describes the results of spike analysis.

4.1 Computer programming

4.1.1 Window discriminator

Windows discriminator is saved in function name "spkcluster". After calling this function, This function will show each of spike properties such as width at half height and height in the coordinate x and y in the left of windows. These properties are widely used in the neuroscience spike classification. User has to selected group of selected spike by click on the group of spike as shown in Figure 4.1. The function will automatically select the minimal boundary of spikes group then show this boundary in the rectangular.

After this program selected the group of spike, it shows spike on another windows. The two windows on the right side shown the first spike picture and its properties such as total spikes in this neuronal signal and total neuronal signal selected. In addition, this window shows an upper limit (the highest boundary of spike

peak), lower limit (the lowermost boundary of spike peak), leftmost limit (the lowest boundary of spike width at half height) and rightmost limit (highest boundary of spike width at half height).

This window also shows the height and width at half height of the shown spike in the msec unit. The bottom window showed the selected spike in the msec unit. The spike train showed only the spike that is selected by window discriminator, instead of other pulse is reduced to zero volts. This signal is used to calculating area under spike using of spikes train.

TTL signal spike train signal, which is the noise free signal converted to 5mv of amplitude, is shown on the new figure window as shown in Figure 4.3. TTL signal is used to calculating the rate count and inter-spike interval (ISI).

For using the window discriminator, user might load the data (mat files which is the recorded neuronal activities in the lateral hypothalamus of conscious sheep) from the directory then pass it through the function. The output of function is array of noise free spike train, signal properties such as height, width at half height, and spike position, TTL spike train, highest spike height boundary, lowest spike height boundary, highest spike width boundary and, lowest spike width boundary.

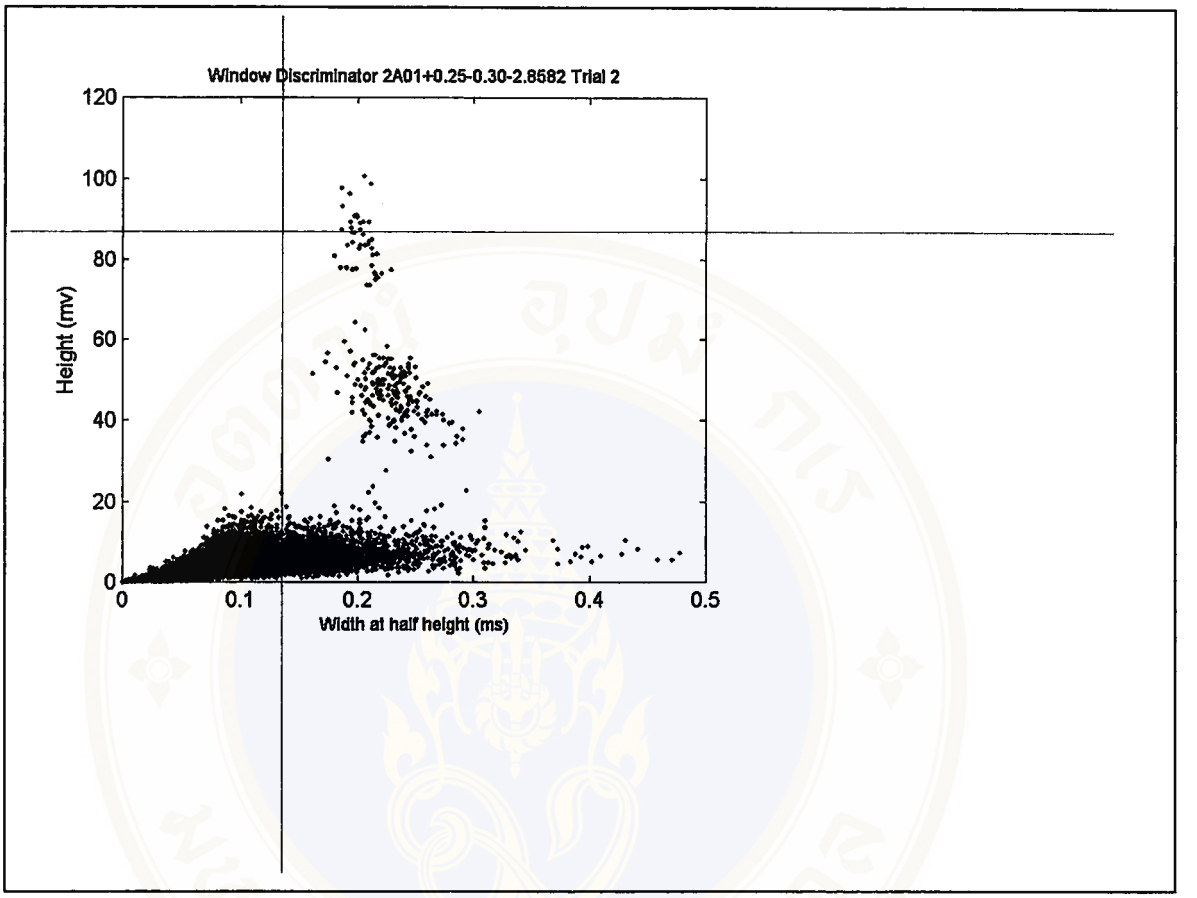

FIGURE 4.1 The neuronal signal of each spike in the height and width at half height before selected spike group and it selected lines.

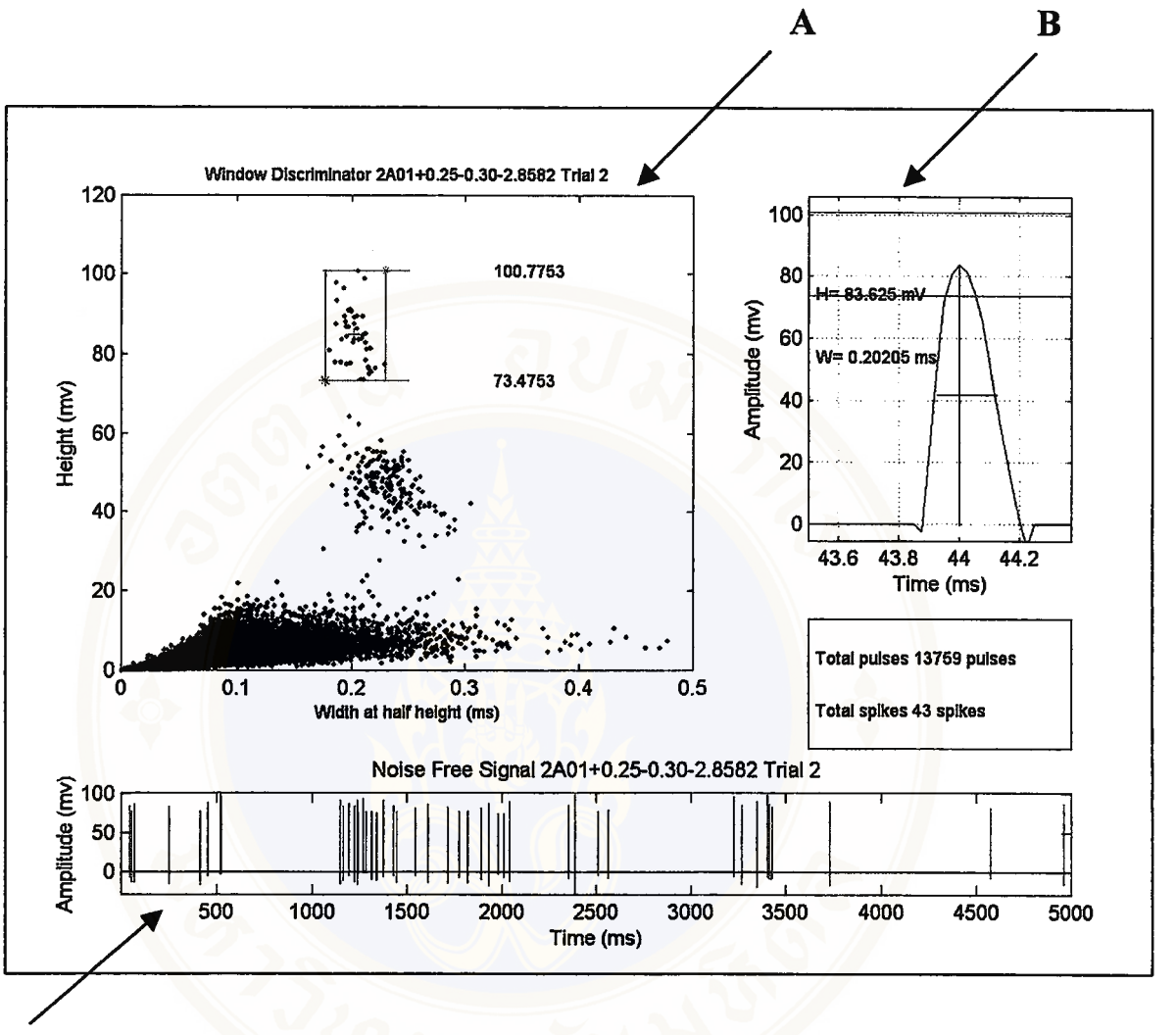

FIGURE 4.2 (A)Window discriminator after selected the group of spike. Left side windows show the spike selected. (B)Right side window shows the first spike, spike height, width at half height, total spike and total pulse. (C)The bottom window shows signal selected after noise reducing.

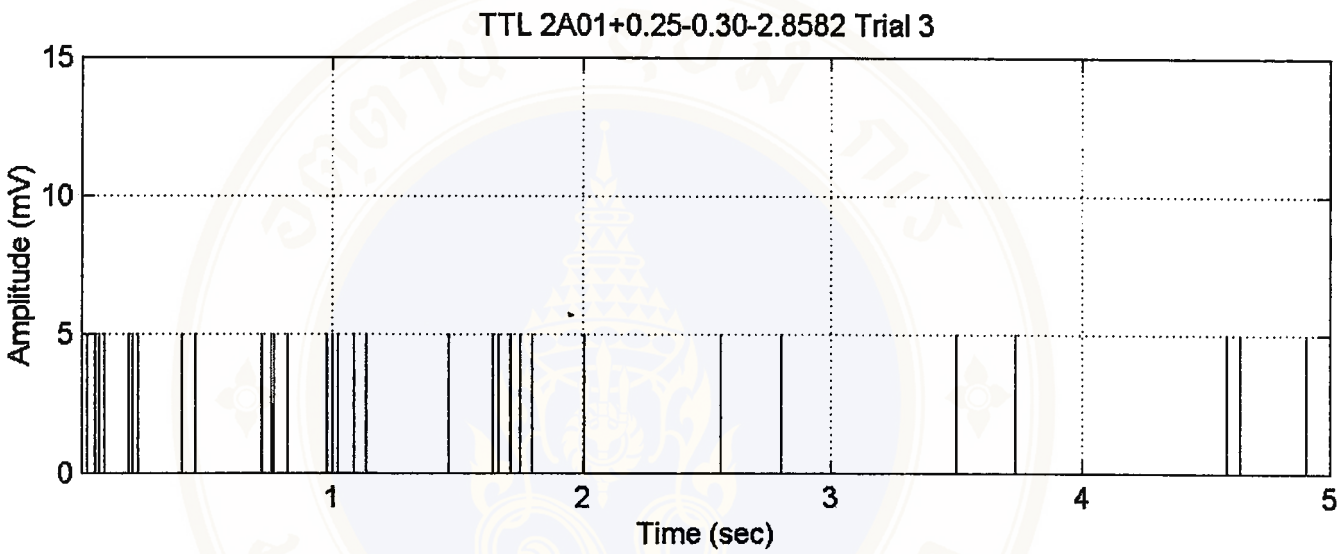

FIGURE 4.3 The TTL of the selected neuronal signals.

4.1.2 Area under spike of spike calculation

This function used to calculate the area under each spike. It is called spikepower. The function calculated the spike area by computing an approximation of the integral of amplitude (y-axis) via the trapazoidal method (Trapezoidal numerical integration). However, trapazoidal function in Matlab can not compute an area beside the spike. These time (x-axis) distance is smaller than one unit of array. A function "interpol" was used to find the distance on x-axis. Therefore, the area under spike is the summing of area calculated by trapazoidal and nearby areas as shown in Figure 4.4.

Spikepower function has 5 input arguments, properties of signal, neuronal signal, highest spike height boundary, lowest spike height boundary, highest spike width boundary, and lowest spike width boundary. After calling function, spikepower not only return a area under spike of each spike but also return the spike starting and stopping point in the same variable.

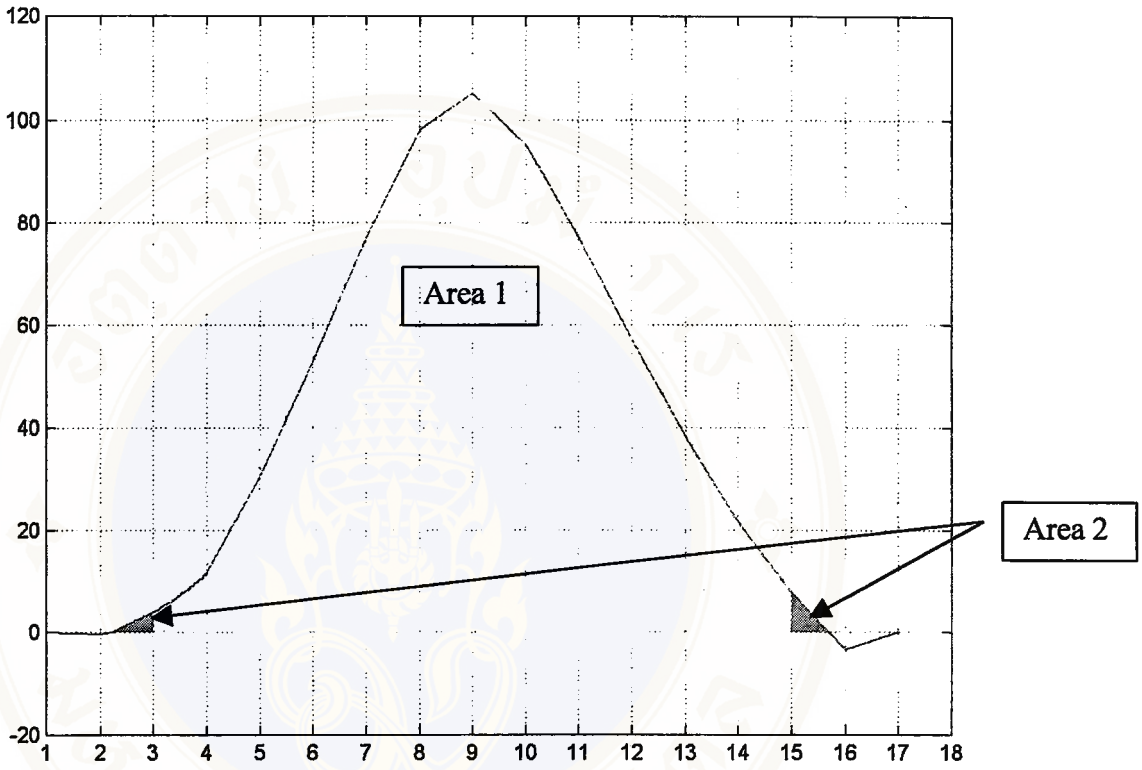

FIGURE 4.4 Area under spike of spike calculation. Total area is the summing of area under spike on area 1 (trapazoidal method) and area 2 (interpolation).

4.2 Neuronal activity signal analysis results

This section presents the results obtained by the analysis of the extracellular signal unit neuronal activity in the lateral hypothalamus (LH) of conscious sheep. The neurons tested responded initially to visual presentation of food but not to water. The experiments were designed to test the hypotheses that are presented in the objective Chapter II.

This result shows that, the firing rate changed in the animal's motivation state from hunger to thirst following intracerebroventricular (icv) injection of Angiotensin II (ANG II) dipsogenic stimuli. The neuron would then respond to the sight of water when the sheep are shown with water. In addition, the area under spike used of neuronal in each of periods is examined.

The neurons are depicted from the 124 neurons, in 7 sheep, were recorded from during 84 electrode penetrations and 2 were selected for detail study as they were stable and initially responded only to the sight in food and not to the sight of water. ANG II dipsogenic was used as a tool to induce sheep from hungry to thirst. Figure 4.5 show (A) raw neuronal signal; (B) noise free signal; (C) spike in TTL form.

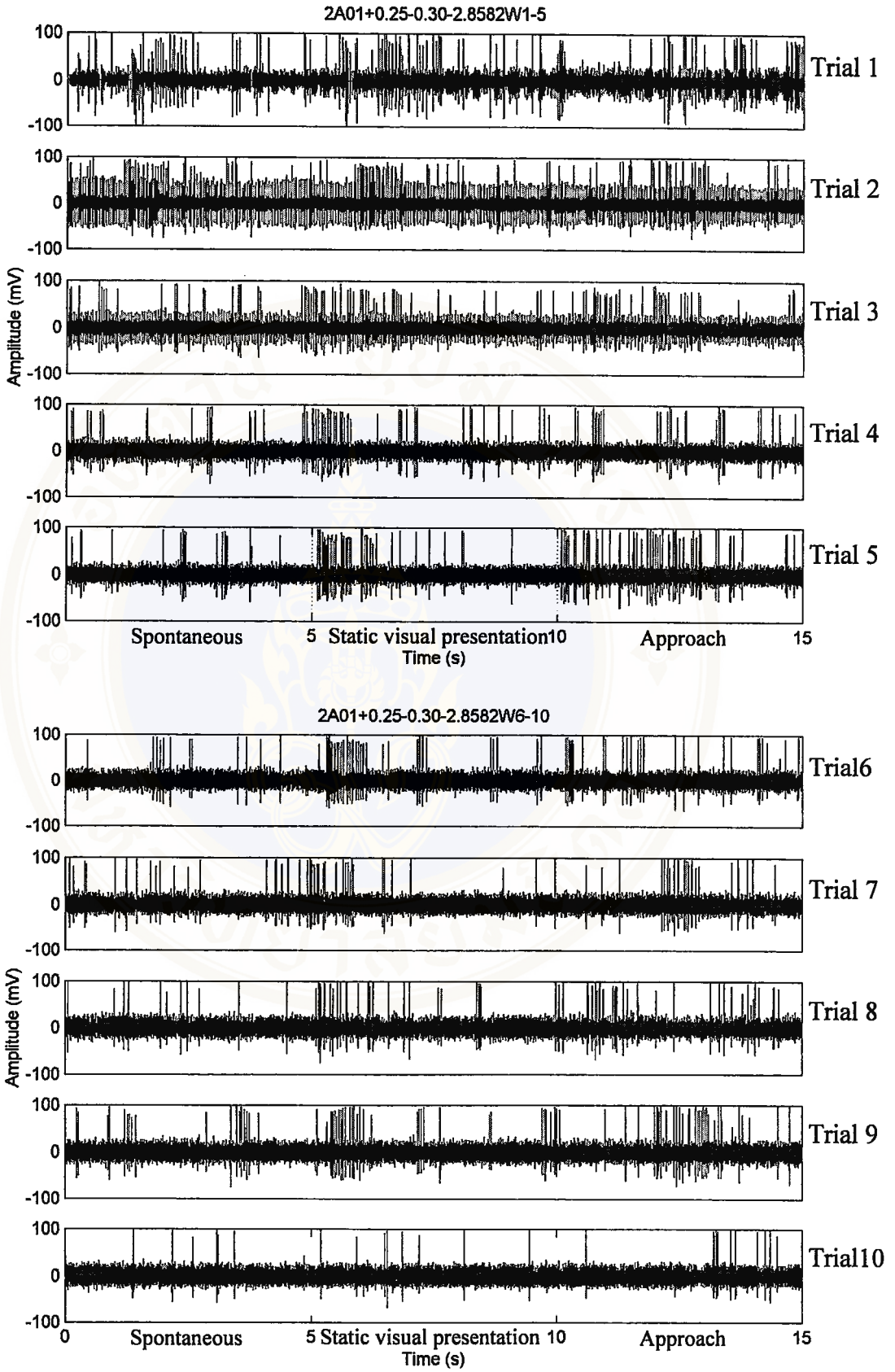

FIGURE 4.5 (A) Neuronal signal recording after ANG II icv injected: neuron coded 2.8582

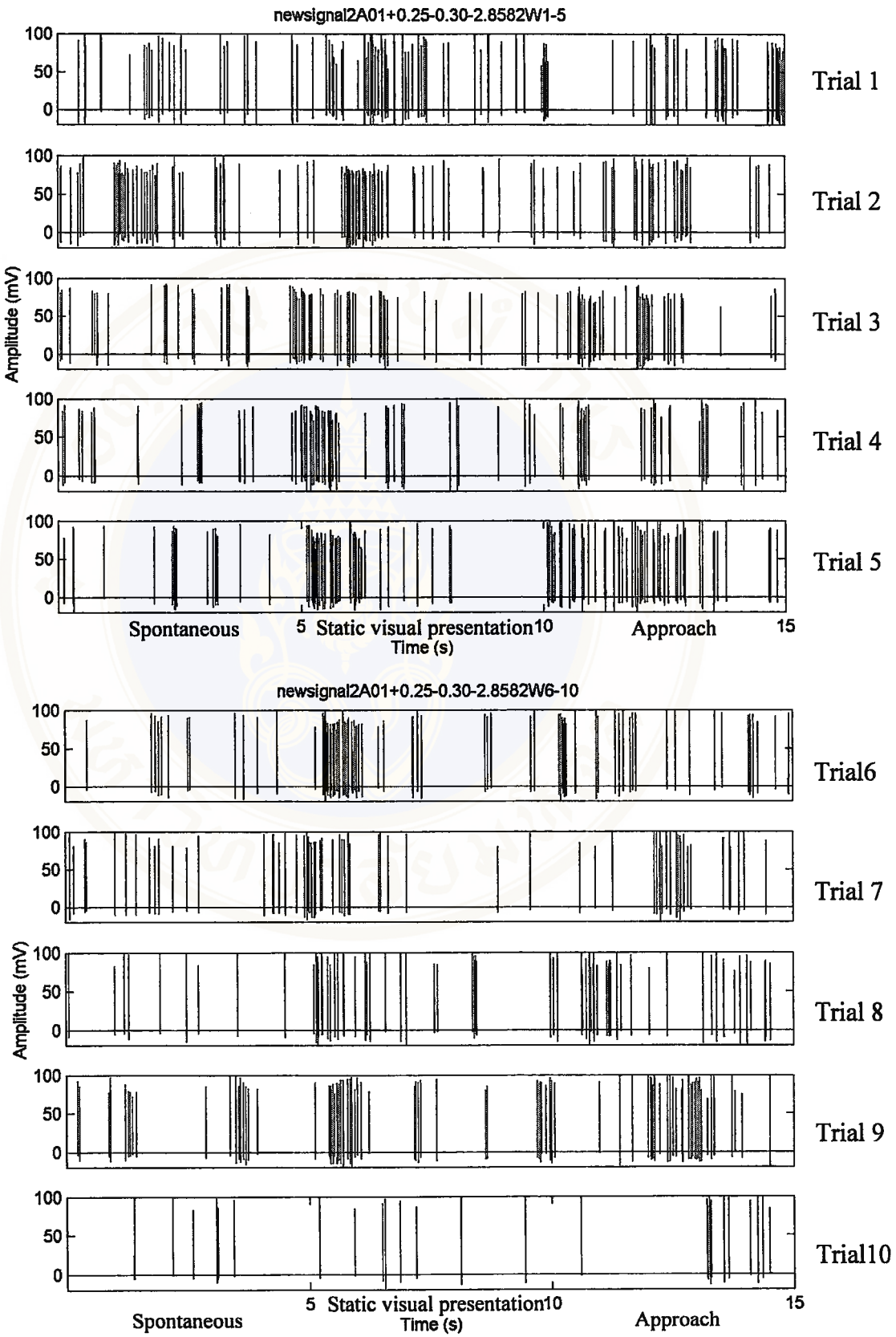

FIGURE 4.5 (B) Noise free neuronal signal recording after ANG II icv injected: neuron code 2.8582

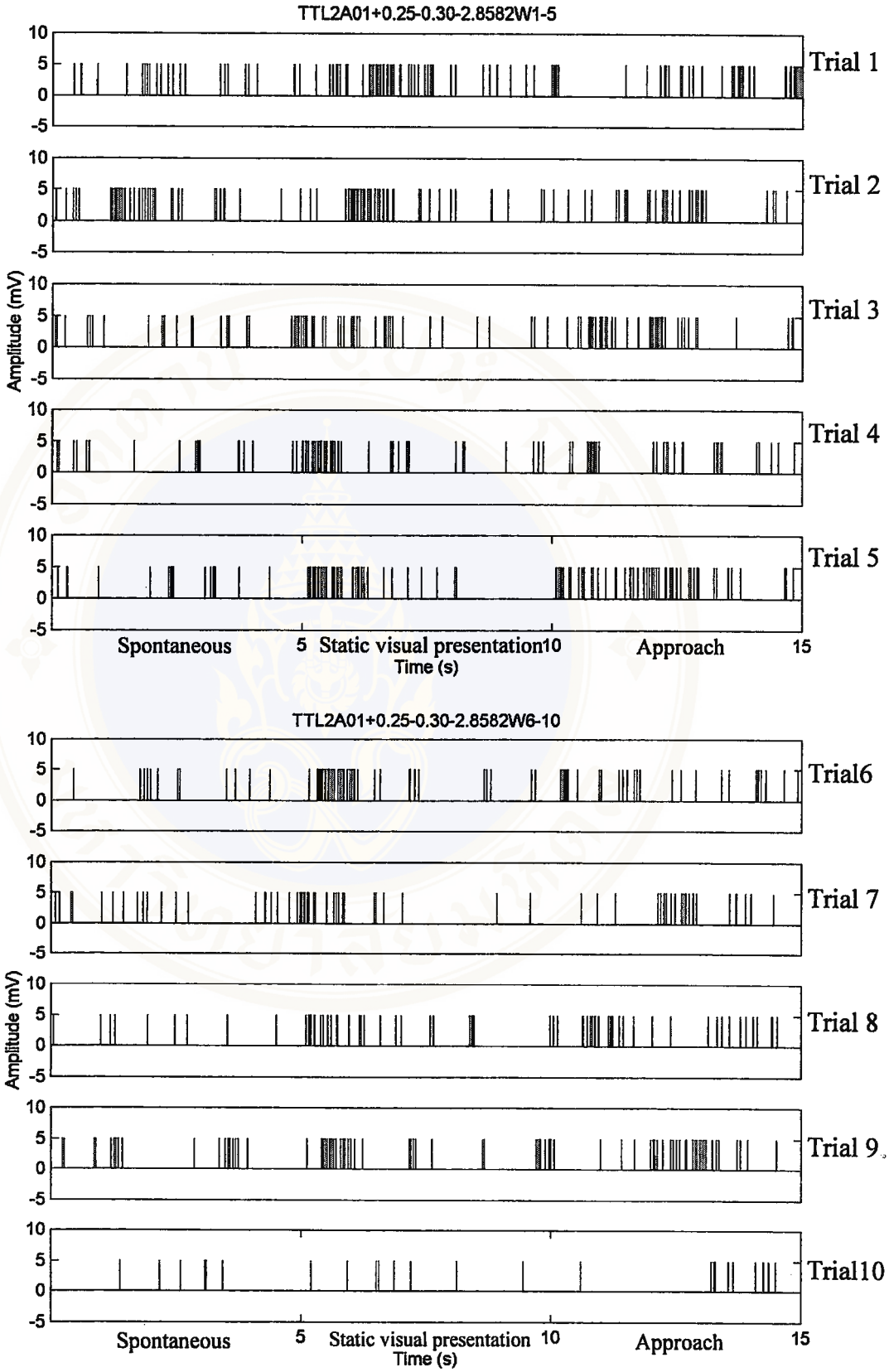

FIGURE 4.5 (C) Neuronal signal TTL after ANG II icv injected: neuron code 2.8582

TABLE 4.1 Rate count (spikes) of neuron code 2.8582

Trial	Rate count 2.8582 (spikes)		
	Control	Static visual presentation	Approach
1	26	51	45
2	43	49	32
3	29	32	40
4	21	39	31
5	20	42	56
6	12	45	29
7	22	21	24
8	9	28	31
9	19	35	34
10	7	8	11
Total =	**208**	**350**	**333**

TABLE 4.2 Rate count (spikes) of neuron code 2.7060

Trial	Rate count 2.7060 (spikes)		
	Control	Static visual presentation	Approach
1	17	17	17
2	29	144	124
3	38	53	65
4	71	55	58
5	34	31	47
6	10	2	0
7	1	9	14
8	19	26	15
9	2	11	20
10	4	3	18
Total =	**225**	**351**	**378**

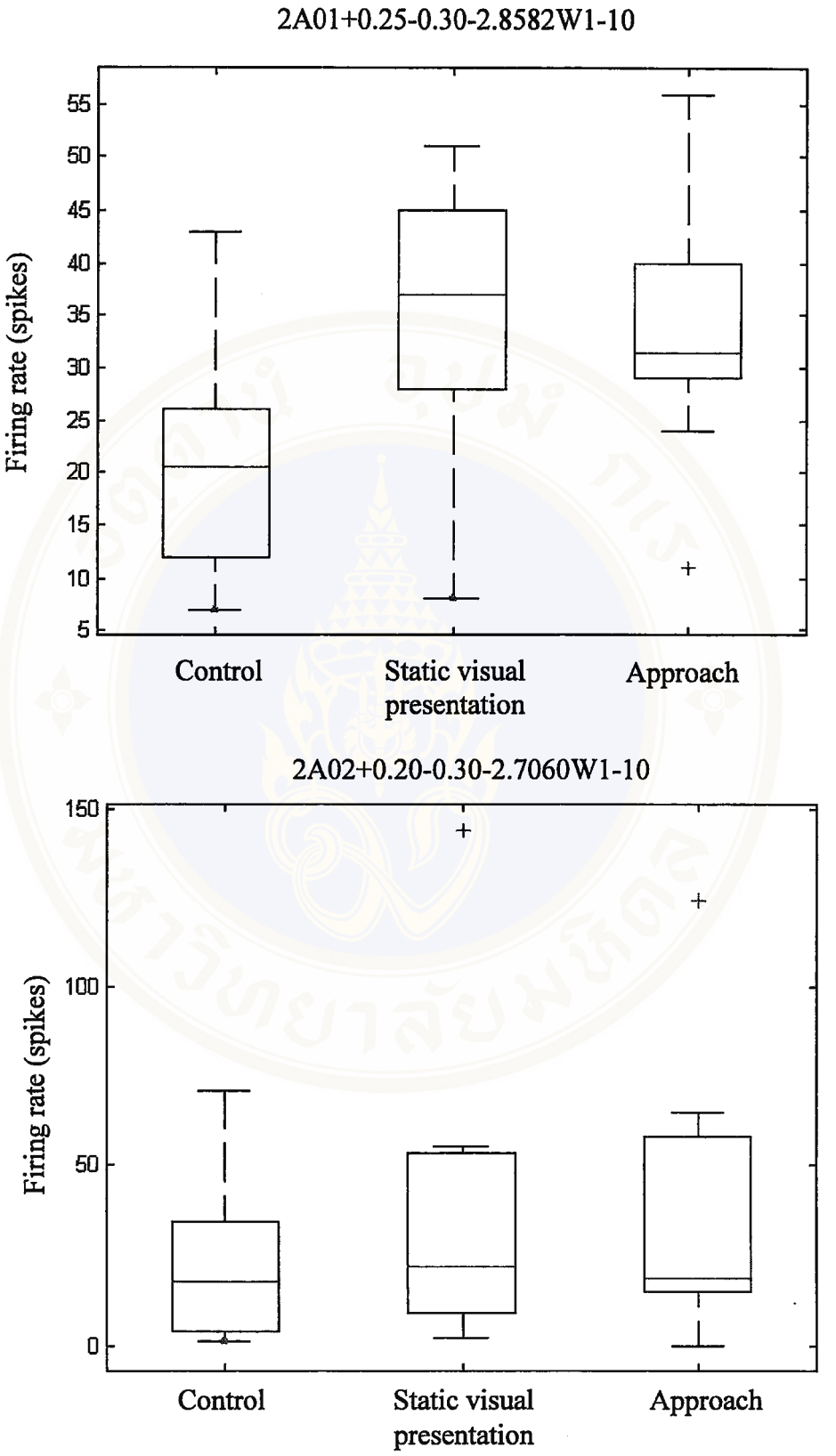

FIGURE 4.6 Boxplot of rate count after ANG II icv injected: (A) Neuron coded 2.8582. (B) Neuron coded 2.7060.

Objective 1

To compare mean interspike interval (ISI) of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the mean ISI in each period related?

TABLE 4.3 Mean ISI (msec) of neuron coded 2.8582 (bin 25 msec)

Trial	Mean ISI 2.8582		
	Control	Static visual presentation	Approach
1	125.0000	98.4694	85.1190
2	84.6154	62.5000	129.1667
3	120.0000	110.7143	84.4595
4	98.4375	69.1176	88.8889
5	101.6667	84.1463	81.9444
6	194.4444	57.3171	133.0000
7	169.7368	95.5882	122.6190
8	191.6667	96.8750	126.7857
9	117.1875	73.3871	108.0645
10	265.0000	250.0000	152.7778

TABLE 4.4 Mean ISI (msec) of neuron coded 2.7060 (bin 25 mssec)

Trial	Mean ISI 2.7060		
	Control	Static visual presentation	Approach
1	218.3333	270.0000	159.6154
2	119.4444	46.5035	53.2520
3	143.2432	96.0784	88.6719
4	83.5714	97.6852	99.1228
5	143.7500	133.0357	121.1957
6	270.0000	-	-
7	-	245.8333	186.3636
8	213.3333	198.9583	193.7500
9	375.0000	203.5714	193.7500
10	-	375.0000	187.5000

Objective 2

To examine the average area under spike of the neuronal signal of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the area under spike in each period related?

TABLE 4.5 Area under spike (mS.mV) of neuron coded 2.8285.

Trial	Average Area Under Spike 2.8285 (mS.mV)		
	Control	Static visual presentation	Approach
1	16.600976	15.931084	16.118223
2	16.745635	16.105250	17.045455
3	16.570485	15.998667	15.447796
4	16.876039	17.079935	17.003195
5	17.354131	16.921270	17.771782
6	17.941900	17.078697	18.244569
7	17.655117	17.569211	17.769801
8	18.371561	18.487737	17.700831
9	16.747815	17.542459	18.012133
10	18.049465	18.247637	18.970126
Average	**17.29131**	**17.09619**	**17.40839**
Variance	**0.451451**	**0.8067**	**1.072809**

TABLE 4.6 Area under spike (mS.mV) of neuron coded 2.7060.

Trial	Average Area Under Spike 2.7060 (mS.mV)		
	Control	Static visual presentation	Approach
1	12.730416	11.737948	12.92886
2	13.365104	13.275601	12.792456
3	12.196564	12.708255	12.692942
4	12.854114	12.833742	12.608932
5	9.862752	9.24854	9.187215
6	17.273016	17.391081	-
7	7.284874	7.554826	7.224925
8	7.625054	7.576364	7.26073
9	7.537277	7.940077	7.454646
10	8.437952	8.060462	7.461714
Average	**10.9167123**	**10.8326896**	9.956936
Variance	**10.8643575**	**10.80353251**	7.400598
Average (Trial 1-4)	12.78655	12.63889	12.7558
Variance (Trial 1-4)	0.230175	0.419959	0.018938

TABLE 4.7 Normalize area under spike of neuron coded 2.8285.

Trial	Normalize Average Area Under Spike 2.8285		
	Control	Static visual presentation	Approach
1	0.9036	0.8617	0.8497
2	0.9115	0.8711	0.8985
3	0.902	0.8654	0.8143
4	0.9186	0.9239	0.8963
5	0.9446	0.9153	0.9368
6	0.9766	0.9238	0.9618
7	0.961	0.9503	0.9367
8	1	1	0.9331
9	0.9116	0.9489	0.9495
10	0.9825	0.987	1

TABLE 4.8 Normalize area under spike of neuron coded 2.7060.

Trial	Normalize Average Area Under Spike 2.7060		
	Control	Static visual presentation	Approach
1	0.9525	0.8842	1
2	1	1	0.9894
3	0.9126	0.9573	0.9818
4	0.9618	0.9667	0.9753
5	0.7379	0.6967	0.7106
6	-	-	-
7	0.5451	0.5691	0.5588
8	0.5705	0.5707	0.5616
9	0.564	0.5981	0.5766
10	0.6313	0.6072	0.5771

Objective 3

To measure the variability associated with interspike interval distribution by using coefficient of variation of the neuronal signal in each period.

TABLE 4.9 Coefficient of variation (CV) of neuron coded 2.8285.

Trial	Coefficient of variance of neuron coded 2.8285		
	Control	Static visual presentation	Approach
1	0.7816	0.9872	1.1732
2	1.0292	1.0357	0.9457
3	1.0524	0.8858	0.8796
4	1.0018	1.1236	0.9961
5	1.3661	1.1734	0.8123
6	0.7088	1.0651	0.9432
7	0.5247	0.9504	0.9684
8	0.3740	0.9103	0.8112
9	1.0443	1.0162	0.9874
10	0.7701	0.4967	0.8466
Average	**0.8653**	**0.9644**	**0.93637**
Variance	**0.2908**	**0.1872**	**0.1084**

TABLE 4.10 Coefficient of variation (CV) of neuron coded 2.7060.

Trial	Coefficient of variance of neuron coded 2.7060		
	Control	Static visual presentation	Approach
1	0.3865	0.4808	0.4660
2	0.9103	0.3459	0.3540
3	0.7875	0.5006	0.3149
4	0.4010	0.4907	0.4225
5	0.6025	0.6132	0.3965
6	0.3065	-	-
7	-	0.6470	0.5497
8	0.4839	0.5032	0.6280
9	-	0.5548	0.4292
10	-	-	0.5333
Average	**0.5540 (n=7)**	**0.5170 (n=8)**	**0.4549(n=9)**
Variance	**0.2242 (n=7)**	**0.09206(n=8)**	**0.1001(n=9)**

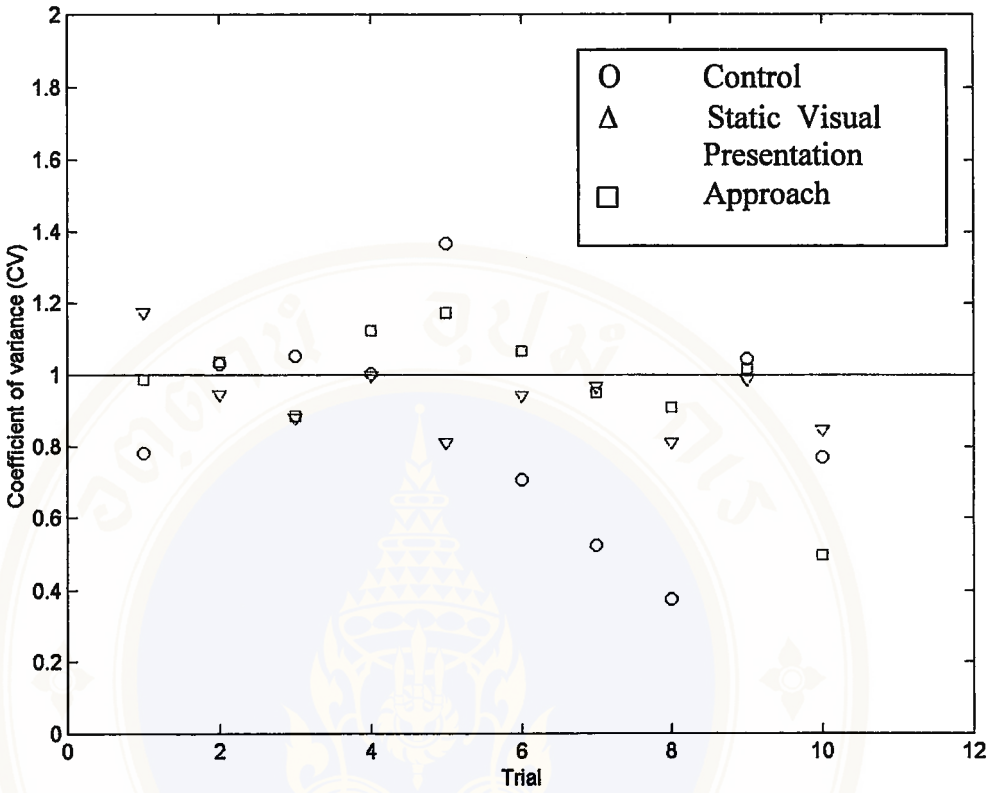

FIGURE 4.7 Coefficient of variance (CV) of neuron coded 2.8285

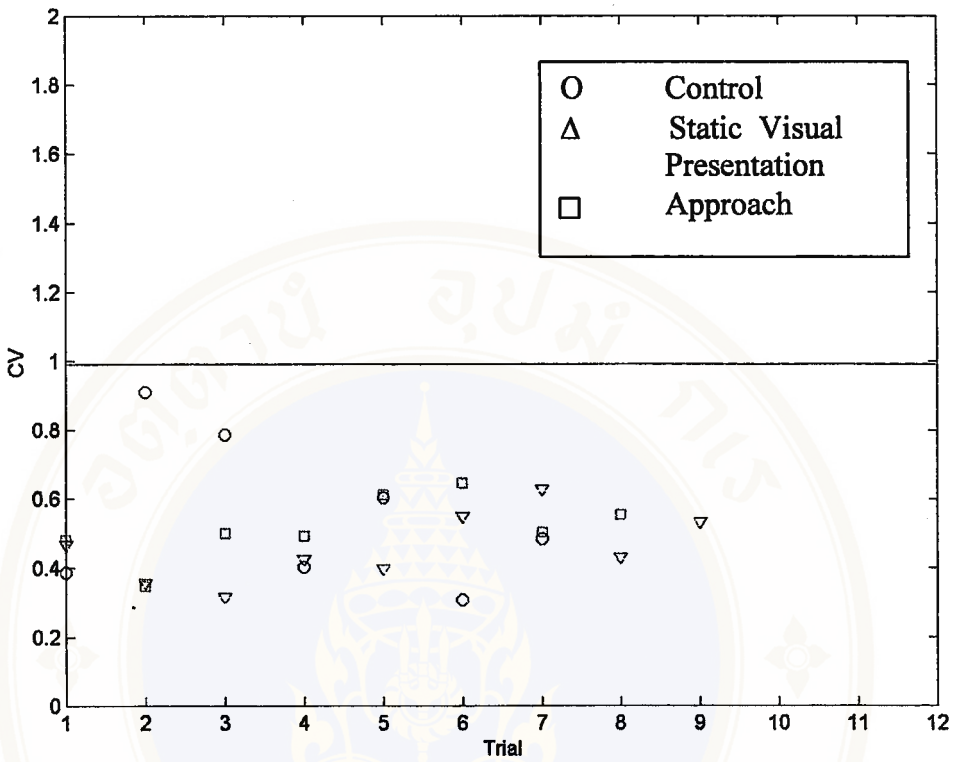

FIGURE 4.8 Coefficient of variance (CV) of neuron coded 2.7060

CHAPTER V

DISCUSSION

This chapter presents the conclusion and discussion of analyzing neuronal signals in the LH, which induced to responded to the sight of water by the icv injection of 200 ng ANG II /ml.

These neurons show that, the effect of icv injection ANG II 200 ng/ml induced neuron to response the sight of water as shown in the firing rate comparison table and boxplot graph (Table 4.1, 4.2 and Figure 4.6)

Objective 1

To compare mean interspike interval (ISI) of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the mean ISI in each period related?

Discussion

From the previous studies, researchers proposed that individual neuron in the lateral hypothalamus and zona incerta, which was known to respond only to the sight or approach of food, but not respond to the sight of water, could be induced to respond to water by the i.c.v. injection of ANG II.(Mungarndee et al. 2001, Weisinger et al.1996.).The criterion of measurement depends on the firing rate in each period.

The result is consistent with this mean ISI difference, which is shown in Table 4.4 and table 4.4, the data of mean ISI in each period and trial of neuron coded 2.8285 and coded 2.7060 demonstrated that mean ISI in neuron coded 2.8582 and in control period is very significantly different ($P<0.01^{**}$) from those in static visual period. For mean ISI in the approach period of the same neuron, it is significantly different ($P<0.05^{*}$) from the control period when compared statistically by pair t-test.

In addition, the mean ISI in neuron coded 2.7076 in control period is considered not significant but mean ISI in the approach period of this neuron, it is significantly different ($P<0.05^{*}$) from the control period when compared again by the method of pair t-test.

This study demonstrated further that the mean ISI of neuronal firing in lateral hypothalamus of sheep with icv 200 ng ANG II dipsogenic agent injection and are also different from spontaneous period in both static visual period and approach period. The presented data also confirms well with previous studies on the effect of the ANG II in the LH to the sight of water after i.c.v. ANG II dipsogenic agent injection. With different criteria, that is, comparing the mean ISI instead of counting the firing rate.

Conclusion

According to the hypothesis, the differences of mean ISI between control period and the other periods may caused from the sight of water after icv injection of ANG 200 ng/ml. The results in neuron coded 2.8285 demonstrate that mean ISI in

control period are different to the other periods (static visual and approach). Unlike neuron coded 2.7060, the comparison of the mean ISI does not show obvious differences, It may come from the sheep's satisfication of water. The results pointed out that mean ISI of neuronal functions of sheep can be used as a tool to indicate the condition (spontaneous, visual, and approach).

Objective 2

To examine the average area under spike of the neuronal signal of neuronal firing between spontaneous and static visual presentation and approach period after icv 200 ng ANG II dipsogenic agent injection. Is the area under spike in each period related?

Discussion

After i.c.v. injection of ANG II 200 ng/ml, a responsive neuron exhibited the response to the sight of water. The changing average area under spike during the static visual presentation and approach water period was considered not significant difference ($P>0.05$) when compared by pair t-test with baseline control period. Data of average area under spike in each period of neuron coded 2.8582 and 2.7060 are shown in Table 4.5 and Table 4.6.

In addition, the data of neuron coded 2.7060 (Table 4.6) shows the average area under spike decreasing in the trial 5-10. It may cause from water satisfaction on sheep. This is satisfaction will reduce the effect of thirst in the neuron. The normalizing of average area under spike (Table 4.7 and Table 4.8) show the

comparison of average area under spike coded 2.8582 and 2.7060. Variance of average area under spike in neuron coded 2.8582 is shown to be small; whereas, it is shown to be high in the other neuron (2.7060).

The averaging area under spike can be used as a criterion for spike neuron clustering. An action potential of the same neuron could have a nearly average area under spike. It could be more than 0.8 of normalizing of average area under spike value.

Conclusion

The results obtained in this experiment demonstrate that the firing of neuron in the LH which response to the sight of water after icv injection of ANG II 200 ng/ml is not significantly different in each period.

Hypothesis 3

To measure the variability associated with interspike interval (ISI) distribution by using coefficient of variation of the ISI neuronal signal in each period.

Discussion

Koch (Koch, Segev 2000) pointed that, the spike distribution is described as Poisson distribution if CV is equal to unity for CV is used as the criteria to identify the distribution function. The coefficient of variation (CV) is not equal to unity, for example, equal to $1/\sqrt{n}$, it is the gamma order n distribution.

According to the CV of the neuronal signal from neuron coded 2.8582, as the result on table 4.9 and figure 4.7, coefficient of variation (CV) in all periods are nearly to unity. Consequently, the distribution of spike coded 2.8582 after icv injection of ANG II in LH can be describe as Poisson spike train distribution. However, the result of neural coded 2.7060 as shown in table 4.10 is nearly equal to 0.5. It is obviously not the Poisson distribution but probably the gamma distribution.

The comparison of CV between spontaneous period and other periods is not significantly different when evaluated by the method of pair-t test. This result point out that the spike train distribution in all period all the same. That is, neuron spike train distribution pattern does not change after visual stimulation. It maintains its distribution although water is presented.

Conclusion

The neurons in LH after icv injection of ANG II in LH could be describe as Poisson spike train distribution but some of neuron could be describe as gamma distribution. Furthermore, visual stimulation effect does not contribute to their pattern distribution as it is proved statistically.

Future works

- Test the others periods (drink, withdraw and removal) with this criteria.
- Used the averaging area under spike as a criterion for spike neuron clustering for water satisfaction on sheep. An action potential of the same neuron could have a nearly average area under spike. It could be more than 0.8 of normalizing of average area under spike value.
- Used a distribution of neuronal signal in lateral hypothalamus to find the lateral hypothalamus model.

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APPENDIX

APPENDIX

```
function[newsignal,zeropointm,ttl,y_up,y_low,x_up,x_low] = spkcluster
(sig,name,trial)
% spike cluster in term of hidth at half height and half hight
%output width,half hight

zero_index=0;
zeropoint=0;
zeropointm=0;
powerdata=0;
sig(length(sig)+1)=0;
for(n=1:length(sig)-1)
  if (sig(n) <= 0) & (sig(n+1) >= 0)
    zero_index=zero_index+1;
    zeropoint(zero_index,1)=n;
    zeropoint(zero_index,2)=n+1;
    zeropoint(zero_index,3)=1;

  end

  if (sig(n) >= 0) & (sig(n+1) <= 0)
    zero_index=zero_index+1;
    zeropoint(zero_index,1)=n;
    zeropoint(zero_index,2)=n+1;
    zeropoint(zero_index,3)=0;
    end
  if (sig(n) == 0) & (sig(n+1) == 0)
     zero_index=zero_index-1;
  end
end
%%%%%%%%%%%%
%SAVE PEAK
%%%%%%%%%%%%
nn=1;
zeropointm=[];
for n=1:length(zeropoint)-1
    if zeropoint(n,3)==1 & zeropoint(n+1,3)==0
    zeropointm(nn,:)=zeropoint(n,:);
    zeropointm(nn+1,:)=zeropoint(n+1,:);
        nn=nn+2;
  end
 end
%%%%%%%%%%
%MAX PEAK & INDEX,(zeropointm(n,4)(y),zeropointm(n,5)(x))
%%%%%%%%%%
```

```
%zeropointm varible
%
%  1     2       3              4                  5             6         7          8
% start\\stop\\direction\\max amplitute\\max direction\\l_ point\\r_point\\length  r-l
%                                          from start   interpol   interpol    point

for n=1:2:length(zeropointm)-1
  [zeropointm(n,4),zeropointm(n,5)]=max(sig(zeropointm(n,1):zeropointm(n+1,1))');
end

%%%%%%%%%%%%
%cluster
%%%%%%%%%%%%

for n=1:2:length(zeropointm(:,5))-1

%%%%%%%%%%%%
%left side interpolate (backword)
%%%%%%%%%%%%

  for nb=0:zeropointm(n,5)-1
    sig_c=sig(zeropointm(n,1)+zeropointm(n,5)-1-nb);
    if sig_c <= zeropointm(n,4)/2
      zeropointm(n,6)=interpol(zeropointm(n,1)+zeropointm(n,5)-1-nb,sig...
(zeropointm(n,1)+zeropointm(n,5)-1-nb),zeropointm(n,1)+zeropointm(n,5)...
-1-nb+1,sig(zeropointm(n,1)+zeropointm(n,5)-1-nb+1),zeropointm(n,4)/2);
      break;
    end
  end

%%%%%%%%
%right side interpolate (forward)
%%%%%%%%
  for nf=0:zeropointm(n+1,1)-zeropointm(n,1)-zeropointm(n,5)+2
    sig_c=sig(zeropointm(n,1)+zeropointm(n,5)-1+nf);
    if sig_c <= zeropointm(n,4)/2
      zeropointm(n,7)=interpol(zeropointm(n,1)+zeropointm(n,5)-1+nf,sig...
(zeropointm(n,1)+zeropointm(n,5)-1+nf),zeropointm(n,1)+zeropointm(n,5)-1+nf-1,...
sig(zeropointm(n,1)+zeropointm(n,5)-1+nf-1),zeropointm(n,4)/2);
      if sig(zeropointm(n,1))==sig(zeropointm(n,2)) & sig(zeropointm(n,1))==sig...
(zeropointm(n+1,1))
        zeropointm(n,7)=zeropointm(n,6);
      end
      break;
    end
  end

end

  zeropointm(:,8)=(zeropointm(:,7)-zeropointm(:,6));
```

```
 for m=1:length(zeropointm)
   if zeropointm(m,8)<0
     end
 end
[newsignal,ttl,y_up,y_low,x_up,x_low]=spkselect(zeropointm,sig,name,trial);
```

```
function[newsignal,ttl,y_up,y_low,x_up,x_low]=spkselect(zeropointm,sig,name,trial)
figure;
cluster_win=axes('position',[0.1 0.35 0.5 0.55]);
 plot(abs(zeropointm(:,8))./40,zeropointm(:,4),'b.');
 hold on
 title(['Window Discriminater ',name,' Trial ',num2str(trial)],'fontsize',8)
 xlabel('Width at half height (ms)','fontsize',8);
 ylabel('Height (mv)');

[limitx,limity]=spikeginput(1);
limitx=limitx*40;
[y_low,y_up]=regioncheck(zeropointm(1:2:length(zeropointm),4),limity,7);
sig1=[];
for m=1:2:length(zeropointm)\
  if zeropointm(m,4) >= y_low
    sig1=[sig1;zeropointm(m,8)];
  end
end
[x_low,x_up]=regioncheck(sig1,limitx,3);
plot(x_up/40,y_up,'m*',x_low/40,y_low,'m*');
x_upc  = ceil(x_up);
y_upc  = ceil(y_up);
x_lowc = ceil(x_low);
y_lowc = ceil(y_low);
y_length=length((y_lowc-1):(y_upc));
x_length=length((x_lowc-1):(x_upc));
plot(x_low*ones(1,y_length)./40,((y_lowc-1)):(y_upc),'r' )  %|
plot(x_up*ones(1,y_length)./40,((y_lowc-1)):(y_upc),'r' )   %|
plot(((x_lowc)-1:(x_upc))./40,y_up*ones(x_length,1),'r' )       %--
plot(((x_lowc)-1:(x_upc))./40,y_low*ones(x_length,1),'r' )      %--
text((x_upc+3)./40,y_up,num2str(y_up),'fontsize',8 )            %--
text((x_upc+3)./40,y_low,num2str(y_low),'fontsize',8 )          %--
[newsignal,ttl]=spkshow(zeropointm,sig,x_low,x_up,y_low,y_up,name,trial);
```

```
function[newsignal,ttl]=spkshow(zeropointm,sig,x_low,x_up,y_low,y_up,name,trial)

t=1/40:1/40:(length(sig))/40;
sp_number=0;
hold on;
single_win=axes('position',[0.7 0.5 0.23 0.4]);
spike_win=axes('position',[0.1 0.09 0.83 0.12]);
data_win=axes('position',[0.7 0.26 0.23 0.15]);
set(gca,'xtick',[]);
set(gca,'ytick',[]);
set(gca,'box','on');


newsignal=zeros(length(sig),1);
ttl=zeros(length(sig),1);
for n=1:length(zeropointm(:,8))
    if zeropointm(n,8) >= x_low & ...
    zeropointm(n,8) <= x_up & ...
    zeropointm(n,4) <= y_up & ...
    zeropointm(n,4) >= y_low
    ttl(zeropointm(n,1)+zeropointm(n,5)-1)=5;
    sp_number=sp_number+1;

newsignal(zeropointm(n,1):zeropointm(n+1,2))=sig(zeropointm(n,1):zeropointm...
(n+1,2));

    if sp_number == 1
      set(gcf,'currentaxes',single_win)
      plot(t(zeropointm(n,1)-15:zeropointm(n+1,2)+6),newsignal(zeropointm...
(n,1)-15:zeropointm(n+1,2)+6));
      hold on
      y_height=0:zeropointm(n,4);
      x_height=t(zeropointm(n,1)+zeropointm(n,5)-1)*ones(1,length(y_height));
      plot(x_height,y_height,'k')

      grid on;
      x_width=t(round(zeropointm(n,6))):.01:t(round(zeropointm(n,7)));
      y_width=(zeropointm(n,4)/2)*ones(1,length(x_width));
      plot(x_width,y_width,'k')
      text((zeropointm(n,1)-14)/40,ceil(zeropointm(n,4)-10),(['H= 'num2str...
(zeropointm(n,4)),' mV']),'fontsize',8);
      text((zeropointm(n,1)-14)/40,ceil(zeropointm(n,4)-30),(['W= 'num2str...
(zeropointm(n,8)/40),' ms']),'fontsize',8);

      plot(t(zeropointm(n,1)-15:zeropointm(n+1,2)+6),y_up*ones(1,length(t...
(zeropointm(n,1)-15:zeropointm(n+1,2)+6))),'r');
      plot(t(zeropointm(n,1)-15:zeropointm(n+1,2)+6),y_low*ones(1,length(t...
(zeropointm(n,1)-15:zeropointm(n+1,2)+6))),'r');
```

```
      axis ([t(zeropointm(n,1)-15) t(zeropointm(n+1,2)+6) min(newsignal
(zeropointm…(n,1)-15:zeropointm(n,1)))-3 y_up+5]);

      xlabel('Time (ms)');
      ylabel('Amplitude (mv)');
    end
  end
end
set(gcf,'currentaxes',data_win)
length(zeropointm)
text(0.025,.7,['Total pulses 'num2str(length(zeropointm(:,1))/2),'
pulses']),'fontsize',8);
text(0.025,.3,['Total spikes 'num2str(sp_number),' spikes']),'fontsize',8);
set(gcf,'currentaxes',spike_win)
plot(t,newsignal);
title(['Noise Reduced Signal ',name,' Trial ',num2str(trial)])
xlabel('Time (ms)');
ylabel('Amplitude (mv)');
axis tight;
figure
t=1/40:1/40:(length(newsignal))/40;
bar(t,ttl,'k');
axis([0 length(t)/40 0 10]);
title(['TTL ',name,' Trial ',num2str(trial)])
xlabel('Time (ms)');
ylabel('Amplitude (mv)');
axis tight;
```

```
function[y_low,y_up]=regioncheck(height,ylimit,level)
le=length(height);
level=level-1;
height=sort(height);
%%%lower
for n=1:0.1:ylimit

  for m=length(height):-1:1
    if height(m) >= ylimit-n & height(m) <= ylimit-n+1
      y_low=ylimit-n;
    end

    yy_low = 0;
    if height(m) >= ylimit-n-level & height(m) <= ylimit-n
      yy_low=1;
      break;
    end
  end

  if yy_low == 0
    break;
  end
end

%%%upper
for n=1:0.1:ylimit

  for m=length(height):-1:1
    if height(m) >= ylimit+n-1 & height(m) <= ylimit+n
      y_up=ylimit+n;
    end

    yy_up = 0;
    if height(m) >= ylimit+n & height(m) <= ylimit+n+level
      yy_up=1;
      break;
    end
  end

  if yy_up == 0
    break;
  end
end
```

```
function [xi] = interpol(x1,y1,x2,y2,yi)
%interpolate xi
%input x1 y1 x2 y2 yi

if y2-y1==0
  xi=((x2-x1)/2)+x1;
else
  xi=((yi-y1)*(x2-x1)/(y2-y1))+x1;
end
```

```
function[powerdata] = spikepower(zeropointm,sig,y_up,y_low)

%,x_up,x_low)
%
%input=spikepower(zeropointm,real signal,y threshold)
%output=power in each spike

%%%%%%%%%%
%POWER
%%%%%%%%%%
nn=1;
for n=1:2:length(zeropointm)
  if (zeropointm(n,4)>=y_low) & (zeropointm(n,4)<= y_up)
    powerdata(nn,1)=interpol(zeropointm(n,1),sig(zeropointm(n,1)),zeropointm...
(n,2),sig(zeropointm(n,2)),0);
    powerdata(nn,2)=interpol(zeropointm((n+1),1),sig(zeropointm((n+1),1)),...
zeropointm((n+1),2),sig(zeropointm((n+1),2)),0);
    b=powerdata(nn,1);
    c=powerdata(nn,2);

    powerdata(nn,3)=trapz(sig(zeropointm(n,2):zeropointm((n+1),1)))+...
                    (0.5*(zeropointm(n,2)-b)*sig(zeropointm(n,2)))+...
                    (0.5*(c-zeropointm(n+1,1))*sig(zeropointm(n+1,1)));
    nn=nn+1;

  end
end
```

BIOGRAPHY

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