PERIPHERAL BLOOD PERFUSION

INDUCED BY ELECTRICAL STIMULATION OF

THE ANTERIOR CINGULATE CORTEX

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by

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ABSTRACT

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In the last decade, much research has implicated the anterior cingulate cortex (ACC) in the modulation of both emotion and visceral functions in primates and rats. Rat studies have shown that electrical stimulation of the ACC suppresses animal's behaviors associated with noxious stimuli, and inhibits spinal cord dorsal horn neuron activity induced by nociception in the periphery. Electrical stimulation of the periaqueductal grey (PAG) is reported to inhibit spinal cord dorsal horn neuron activity induced by nociception, while it generates dorsal root reflex (DRR). This is an important contributor to peripheral vasodilatation, i.e. leading to increased blood flow. The purpose of the present study is to investigate whether electrical stimulation of the ACC will evoke DRR, which would provide some evidence of communication between the ACC and the PAG involved in inhibition of nociception. Preliminary experiments

indicated no change of DRR after electrical stimulation of the ACC. However, a bi-phase change of cutaneous blood perfusion was associated with the stimulation. During the 60 second stimulation and right after the stimulation (around 100 seconds), there was a decrease in blood perfusion, followed by a long-term increase which could last up to 2 hours. The current experiment seeks to validate the possible contribution of lateral hypothalamus (LH) to the bi-phase change of blood perfusion induced by electrical stimulation of the ACC. The influence of peripheral sympathetic fibers might be involved, because 1) in skin, sympathetic fibers are the major factor on vasoconstriction, i.e. leading to decreased blood flow; 2) in intact animals, sympathetic fibers help maintain the volume of blood vessels by means of conducting spontaneous action potentials. In the rat brain, much of the literature suggests that the LH is a core region involved in regulating the autonomic nervous system, including the sympathetic branch. Therefore, the hypothesis is that the change of cutaneous blood perfusion induced by electrical stimulation of the ACC is attenuated or diminished in unilateral-LH-lesion rats. Result of the experiment fails to detect significant difference in the bi-phase peripheral blood perfusion between intact and lesion groups. This suggests that 1) unilateral LH lesion might not have sufficient influence on the change of blood perfusion induced by the ACC stimulation; 2) electrical stimulation of the ACC might evoke distinct neural pathway from the PAG stimulation; 3) direct projection from the ACC to the rostral ventrolateral medulla (rVLM), and peripheral interaction between primary afferent fibers and sympathetic fibers might account for the bi-phase change of blood perfusion induced by electrical stimulation of the ACC.

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LIST OF ABBREVIATIONS

ACC: anterior cingulate cortex

ACd: dorsal anterior cingulate cortex

ACh: acetylcholine

ACv: ventral anterior cingulate cortex

AI: anterior insular

BK: bradykinin

BP: blood perfusion

Cg1: cingulate area 1

Cg2: cingulate area 2

Cg3: cingulate area 3 (also called PrL or PL)

CGRP: calcitonin-gene related peptide

DLPAG: dorsal lateral periaqueductal gray

DMPAG: dorsal medial periaqueductal gray

DRR: dorsal root reflex

ECG: electrocardiograph

Fr2: frontal area 2

GABA: gamma-aminobutyric acid

IL: infralimbic cortex

IML: intermedial lateral column

LH: lateral hypothalamus

LPAG: lateral periaqueductal gray

mPFC: medial prefrontal cortex

MAP: mean arterial pressure

MD: medial dorsal thalamus

MO: medial orbital cortex

MPO: medial preoptic nucleus

NE: norepinephrine

NK1: neurokinin type 1 receptor

NMDA: N-methyl-D-aspartate

NTS: nucleus tractus solitarii (also called nucleus of the solitary tract)

PAG: periaqueductal gray

PrL: prelimbic cortex (also called Cg3 or PL)

rVLM: rostral ventrolateral medulla

SP: substance P

Stim: stimulation

VLPAG: ventrolateral periaqueductal gray

VO: ventral orbital

CHAPTER 1

INTRODUCTION

1.1 Functional and Anatomical Features

The anterior cingulate cortex (ACC), the periaqueductal gray (PAG), and the hypothalamus all play important roles in controlling autonomic functions. Electrical or chemical stimulation in the anterior tip of the cingulate cortex, also called the infralimbic area, causes the changes in blood pressure (Kandel, Schwartz, & Jessell, 2000). Functionally, the PAG is highly related with the arterial pressure, regional vasoconstrictor tone etc (Beitz, 1994). Recent researches disclosed strong relations between lateral hypothalamus (LH) activity and blood pressure. Abrao reported that LH played an important role on balancing inhibition and releasing nitric oxide into medial preoptic nucleus (MPO), in which the inhibition of synthesizing nitric oxide by microinjecting of N(W)-nitro-L-arginine methyl ester (L-NAME) produced increase of mean arterial pressure (Abrao et al., 2004). Pajolla found that microinjections of L-glutamate into LH caused dose-dependent hypotension (Pajolla, Pelosi, & Correa, 2005). Evidence from Montanaro indicated a discrete set of hypothalamic pathways that might underlie the involvement of leptin in obesity-induced hypertension (Montanaro, Allen, & Oldfield, 2005). Detailed anatomical introductions for the three brain areas will be expanded in the following paragraphs.

1.1.1 Structure of the ACC

The ACC is part of prefrontal cortex, locating medially in each of hemispheric brain. In detail, it occupies a cubic area roughly from Bregma 4.00mm to -1.40mm in length, with 1 to 3mm in depth, and 1mm towards the lateral, i.e. 2mm in width for both sides. Figure 1 shows the 3 dimension shape of a typical rat brain in three perspectives. Figure 2 illustrates a stereotaxic drawing of the rat brain at a coordinate of Bregma -0.26mm (Paxinos, 1998), in which the upper left inset is sagittal view indicating the position of coronal plane of stereotaxic drawing. The region highlighted in Figure 2 by means of rectangle is enlarged in Figure 3, where the ACC locates within the red line, noted as Cg1 and Cg2, representing cingulate area 1 and cingulate area 2 respectively. Note that only one side of ACC is marked by red line, and the other distributes symmetrically to the sagittal plane.

The afferent and efferent connections between the ACC and hypothalamus are summarized in the Table 1. The dense reciprocal projections between ACC and lateral hypothalamus were identified by many studies. The perifornical neurons in tuberal lateral hypothalamic (LHAt) region, around the mammillothalamic tract, innervated the medial frontopolar, prelimbic, infralimbic, and anterior cingulate cortex (Saper, 1985). By injections of the retrograde pathway tracer, wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP), into various of areas of brain, midbrain, and spinal cord, approximately 27.4% layer 5 pyramidal cells in infralimbic/prelimbic/dorsal anterior cingulate (IL/PL/ACd) were found to project to LH (Gabbott, Warner, Jays, Salway, & Busby, 2005).

1.1.2 Structure of the PAG

The PAG is in the midbrain, one of the subdivisions of the brain stem. It is also called midbrain central gray (Beitz, 1994). Figure 4 is a coronal drawing at Bregma -7.80mm, indicating a 7.80mm from Bregma point caudally. Figure 5 is the highlighted area selected from Figure 4, in which it shows a clear view of PAG with regards of several sub nuclei, such as dorsomedial (DMPAG), dorsolateral (DLPAG), lateral (LPAG), and ventrolateral (VLPAG) (Paxinos, 1998). In three dimensions, PAG ranges from Bregma -5mm to -8.5mm in length, 2mm both in width and in height, surrounding the central aqueduct, the black area noted by Aq in the center of Figure 4 and Figure 5.

In addition, much of the literature indicates the reciprocal connections between PAG and some particular nuclei in hypothalamus, summarized in Table 1. Early retrograde tracing study by using horseradish peroxidase injected in PAG reported the connections from the MPO, ACC, and lateral hypothalamus to PAG (Marchand & Hagino, 1983). Injections of WGA-HRP and Phaseolus vulgaris leucoagglutinin (PHA-L) in PAG produced both strong anterograde and retrograde labeling in MPO (Rizvi, Ennis, & Shipley, 1992), indicating a reciprocal connections between PAG and MPO. Furthermore, Mihaly *et al.* used PHA-L as a tracer injected in ventrolateral PAG to investigate the tract involved prothyrothropin-releasing hormonal (proTRH) neurons, and found scattered double-labeled fibers in LH (Mihaly, Legradi, Fekete, & Lechan, 2001). There is also evidence that PHA-L injected in MPO showed extensive projections to PAG and LH (Simerly & Swanson, 1988).

1.1.3 Structure of the hypothalamus

In general, the hypothalamus is the ventral part of brain area, bordered caudally with PAG. Figure 6 and 7 illustrate a horizontal and a sagittal view of the rat brain 0.40mm laterally, respectively (Paxinos, 1998). There are many hypothalamic compartments, each of which has a specific projection to a particular brain area almost involved the whole brain area, and of course plays a certain role of brain function (Simerly, 1994). The medial preoptic nucleus (MPO or called MPN) and lateral hypothalamus (LH) are concentrated in the autonomic function. The efferent and afferent pathways between the MPO and PAG, and pathways between the ACC and LH have been recognized as the major structures involved in physiological mechanism of the autonomic regulation, such as blood pressure, and sexual behavior (Gabbott et al., 2005; Saper, 1985; Rizvi et al., 1992; Mihaly et al., 2001; Marchand et al., 1983; Simerly et al., 1988). The MPO and LH are also shown in Figure 6 in horizontal plane, where the black rectangle bounds the hypothalamus and MPO was pointed rostrally and LH places laterally (Paxinos, 1998).

Another point worth mentioning is that although there has no firm evidence to support a strong and direct connection between PAG and LH, whereas PAG has dense input from and output to MPO, the heavy projection from MPO to LH has been recognized (Simerly et al., 1988; Gritti, Mainville, & Jones, 1994).

1.1.4 Topography of hypothalamus with connections with ACC and PAG

As shown in Figure 8, LH has strong and direct reciprocal projections with ACC, and dense indirectly connections with PAG, according to the previous discussions.

Current studies indicated that hypothalamus serves integrative function of regulating basic physiological needs, including blood pressure and blood flow to muscle and other tissues (Kandel et al., 2000). The nucleus of the solitary tract not only projects to neurons forming brain stem and spinal circuits that control simple autonomic responses, but also integrate autonomic functions, and then descend to autonomic terminals, such as organs (Kandel et al., 2000). Recent literatures indicate that LH plays a key role in blood pressure modulation as a central structure to affect autonomic nervous system. In Abrao's study, the significant increase of mean arterial pressure (MAP) occurred in the group of rats with N w-nitro-L-arginine methyl ester (L-NAME) treatment, a nitric oxide synthase inhibitor (NOSI), whereas the LH-lesioned groups had little increase of MAP and no significant difference with control group and group with FK 409 treatment, which is a nitric oxide donor (Abrao et al., 2004). It is reported that microinjections of L-glutamate (L-glu) in LH of unanesthetized rats caused a hypotensive response, and the pretreatment with NMDA receptor antagonist AP-7 and LY235959 significantly reduced the hypotensive, whereas the pretreatment with AMPA-receptor antagonist NBQX and saline did not affect the hypotensive response (Pajolla et al., 2005). In addition, microinjection of leptin, a peptide hormone, into the LH increased lumbar sympathetic nerve activity (Montanaro et al., 2005). Basically, leptin has been shown to influence nitric oxide production and natriuresis, and along

with chronic sympathetic activation, which might lead to systemic vasoconstriction, and blood pressure elevation (Bravo, Morse, Borne, Aguilar, & Reisin, 2006).

1.2 Neurogenic Inflammation

Inflammation is characterized by redness and warmth (secondary to vasodilatation), swelling (secondary to plasma extravasation), and hypersensitivity (secondary to alterations in the excitability of certain sensory neurons) (Richardson & Vasko, 2002). Inflammation triggered by substances released from primary afferent fiber terminals is referred as "neurogenic inflammation" (Willis, Jr., 1999). Antidromic action potential propagations via primary afferent fibers contributing to neurogenic inflammation in skin, including vasodilatation, plasma extravasation, and hyperalgesia, tend to be by means of axonal reflexes (Lewis, 1927), and dorsal root reflexes (Lin, Wu, & Willis, 1999; Rees, Sluka, Westlund, & Willis, 1995; Garcia-Nicas, Laird, & Cervero, 2001; Szolcsanyi, 1988; Weng & Dougherty, 2005). Release of Substance P (SP) and calcitonin-gene related peptide (CGRP) from primary afferent fiber endings are shown to be heavily involved in edema formation, protein plasma extravasation and vasodilatation. More specifically, SP causes plasma extravasation (Chahl, 1979; Lembeck & Holzer, 1979; Foreman, Jordan, Oehme, & Renner, 1983; Saria, Lundberg, Skofitsch, & Lembeck, 1983; Fuller, Conradson, Dixon, Crossman, & Barnes, 1987; Wallengren & Hakanson, 1987; Jacques, Couture, Drapeau, & Regoli, 1989) by binding on NK1 receptors (Iwamoto & Nadel, 1989). CGRP produces vasodilatation via multiple mechanisms (Bell & McDermott, 1994; Brain & Cambridge, 1996; Marshall,

1992; Brain & Grant, 2004). However, due to the interactions between SP and CGRP, to some extent, both of the two neurotransmitters are making contributions to plasma extravasation and vasodilatation (Gamse & Saria, 1985; Escott & Brain, 1993; Campos & Calixto, 2000).

1.3 Three hypotheses on BP change induced by ACC stimulation

The blood perfusion in the hind paws is the balance between vessel dilation and constriction. Generally, vasodilatation can cause the increase in blood flow whereas vasoconstriction leads to a decrease in blood flow. First, in sympathetic nervous system, efferent mediation originating from brain area arrive at the intermedial lateral column (IML) in spinal cord through the solitary tract, which causes pregangalionic neurons releasing acetylcholine (ACh) to evoke postganglionic fibers secreting norepinephrine in peripheral which activate adrenoreceptor on smooth muscles and cause the blood vessel constriction (Goldstein, 2006). Second, the ACC stimulation can cause the inhibition of dorsal horn neuron activity (Senapati, Lagraize, Huntington, Wilson, Fuchs, & Peng, 2005), likely through activation of PAG, since stimulation of both of them reduced the responses to nociceptive stimuli (pressure and pinch applied on the paws). In descending sensory pathway, activation of the PAG induces release of GABA which has been shown to be involved in generation of dorsal root reflex (DRR) (Peng, Wu, Willis, & Kenshalo, 2001). DRRs in unmyelinated and thinly myelinated primary afferent fibers may contribute to release SP and CGRP, resulting in plasma extravasation, and vasodilatation, as mentioned earlier. The above two phenomena are

short term regulations of blood perfusion, compared with the hormone effect illustrated in Figure 9. The third potential mechanism is that activation of hypothalamus that controls the pituitary gland leads to secreting vasopressin to cause vasoconstriction (Kandel et al., 2000), while the sympathetic nervous system also evoke adrenomedulla to induce the high levels of adrenaline in the blood stream associated with the constriction of skin blood vessels (Goldstein, 2006).

My ***central hypothesis*** is that the combination of three possible influences contributes to the blood perfusion, as illustrated in Figure 9, which speculates three mechanisms that impact blood perfusion in peripheral sites, such as hind paws. In details, the stimulation in ACC might activate LH and send signals down to spinal cord. The activity in LH can lead pituitary gland releasing peptides, for example vasopressin, into blood circulation system to regulate autonomic function throughout the body, which is noted as dashed blue arrow in Figure 9. This influence caused by hormone is long term, which means a long duration of effect and a long time to response the hormone after it is generated. The sympathetic system can be activated by LH. The postganglionic neurons project to local targets in periphery, such as the skin, as noted by blue arrows, indicating a short term impact. Finally, ACC stimulation can indirectly cause DRRs via descending pathway, and increase the release of substance P and CGPR in peripheral position that causes increase of blood perfusion. It is also a short term influence, due to the direct electrophysiological influence in periphery by means of neurons, rather than hormones via circulation.

To validate my hypothesis, the following specific aims are proposed.

Specific Aim 1: Determine the effect of electrical stimulation of ACC on change of blood perfusion in the paws.

Specific Aim 2: Determine the effect of electrical stimulation of ACC on elicitation of DRRs.

Specific Aim 3: Determine the effect of LH lesion on ACC evoked change of peripheral blood perfusion.

CHAPTER 2

METHODS

2.1 Animal Preparation

Thirteen male Sprague-Dawley rats at age around three months old were used in the experiments. All surgical procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee. The procedures were in accordance with the guidelines published by the Committee for Research and Ethical Issues of the International Association for Study of Pain (Zimmermann, 1983). Initially, all of them were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) for surgery and a catheter was inserted into the jugular vein for continuous anesthesia by administration of sodium pentobarbital (5 mg/kg, i.v.) at a rate of 0.02 ml/min throughout three-hour-long experiment. A cannula was placed in the trachea following tracheotomy for artificial respiration in case needed. The lumbasacral part of spinal cord was exposed by laminectomy of 4 cm in length and immobilized in a stereotaxic frame. Mineral oil is used to cover the spinal cord to keep the moisture and isolation. The body temperature was maintained at 37 degree centigrade by a feedback controlled heating blanket.

Craniotomies were performed in each rat to make two holes. Two electrodes were positioned in unilateral ACC and LH, respectively. The coordinates of ACC are anterior Bregma 1.6 – 2.5 mm with 0.5 mm left lateral and 2.5 - 3.0 mm in depth; the

coordinates of LH are 1.4 mm posterior to Bregma, 1.8 - 2.0 mm left lateral, and 8.0 mm deep.

Two bended syringe needles were punctured through the skin on two sides of underarm, respectively, both pinpoints of which were contacted with sampling device, CED 1401 Plus (Cambridge Electronic Designed Limited, UK), for electrocardiogram (ECG) recording.

2.2 Dorsal Root Reflex Recording

A tiny filament of the L4 or L5 dorsal roots was teased off proximally from the main trunk, and the side connecting with spinal cord was wrapped around a silver hook electrode for recording.

The first ACC stimulation was delivered at 10 v, 0.1 ms impulse duration, 60 s stimulating duration (in control group, the frequency was 100 Hz in continuous mode; in lesion group, the frequency is 300 Hz in train mode), after the baseline activity was recorded for 5 - 10 min. Then responses to intensity-coded mechanical stimulation (brush, pressure, and pinch), dorsal root reflexes, and electrocardiogram were simultaneously collected by means of a computer software program SPIKE2 and a multi-channel data acquisition system, CED 1401 Plus (Cambridge Electronic Designed Limited, UK). One hour later, in a group of thirteen rats (named as lesion group), a lesion discrete current (2.0 mA; 10 s duration) was introduced into LH; whereas the rest eleven rats (as control group) did not receive anything to LH,. Another one hour sampling later, the second ACC electrical stimulation (10 v, 0.1 ms impulse duration,

60 s stimulation duration; in control group, the frequency was 100 Hz in continuous mode, in lesion group, the frequency is 300 Hz in train mode) was applied again. The recording continued for one more hour. In total, there were more than three hours of electrophysiological recording for each rat.

2.3 Recording for Blood Perfusion

In blood perfusion recordings, a laser Doppler imager (product of Perimed AB, Sweden) was used to measure blood blow in two hind paws, and with the help of software (LPIwin), successive three-hour-long scanning results were stored in computer for further analysis. Laser Doppler imager is able to detect cutaneous blood perfusion in a two dimension flat area. During recording, the computer is monitoring the measure of blood perfusion in target area in terms of colors. The colorized images could be converted into numeric data for statistic analysis.

2.4 Histology

In order to verify the stimulation and lesion regions and inspect the correlation between second ACC stimulation effect and lesion information, such as size, position, the brains of rats were removed for histology. Each animal was sacrificed by intravascular injection of overdose sodium pentobarbital. The brains were removed and immersed in 10% formaldehyde solution. The coronal slices (80 or 120 μm thick) of brains were stained with thionin twenty four hours after the tissues were mounted on slides.

2.5 Data Analyses

Both electrophysiological and image data were analyzed off-line. Statistical significance was testified by student t test or ANOVA for difference and Pearson correlation coefficients (r) for trend. All values except r represented were formatted as mean ± SEM (standard error of mean). The analysis was focused on the blood perfusion change and dorsal root reflex when the first and second ACC stimulation were delivered. The significant level adopted is 0.05; P value indicates the possibility of estimate of variable greater that zero.

CHAPTER 3

RESULTS

3.1 Mathematic Tools

Before the presentation of experimental data, some mathematic tools are needed to introduce. Basically, there are three fragments of recording, including the first ACC stimulation, one hour recording/LH lesion, and the second ACC stimulation (in control group, there is a one hour recording between the stimulations; in lesion group, an LH lesion applied between the stimulation). Each fragment took about one hour, including around forty images. Therefore, in each fragment, both macro-information, i.e. the trend of change during one hour, and micro-information, i.e. change between two cohere images, are needed to investigate.

For macro-information, four types of analyses would be applied.

1) The raw data plotting could tell a range of blood perfusion cross time, in which the mean, and standard deviation would express the range of blood perfusion at individual image, for instance, the 1st, 2nd, and so forth, corresponding to a certain time slot. For example, in the first ACC stimulation paradigm in control group, the first data point measured on left paw is 0.1265 ± 0.073 with arbitrary unit during around 100 seconds (which varied from animal to animal, because of the size of paws), indicating that the mean blood perfusion measurement in seven left paws from distinct rats is 0.1265 with standard deviation of 0.073.

2) The percentage change is converted from raw data to minimize the individual differences. Figure 10 illustrates the individual difference. The rationale of this conversion is to normalize each data point from the same subject. For a particular rat, the first five images are treated as the baseline. Each image after those five will be compared with the baseline value (the average of the first five measurements) and resulted in percentage value, indicating the normalized change.

3) The Pearson correlation coefficient (r) could be used to detect the trend of change in terms of blood perfusion cross time, in which one variable could be the blood perfusion measurement, and the other could be the number of image. For example, (1, 0.0296) means that at the first image the measurement is .0296. If the two variables are x and y, the equation of correlation between variables x and y is listed below,

$$r_{xy} = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n\sum x_i^2 - (\sum x_i)^2}\,\sqrt{n\sum y_i^2 - (\sum y_i)^2}}.$$

Under the normal distribution assumption, the Pearson correlation could be able to illustrate the extent of linearity between two variables. In other words, if x is indicating the x-axis, when the absolute value of r is closed to 1, the maxim value, y has very strong linear trend on the coordinates, i.e. either increase or decrease in a straightforward manner.

4) The Pearson correlation coefficient (r) could also tell the correlation between the blood perfusions in left paw and right paw. The calculation equation is the same as described previously. At this time, the two variables are blood perfusion measurements

in left paw and right paw. The greater r denotes the more likelihood of the blood perfusions in two paws going up and down together.

For micro-information, i.e. the change in every single time slot, the slope analysis is going to be employed. Theoretically, the slope is the ratio between the vertical side and horizontal side in a right triangle, indicating the steepness. The greater the slope is, the steeper the change is. The negative value indicates decrease, whereas the positive indicates increase. In Figure 11, the slope of empty dot is the length of vertical line (dash line) in the right triangle divided by the length of the horizontal side (solid line). Because x axis indicates the number of image, and the time interval between two cohere images is consistently identical, only vertical side (dash line) could represent the extent of steepness. Thus, the length of vertical side is treated as slope. In analysis, the slope is normalized into percentage format. In Figure 11, the normalized slope is the dash line in triangle divided by the dash line out of triangle, which means how much increase or decrease, compared with the previous data point, in terms of percentage.

3.2 Histology

3.2.1 The Electrical Stimulation Sites in ACC

There are two groups of rats. In control group, 7 rats were treated with electrical stimulation of ACC, including from 3 rats the sites were in cingulate cortex sub region 2 (Cg2), 1 on the border between prelimbic cortex (PrL) and infra-limbic cortex(IL), 1 in PrL, and 1 on the border between prelimbic cortex (PrL) and cingulate cortex sub

region 1(Cg1). In lesion group, 6 rats were treated with ACC stimulation, in which, more specifically, 3 sites were in Cg1, 1 in Cg2, 1 in PrL, and 1 on the border between Cg1 and PrL, and all of them had unilateral (in left hemisphere) adequate lesion. Every site is also illustrated on Figure 12.

3.2.2. The Lesion sites in LH

The lesion centers (the position of tip of lesion electrode, and also the position of the largest lesion area) were located between Bregma -1.30 to -1.50 mm. The rough estimate of length (number of tissues on slides times thickness of tissues) was between 1.70 mm to .50 mm (summarized in Table 2). Six rats were received accurate ACC stimulation and enough unilateral lesion (shown in Figure 12, and Figure 13).

3.3 Laser Doppler Images Analyses

3.3.1 Schematic illustration of data acquisition

Figure 14 depicts the manipulations between two distinct groups, control and lesion. There are three fragments in each group, including the 1st ACC stimulation, one hour recording (or LH lesion), and the 2nd ACC stimulation. In each fragment, the recording lasted one hour or a little bit more. Because the scanning duration varied from animal to animal, due to the size difference in paws, the last number of image in one hour recording was a variable, shown as N in Figure 14. In order to get an equal sample size in statistical model, the image data involved in analyses were chosen from the 1st to 26th.

Figure 15 represents the real and typical images scanned on two paws from one rat. The color denotes the intensity of speed of blood perfusion, which means the lighter the faster, the darker the slower.

3.3.2 *No difference of blood perfusion between left and right paw from the same rat*

In Figure 16, in A, B, C, D, E, and F, the blood perfusions in left and right paws are highly overlapped with each other in terms of both raw and percentage data, and these relations are robust by statistical tests on percentage data. In control group, the Pearson correlation coefficients are .938, .942, and .929, between two paws, in 1st ACC stimulation, one hour recording, and 2nd ACC stimulation, respectively. The lesion group has the similar features, shown in Figure 17. In other words, the left and right paws are going up and down together. Therefore, in slope analyses, there is no need to separate the left and right paws.

3.3.3 *The short-term and long term changes of blood perfusion induced by the ACC stimulations*

3.3.3.1 The blood perfusion change in control group

In Figure 16 A, B, C show the means of blood perfusion measurements at each of 26 images, which cover around 40 minutes' recording, in 1st ACC stimulation, one hour recording, and 2nd ACC stimulation, respectively. D, E, and F illustrate the percent change of blood perfusion corresponding to the same 26 images shown above in three fragments of experiment. G, H, and I indicate the slope distributions of the 26 images in three distinct manipulations.

It is clear to notice that, in raw data plotting, i.e., A, B, and C, the average blood perfusion level increases from around .1 (the first point in A) to around .5 (the 26th in C). The specific change, such as in a linear manner or step-like manner, and detailed status at a particular position (at a certain time bin) could be detected by means of Pearson correlation coefficients and Students' t test. In the following paragraphs, the correlation coefficients and t test are about to be applied into percent data and slopes.

For the 1st ACC stimulation, in graph D in Figure 16, the Pearson correlation coefficients (r) tell a strong linear increment pattern for both paws (left paw r = .746; right paw r = .700). In graph G, the slope analyses disclose a more detailed manner of prompt declining followed by rising-up. It is because that there is a significant negative value (t(13), one-tailed, p = .003) at the stimulation image (the 6th), indicating the decrease of blood perfusion during electrical stimulation of ACC, and in addition, after stimulation (from the 7th to 26th), 13 out of 20 images' slope are significantly positive (t(13), one-tailed, p = .017, .007, .013, .001, .031, .013, .006, .005, <.001, .03, .039, .034, .002), and no significant negative point exists at all, which in short suggest a short-term decrease blood perfusion during stimulation and a long-term monotonic increase blood perfusion after stimulation.

For the one hour recording after 1st ACC stimulation, in general, the correlation coefficients suggest somewhat positive linear feature, since the r for left paw is .565, and r for right paw is .497. More specifically, 6 of the first 8 images show increase of blood perfusion (t(13), one-tailed, p = .007, .048, <.001, .001, .008, .016), and only 2 of the rest 17 show significant increase (t(13), one-tailed, p = .004, <.001), while no

significant negative slope could be found at .05 level. In sum, there is monotonic increase of blood perfusion in one hour recording section, in which the intense increase occurs in the first one third of recording and the blood perfusion level maintains a smooth and slow increase manner in the last two thirds, i.e. more likely to be in a step-shaped manner, rather that a straight linear manner.

For the 2nd ACC stimulation, there is a significant decrease of blood perfusion during stimulation (t(13), one-tailed, p = .007), and quick increase occurs at the second image after stimulation (the 8th), since 4 of 6 slopes (from the 8th to 13th image) are significantly positive (t(13), one-tailed, p = <.001, .001, .047, .045). Neither of paws' overall correlations is strong (left paw r = .236; right paw r = .337). However, the seven images after stimulation (in 6 of 7 rats from 7th to 13th) express a very robust positive linear trend (left paw r = .652; right paw r = .631). Furthermore, in the rest (7 images), 2 points show decrease of blood perfusion at 18th (t(13), one-tailed, p = .048) and 24th (t(13), one-tailed, p =.042) , whereas 22nd and 23rd show increase (t(13), one-tailed, p = .022, .021). Those indicate a fluctuation pattern of blood perfusion change. Therefore, the second ACC stimulation produces a transient decrease of blood perfusion by the electrical current, and an increase pattern occurs and lasts about 7 images' recording, and then, the blood perfusion goes to a fluctuation status.

To sum up with the three sections' results from control group described above, the electrical stimulations of ACC induce a short-term (only in occurrence of stimulation) decrease of blood perfusion, and a long-term increase, in which the first stimulation produces a monotonic increase lasting about two hours (from the end of the

first stimulation to the start of second), and second stimulation produces a monotonic increase in the first 20 min which is followed by intense fluctuation.

3.3.3.2 The blood perfusion change in lesion group

A long-term increase of blood perfusion is also qualitatively obvious, illustrated in raw data plotting, i.e., A, B, and C in Figure 17, in which the average blood perfusion level increases from around .2 (the first point in A) to around .6 (the 26th in C). The range is heavily overlapped with the range in control group.

For the 1st ACC stimulation, in percent data, illustrated in sub D, a strong linear trend is verified with correlation coefficients in both paws (left paw r = .607; right paw r = .597). In slopes, i.e., in sub G, there are two significant negative slopes (t(11), one-tailed, p = .04, and <.001) during and right after the stimulation (the 6th and 7th), indicating the decrease blood perfusion during and one image after electrical stimulation of ACC. In addition, after stimulation (from 8th to 26th), 13 out of 19 images' slope are significantly positive (t(11), one-tailed, p = .002, .04, .003, .007, .047, .029, .04, .013, .025, .004, .015, .004, .008), and no significant negative slope occurs at all, which in short denotes a short-term decrease blood perfusion during and one image after stimulation and a long-term monotonic increase blood perfusion after stimulation.

For the lesion section, only 3 of all 25 slopes show increase of blood perfusion (t(13), one-tailed, p = .007, .019, .009) at 3rd, 8th, and 17th, and none of the rest show significant difference from zero at .05 level, indicating no consistent change. In sum, the unilateral lesion of LH does not evoke consistent change in terms of blood perfusion in two paws.

For the 2nd ACC stimulation, there is significant decrease of blood perfusion one image after stimulation (t(11), one-tailed, p = .004), and quick increase occurs two images after stimulation, since 4 of 6 slopes starting from the 8th image are significantly positive (t(11), one-tailed, p = <.001, .002, .002, .043). Furthermore, in the rest (13 images, roughly 20 min) of time, 3 points show increase of blood perfusion at 18th (t(11), one-tailed, p = .003), 24th (t(13), one-tailed, p = .045) , and 26th (t(11), one-tailed, p = .002), whereas 23rd show decrease (t(11), one-tailed, p = .049). That indicates a fluctuation pattern in terms of blood perfusion change. Therefore, the second ACC stimulation produces a short-lasting decrease of blood perfusion one image after the electrical current, and an increase pattern occurs and lasts 7 images' recording, and then, the blood perfusion goes to a fluctuation status.

To sum up with the three section results described above, the electrical stimulations of ACC induce a short-term (only in occurrence of stimulation and one image after) decrease of blood perfusion, and a long-term increase, in which the first stimulation produces a monotonic increase, unilateral LH lesion does not have consistent influence on blood perfusion, and second stimulation produces a monotonic increase in the first 20 min followed by intense fluctuation.

3.3.3.3 Comparisons of blood perfusions induced by ACC stimulations between two groups

As mentioned above, in control group, both of the first and second ACC stimulations produce bi-phases influences on blood perfusion, which is the decrease in blood perfusion during stimulation followed by a linear increase lasting 6 images (at

even longer for 1st ACC stimulation) after that. In lesion group, both of the first and second ACC stimulations induce a similar bi-phases change of blood perfusion, which is the decrease of blood perfusion one image after stimulation followed by a linear increase lasting 6 images (even longer for 1st ACC stimulation).

Taking a close look at the analyses between two groups, the percent data of control group differ from lesion group in two ways. First, the overall steepness in 1st ACC stimulation in control group (the mean at 26th is most likely over 100, i.e. one fold) is higher than the overall steepness in lesion group (the mean at 26th is most likely around 60). Second, the decrease of blood perfusion occurs at one image after stimulation in lesion group in both 1st and 2nd ACC stimulation paradigms, whereas the decrease only happens during stimulation in control group in two stimulation paradigms. This might be due to the parameter difference applied to the distinct groups. In control group, the electrical stimulation was in continuous mode with 100 Hz in frequency; in lesion group, the current is in train mode with 300 Hz in frequency, in which there was a two second interval between two-second-duration electrical impulses. It is also worthy to mention that the energies of the electrical stimulation for control group and lesion group were similar.

Despite of the differences between groups, in each group, the bi-phases pattern shows up identically in the first and second stimulation paradigms as described in the summary. In other words, as a conclusion, the unilateral (left hemisphere) LH lesion failed to change either the decrease or linear increase of blood perfusion in bilateral paws.

3.4 Electrophysiological Recording Analyses

3.4.1 No long term linear trend in terms of DRRs

Figure 18 illustrates a typical single neuron recording in terms of DRRs lasting near 3725 second. Each blue bar in the middle graph represents an action potential, which is highlighted on the right bottom corner. The red strip indicates the start point and duration of ACC stimulation current. The upper graph counts the number of action potentials in every second from the middle graph; whereas the bottom graph reckons the number of action potentials in every time slot while one image is scanned. This example (from the first ACC stimulation on rat #102806) shows somewhat increasing trend. However, taking into account multiple neurons' activities, the correlation coefficients in both groups and in all three fragment recordings fail to indicate linear trend, illustrated in Table 3. Therefore, the long term linear trend in blood perfusion induced by ACC stimulations might not have strong linear relation with DRRs.

3.4.2 No short term consistent change of DRRs induced by ACC stimulations

As mentioned earlier, the first ACC stimulation produces short-term decrease in blood perfusion in both control group and lesion group. The slope analyses disclose that this decrease pattern starts with the delivery of stimulation. Figure 19 shows the means and their standard deviations in terms of action potential frequencies in 60-second before stimulation, during the 60-second stimulation, and 60-second after stimulation, respectively, which are .132±.041, .141±.030, .140±.027 (impulse/second). ANOVA (group as a between subject factor, either control or lesion; time as a within subject

factor with three levels, including 60-second before stimulation, within stimulation, and 60-second after stimulation) tests fail to verify any significant difference, either between control and lesion group (df = 1, 38; p = .225; NS), or between the three 60-second measures (df = 2, 76; p =.936; NS), or in interaction between group and time (df = 2, 76; p =.554; NS), shown in Figure 19.

The decrease in blood perfusion induced by the 2nd ACC stimulation slightly differs in start time between control group and lesion group. In control group, the decreasing occurs at the image with stimulation (6^{th}); whereas, in lesion group, this phenomenon postpones to one image after stimulation (7^{th}). Therefore, there are two distinct ANOVAs. No significant difference can be detect at .05 level between 60-second before stimulation, within 60-second stimulation, and 60-second after stimulation in control group (df = 2, 42; p = .521; NS), shown in Figure 20.

In another test for lesion group, rather than three comparable levels in terms of the mean of action potential frequency, there are four, including 60-second before stimulation, within 60-second stimulation, 60-second after stimulation, 60-120 second after stimulation. Because each image takes 100.4±1.9 second (i.e., the 7th image roughly starts at the 40^{th} second after the one-minute-long stimulation), the 60-120 second time slot is completely nested within 7th image (roughly 40-140 second after stimulation). Similar with the other tests for either the first ACC stimulation, or the second stimulation, no significant difference could be found at .05 level between any two pairs from the four time slots (df = 3, 48; p = .192; NS), shown in Figure 21.

To summarize, in a macro-view, there is no consistent change pattern in terms of DRRs, while blood perfusion has linear trend associated with ACC stimulation; in a micro-view, there is no significant change in DRRs between 60-second before stimulation, within stimulation, and 60-second (or 120-second for the 2nd ACC stimulation in lesion group) after stimulation, while blood perfusion has robust reduction during ACC stimulation (except for the 2nd ACC stimulation in which the reduction is postpone to one image after stimulation). Therefore, it is safe to draw the conclusion that DRR might have no relation with blood perfusion in hind paws in either long-term or short-term perspective.

3.5 Electrocardiograph (ECG) Analysis

ECG could be a good indicator to sympathetic activity. The change in ECG is heavily associated with activation of sympathetic system. 5 of 7 rats in control group were enrolled with ECG recording. Qualitatively, Figure 22 reveals the ECG in instant frequency with the help of Spike II. The instant frequency is the reciprocal of the length in time between two adhering heartbeats, which is the most accurate estimate of heartbeat. In some cases, during stimulation (limited in red squares), there are apparent changes in instant frequency, i.e. measures of heartbeat, such as in the first two rows (i.e. two rats in two stimulation paradigms) and in the 2nd ACC stimulation for the fourth row. In other cases, visually, no change could be observed. Thus, the correlations between heartbeat, DRRs, and blood perfusion in hind paws are very vague. No further

quantitative investigation has been made with the data and no further ECG recording has been applied on animals.

CHAPTER 4

DISCUSSIONS AND CONCLUSIONS

4.1 Discussions

This study is to investigate the influence on peripheral blood flow, especially in hind paws, by electrical stimulation of anterior cingulate cortex (ACC) on intact and anesthetized rats. The original hypothesis is that lateral hypothalamus (LH) and primary afferent fibers play important and separate roles on regulation of cutaneous vascular bed. However, according to the result, the unilateral lesion of LH did not make significant change of hind paw blood perfusion induced by electrical stimulation of ACC, compared with the non-lesioned rats, i.e. rats from control group. Furthermore, no significant change could be detected in terms of dorsal root reflexes (DRRs) either in a long-term perspective (linear trend is not robust in 40-minute recording), or in a short-term perspective (no significant change between 60-second before, during, and 60-second after electrical stimulation). The study shows a bi-phase blood perfusion change induced by ACC stimulation (both the first stimulation and second stimulation). The decrease in blood perfusion occurs either during the delivery of stimulation or short time after that (100 seconds, equivalent to one image scanned), followed by a long-term increase pattern in blood perfusion. The first ACC stimulation could hold the pattern up to two hours; whereas the second stimulation could lead the increase up to 700 seconds. The different effects might be due to ceiling effect. Therefore, the conclusion could be

drawn that LH might not play a critical role of regulating cutaneous vascular bed on intact animal under deep anesthesia, and the change of blood flow on hind paws induced by electrical stimulation of ACC should have nothing to do with DRRs.

The results suggest that the unilateral LH lesion might have no influence on the change of blood perfusion in hind paws induced by ACC stimulation. Literature research results in another potential mechanism, which is that in supraspinal structure, the sub nuclei in anterior cingulate cortex have multiple pathways to brainstem; at spinal cord and brainstem level, the activation of brainstem structures, such as the rostral ventrolateral medulla (rVLM) innervates the pre-ganglionic sympathetic fibers, which evoke the post-ganglionic sympathetic fibers spreading to periphery; at peripheral level, the interaction between peripheral terminals of primary afferent sensory fibers and sympathetic fibers produces the bi-phase change in blood perfusion. In the following text, detailed interpretation will be presented.

The prefrontal cortex has been studied as an important brain area with both cognitive and emotion functions, especially in primates, e.g. human, macaques (Barbas, Henion, & Dermon, 1991; Barbas, Ghashghaei, & Xiao, 2002; Antonio R.Damasio, 1994; Fuster, 1997; Miller & Wallis, 2002). Distinct sub nuclei are believed to be differentiated due to evolution in to order to implement complex cerebral functions (Preuss & Kass, 1999). The conventional criterion of differentiation and specialization was the thalamocortical connection particularly to medial dorsal (MD) nucleus of thalamus (Rose & Woolsey, 1948), which has been intensively criticized by the abundant evidences that other thalamic nuclei are also connected to prefrontal cortex,

and in addition, MD projects to other cortical area besides prefrontal cortex (Berendse & Groenewegen, 1991; Groenewegen, Wright, & Uylings, 1997; Groenewegen, 2004; Shibata, 1992). Based on the decades' researches, prefrontal cortex is considered to involve frontal area 2 (Fr2), dorsal anterior cingulate (ACd, also called Cg1), ventral anterior cingulate (ACv, also called Cg2), prelimbic (PL, also called PrL, or Cg3), infralimbic (IL), medial orbital (MO), ventral orbital (VO), and anterior insular (AI), some of which, however, are on debate to be accepted in prefrontal cortex (Paxinos, 1998), shown in Figure 23. The novel and acceptable differentiation criteria take into accounts both functional differences as well as anatomical features (Uylings, Groenewegen, & Kolb, 2003). According to that, dorsal medial prefrontal cortex (mPFC) including ACd and dorsal PL process cognitive information; whereas ventral mPFC including ventral PL, IL modulate autonomic functions, especially, cardiovascular and respiratory activities (Hardy & Mack, 1990; Neafsey, 1990; Loewy, 1991; Neafsey, Terreberry, Hurley, Ruit, & Frysztak, 1993; Spyer, 1994; Fisk & Wyss, 2000; Heidbreder & Groenewegen, 2003). The stimulation sites involved in my study are mainly located around the border of ACd and PL, or in ACv.

The stimulation in either ACv, ACd, or PL might have the similar influence due to the interconnections among those three areas (Beckstead, 1979; Vogt & Peters, 1981). The stimulation of the ventral PL and IL is known to influence arterial blood pressure and blood flow (Owens & Verberne, 1996), suggesting the interaction between vPL and IL. In addition, descending projections from IL innervate catecholaminergic neurons in rVLM and NTS (Gabbott, Warner, & Busby, 2007; Zagon, Totterdell, &

Jones, 1994). Therefore, there could be one possible descending pathway modulating autonomic function without relay on LH, stemming from cortex, especially prefrontal cortex. Actually, there could be multiple ways with direct or indirect connection from prefrontal to brainstem, for example amygdaloid complex is proved to be a critical relay between prefrontal cortex and brainstem (Uylings et al., 2003; Salome, Viltart, Leman, & Sequeira, 2001).

In brainstem, there might be direct and indirect connections from NTS to rVLM (Dampney et al., 2002; Guyenet, 2006). The rostral C1 neurons, majority of rVLM cells, are proved to project directly to sympathetic pre-ganglionic neurons in the intermediolateral cell column (IML) of the thoracic spinal cord (Guyenet, Haselton, & Sun, 1989; Reis, Morrison, & Ruggiero, 1988; Schreihofer & Guyenet, 1997), and post-synaptic transmission to pre-ganglionic neurons is mediated with NMDA type glutamate receptors (Aicher, Hahn, & Milner, 2000). The post-ganglionic activity could be enhanced by glutamate receptor located in the L4 and L5 sympathetic gray rami (Coggeshall & Carlton, 1999).

In periphery, due to the activation of postganglionic fibers, the peripheral endings might release norepinephrine (NE) that regulates vascular smooth muscle which might cause vasoconstriction in cutaneous bed (Shepherd & Vanhoutte, 1985). Recent literatures show the direct contribution to vasoconstriction of NE both in human (Ali, Raja, Wesselmann, Fuchs, Meyer, & Campbell, 2000) and rats (Kakizoe, Kobayashi, Shimoura, Hattori, & Jidoi, 1992). That might be the reason of short-term decrease in blood perfusion induced by the ACC stimulation. Meanwhile, the NE could

also bind on alpha-2-adrenoreceptors on primary afferent axons which induces nociception (Abdulla & Smith, 1997; Chen, Michaelis, Janig, & Devor, 1996; Honma, Yamakage, & Ninomiya, 1999; Leem, Gwak, Nam, & Paik, 1997; O'Halloran & Perl, 1997; Sato & Perl, 1991; Sato, Suzuki, Iseki, & Kumazawa, 1993). The activation of peripheral nociceptors via axonal reflexes induced by antidromic electrical stimulation of nociceptive fibers could produce increase of blood perfusion in peripheral (Lisney & Bharali, 1989; Kauppila, Kontinen, Wei, Jyvasjarvi, & Pertovaara, 2002), which is proved to be mediated by the calcitonin gene-related peptide (CGRP) (Sato, Sato, & Uchida, 1994). However, the peripheral mechanisms might not be that easy as described before. There are supposed to be multiple mechanisms involved in the long-term increase in blood perfusion induce by the ACC stimulation. It has been established that SP exhibits higher affinity for the NK1 receptor (Maggi, 1995; Regoli, Boudon, & Fauchere, 1994; Mussap, Geraghty, & Burcher, 1993). NK1 receptors are shown to be expressed on the unmyelinated axons in glabrous skin of the rat hindpaws (Carlton, Zhou, & Coggeshall, 1998), which indirectly provides an evidence of a positive feedback loop to augment the release of SP. That might lead to vasodilatation, i.e. an increase in BP. Furthermore, bradykinin (BK) has been demonstrated to have crosstalk with primary sensorial neurons in terms of release of SP and CGRP (Holzer, 1998; Hall & Morton, 1997; Geppetti, 1993; Campos & Calixto, 1995). Besides, BK has direct influence on vasodilatation, because that its receptors, B1 and B2, have been found in both endothelium and smooth muscle cells (Regoli, Dion, Rhaleb, Drapeau, & D'Orleans-Juste, 1990). The overall illustration can be seen in Figure 23.

4.2 Conclusion

The electrical stimulation of the ACC, including ACv, ACd, PL, and IL, could induce bi-phases change of blood perfusion in periphery, which is short-term decrease in blood flow followed by long-term increase. This phenomenon might not be mediated by the LH.

APPENDIX A

TITLE OF APPENDIX HERE IN ALL CAPS

Appendix A Content

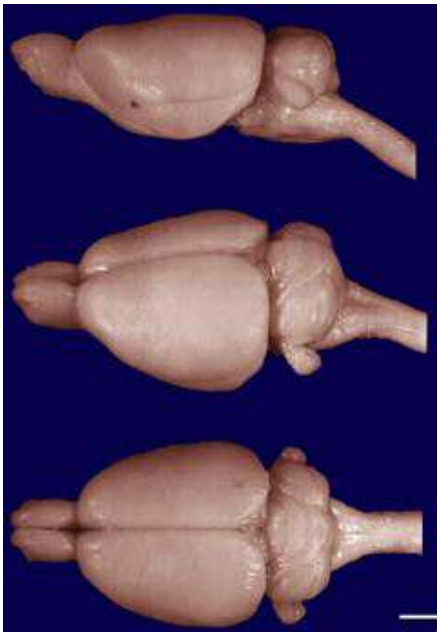

Figure 1. Three pictures of rat brain from lateral, roughly 45 degree lateral, and dorsal perspectives (from top to bottom), respectively, from http://www.econ.nyu.edu/user/bisina/brain_rat.jpg.

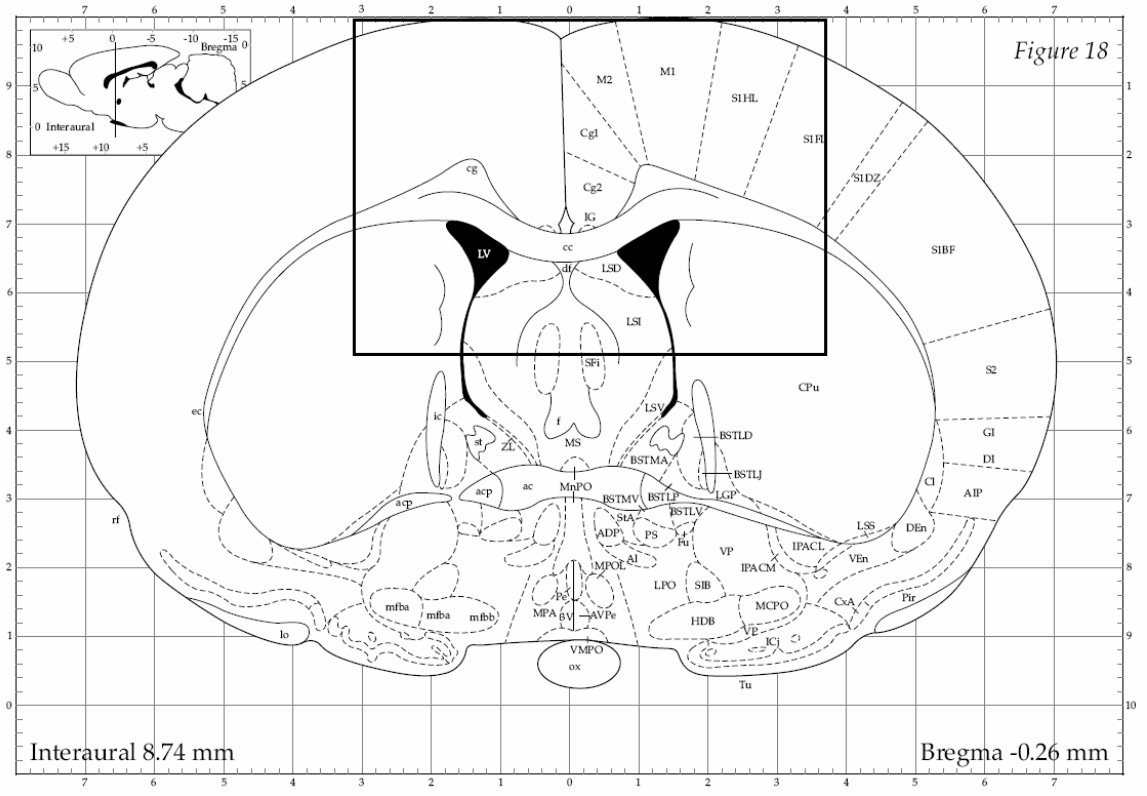

Figure 2. The coronal drawing indicates the different functional areas at the position of Bregma -0.26mm, cited from The Rat Brain, software edition (Paxinos, 1998). The bold black rectangle denotes a region being enlarged in Figure 3.

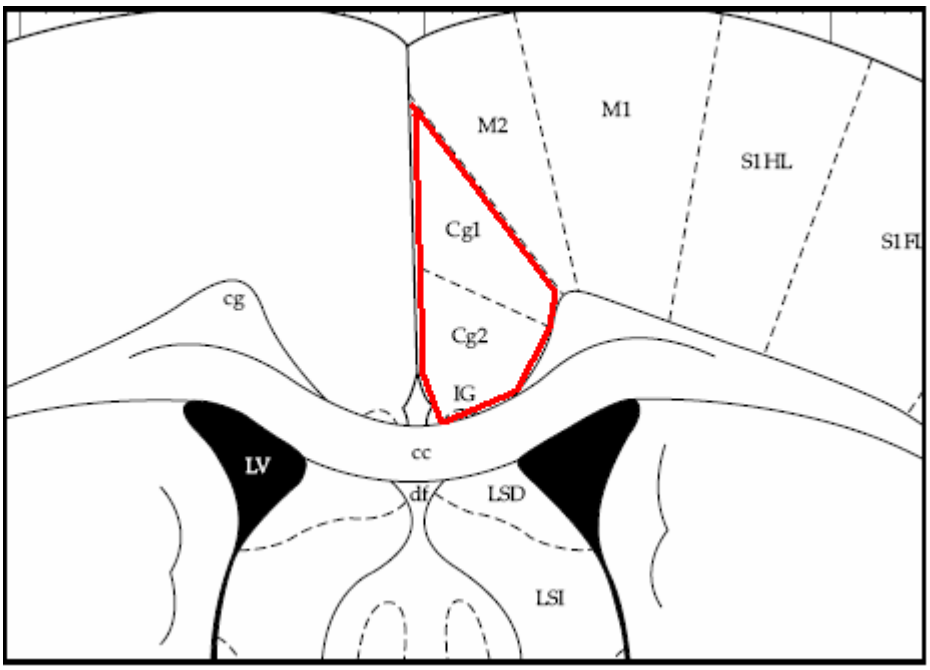

Figure 3. The region inside the red border is anterior cingulate cortex (ACC) in one hemisphere of rat brain (Paxinos, 1998).

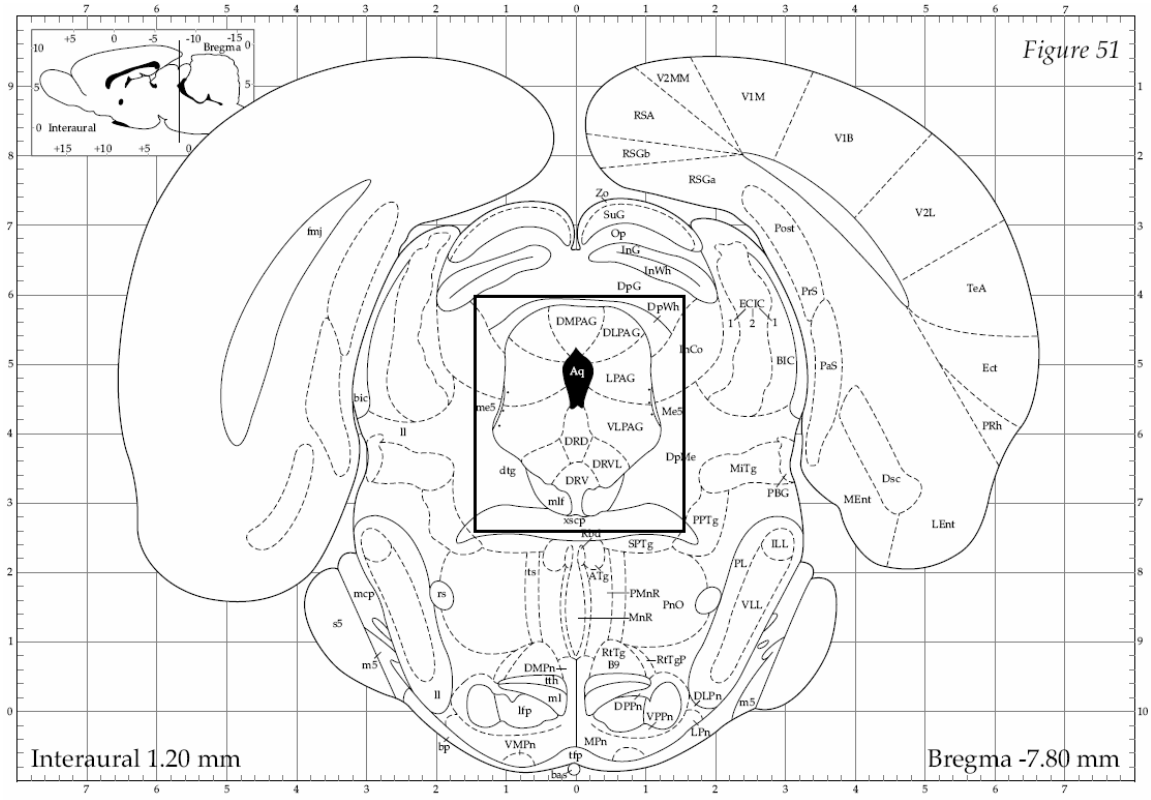

Figure 4. The coronal drawing indicates the different functional areas at the position of Bregma – 7.80mm (Paxinos, 1998). The bold black rectangle denotes a region being enlarged in Figure 5.

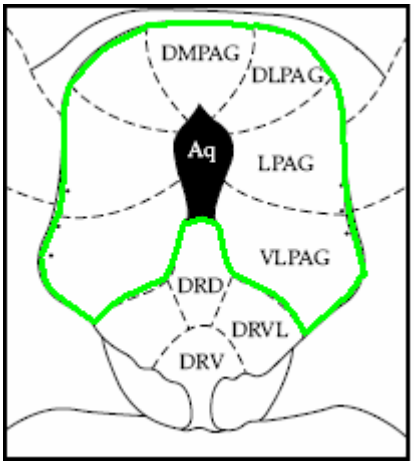

Figure 5. The region inside the green border is PAG, including some nuclei, such as dorsomedial (DMPAG), dorsolateral (DLPAG), lateral (LPAG), and ventrolateral (VLPAG).

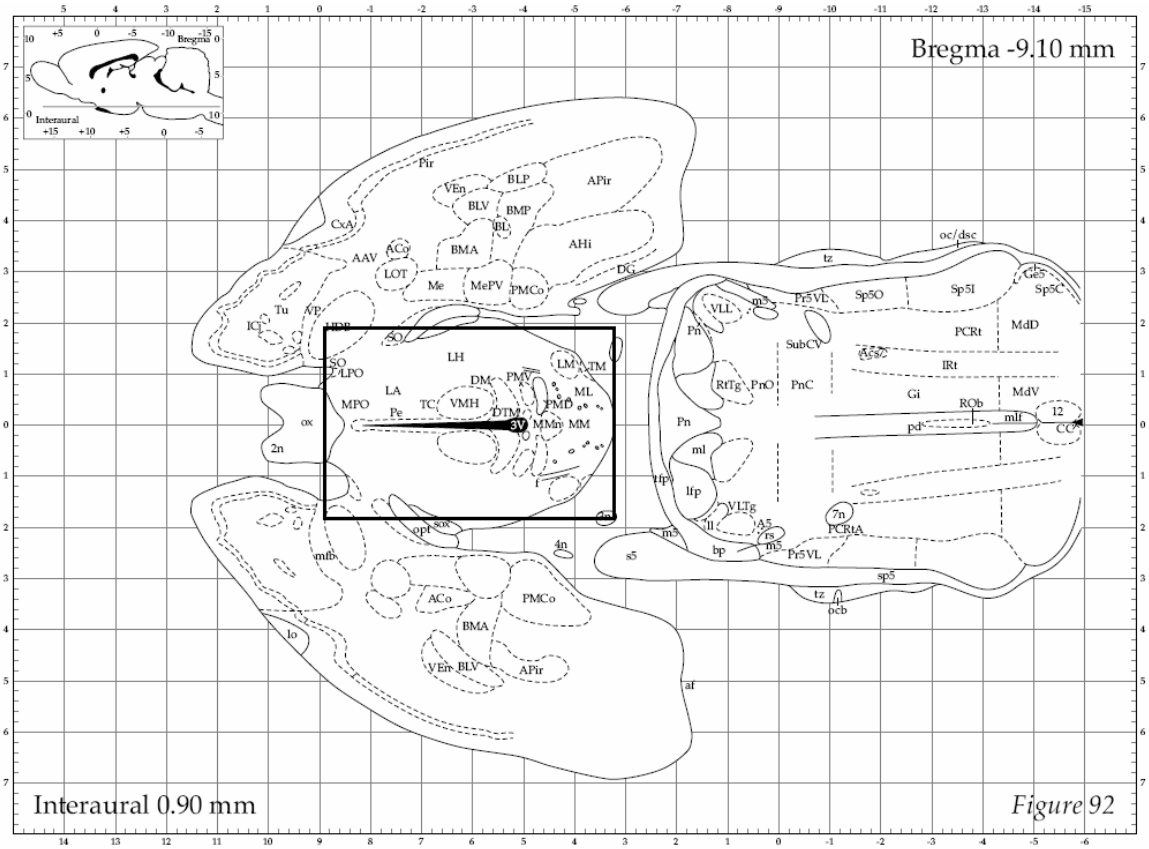

Figure 6. In the horizontal drawing, the blue border indicates the hypothalamus at 0.40mm laterally (Paxinos, 1998).

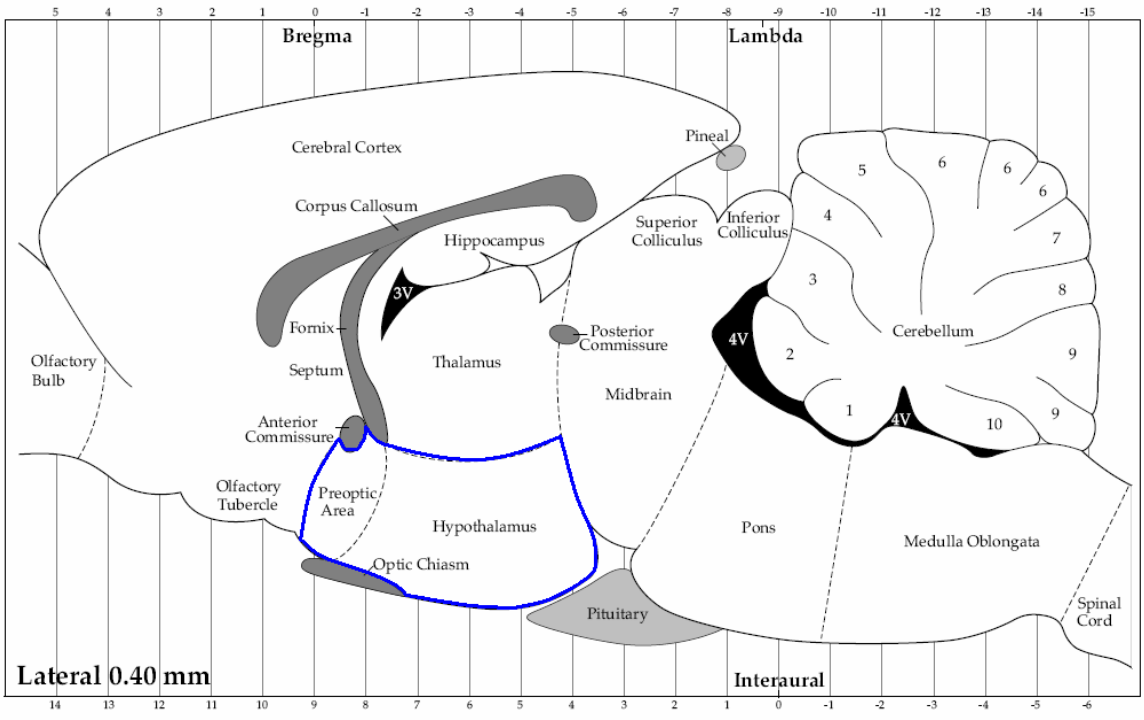

Figure 7. The sagittal drawing was generated at a ventral position of rat brain (Paxinos, 1998). The bold black rectangle illustrates the hypothalamic nuclei, such as lateral hypothalamus (LH), medial preoptic nucleus (MPO), ventromedial hypothalamus (VMH), lateral preoptic area (LPO), etc.

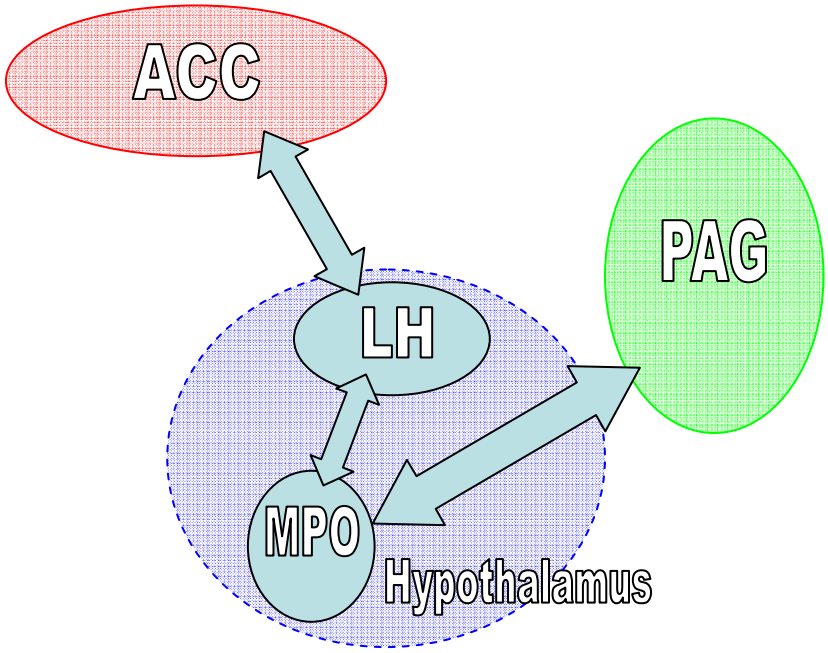

Figure 8. The topology of ACC, PAG, and hypothalamic nuclei, including MPO and LH.

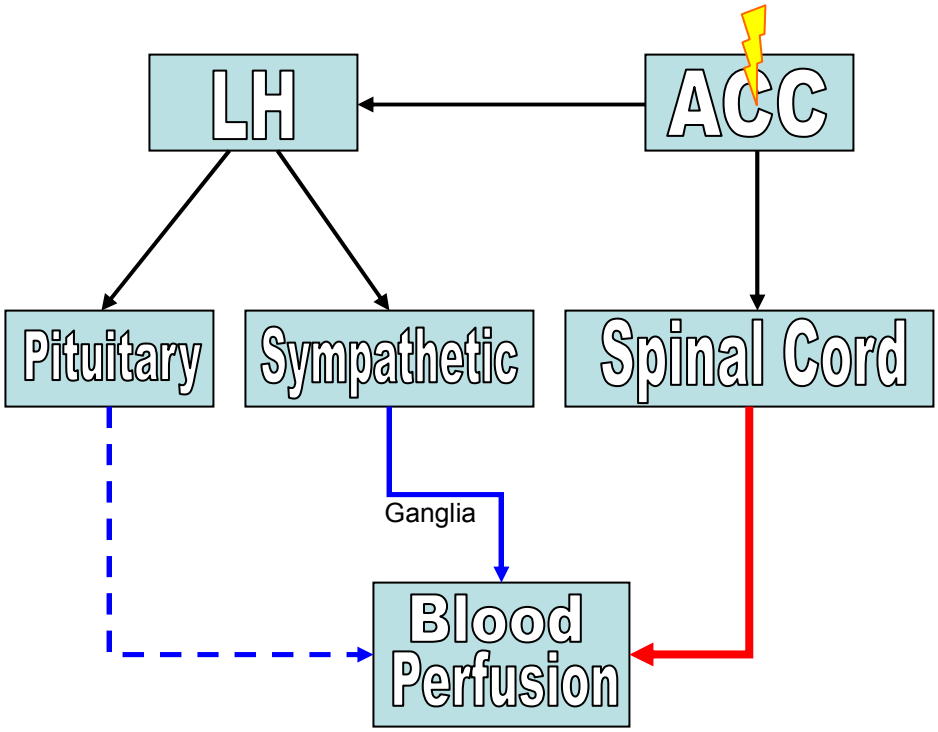

Figure 9. Three mechanisms impact the ACC-stimulation-induced blood perfusion change in hind paws, which are hormone, sympathetic nervous system, and dorsal root reflex. The black lines show the pathways. The red line means vasodilatation, whereas the blue lines (both bold and dashed) show vasoconstriction. The dash indicates hormonal effect.

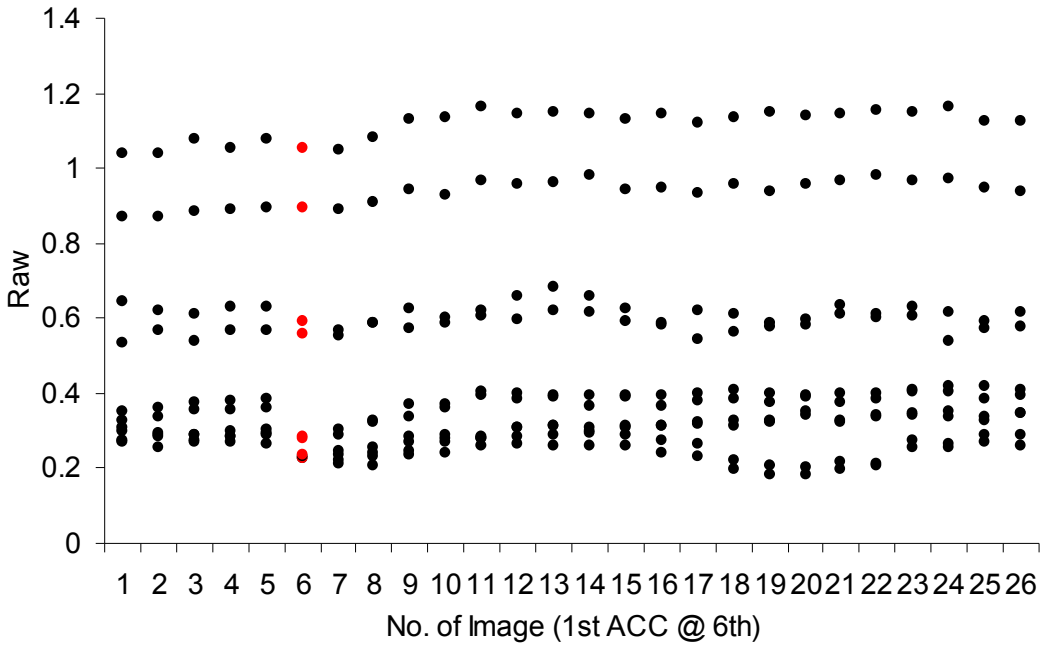

Figure 10. Raw data plotted as an illustration of individual .differences. The red dots represent the BP level during electrical stimulation of the ACC.

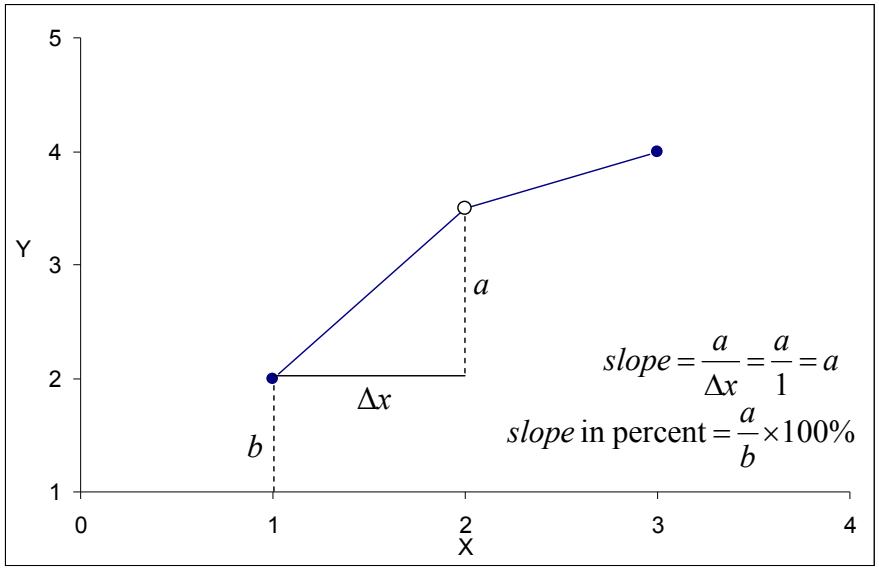

Figure 11. Definition of slope, and its relevant calculation.

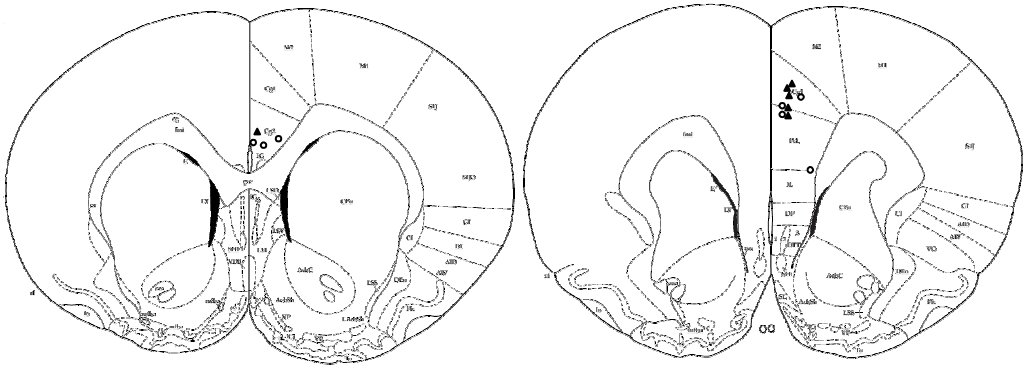

Figure 12. Electrical stimulation sites in ACC. Left side shows the most caudal position (Bregma 1.6 mm) (Paxinos, 1998). Right side shows the most rostral position (Bregma 2.5 mm) (Paxinos, 1998). Every stimulation site is included in this range, however, the real stimulation site might not be at either of the coordinates shown here. Circles represent the control group stimulation sites; whereas solid triangles depict the lesion group sites.

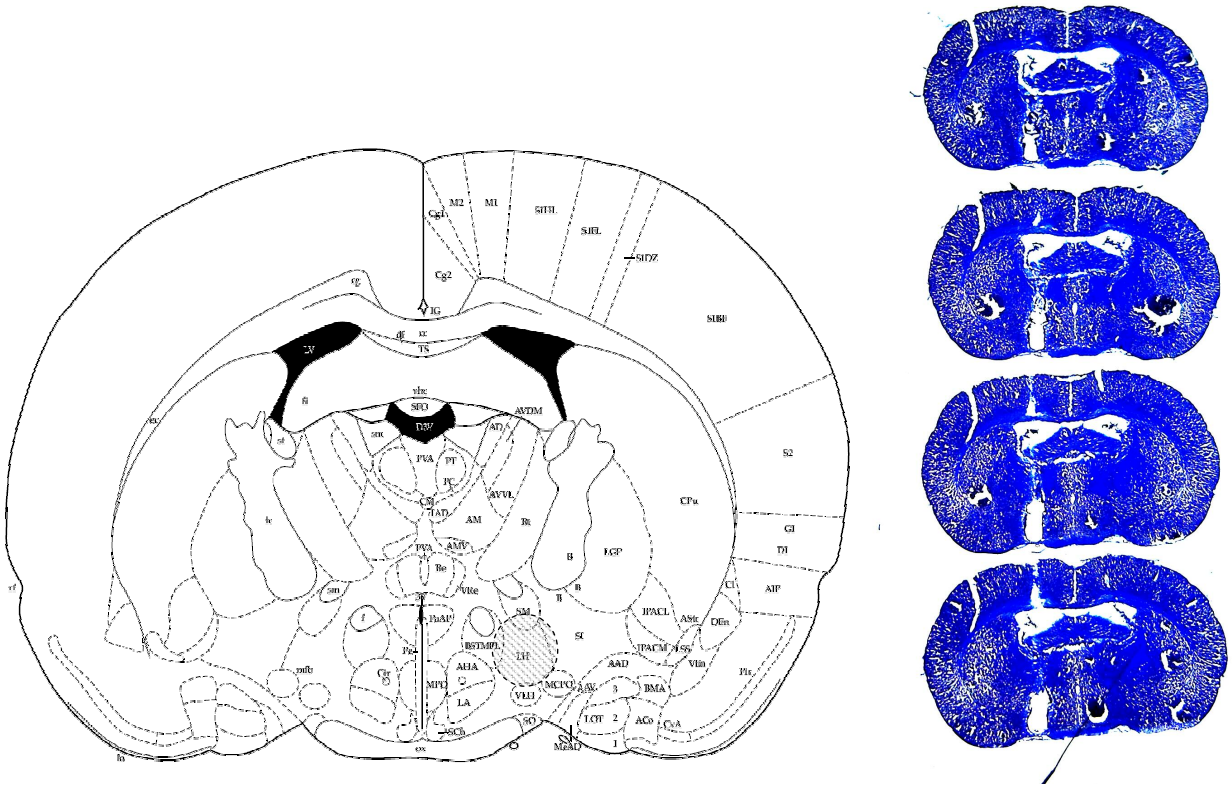

Figure 13. Left: the stereotaxic illustration of lateral hypothalamus, at Bregam -.40 mm (Paxinos, 1998). Right: the histological slides represent the lesion size, thickness 80 micrometer per tissue.

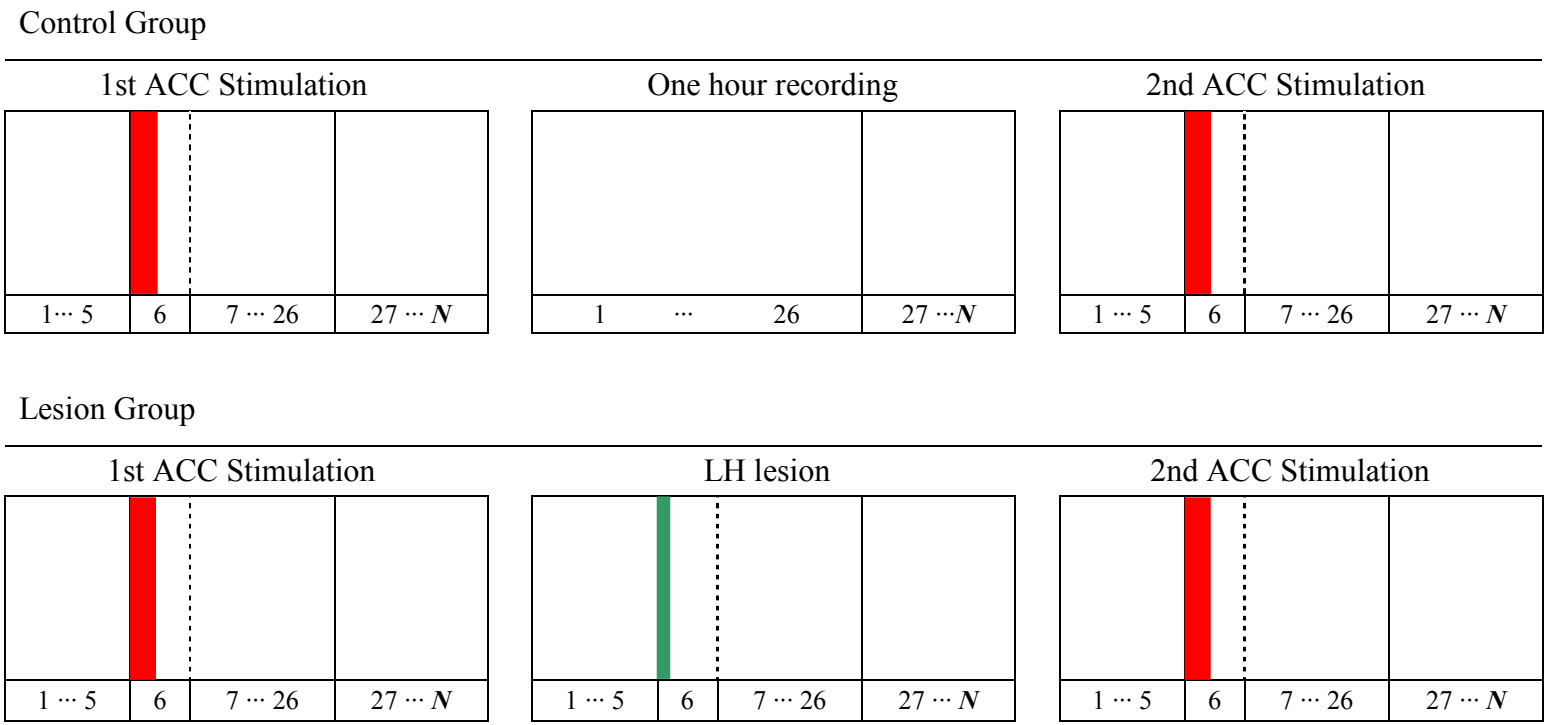

Figure 14. Schematic explanation of manipulations for lesion group and control group. The red strips indicate the electrical stimulation, and the green strip indicates the lesion process. The N indicate the last image in BP recording, which depended on the size of paws. Only 26 images were taken for data analyese.

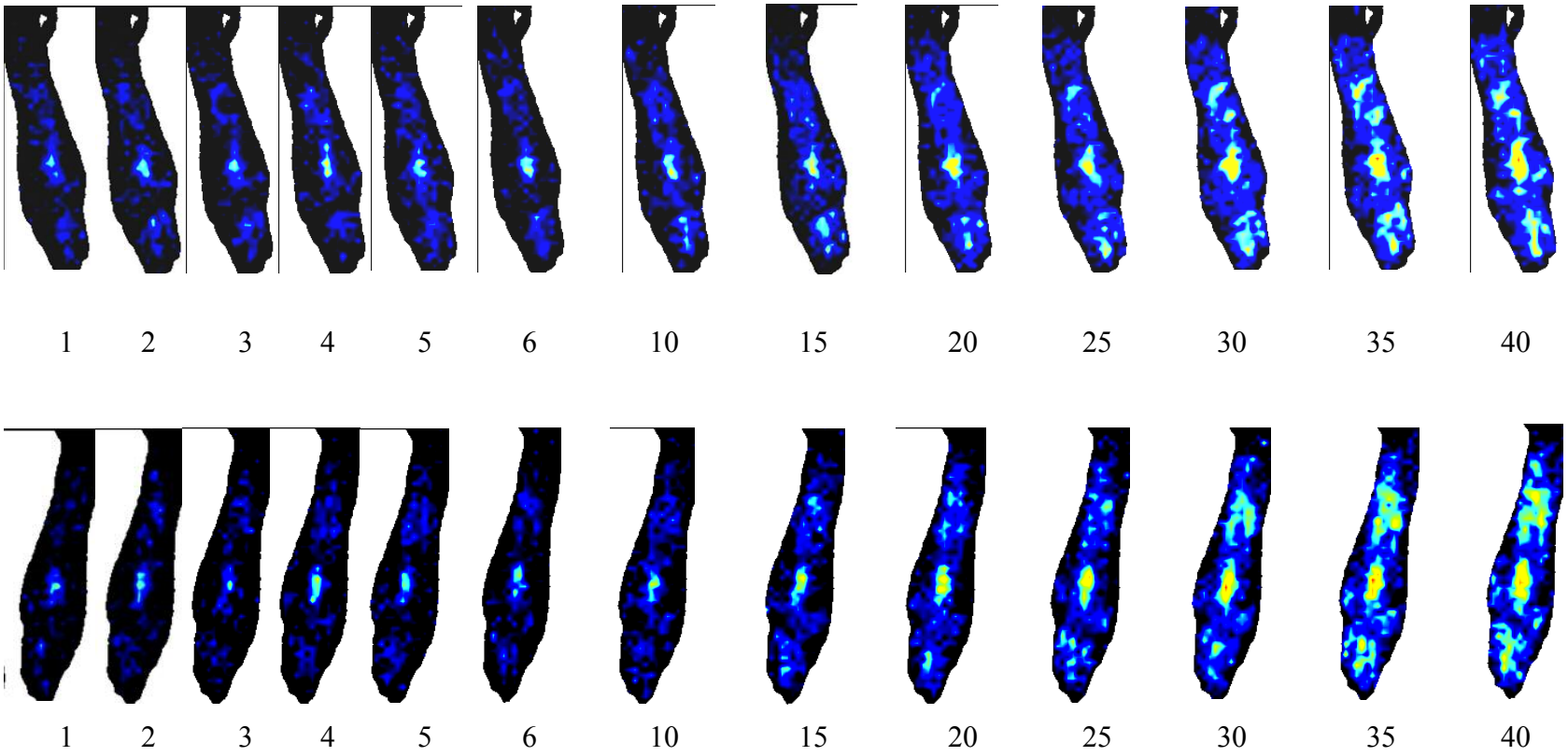

Figure 15. Laser Doppler Imager outputs denotes the speed of blood perfusion in cutaneous sites of hind paws. Upper: left paw; Bottom: right paw; Numbers represent the time course. First 5 images were treated as baseline. The stimulation was delivered at 6th. Images were all continuously recorded.

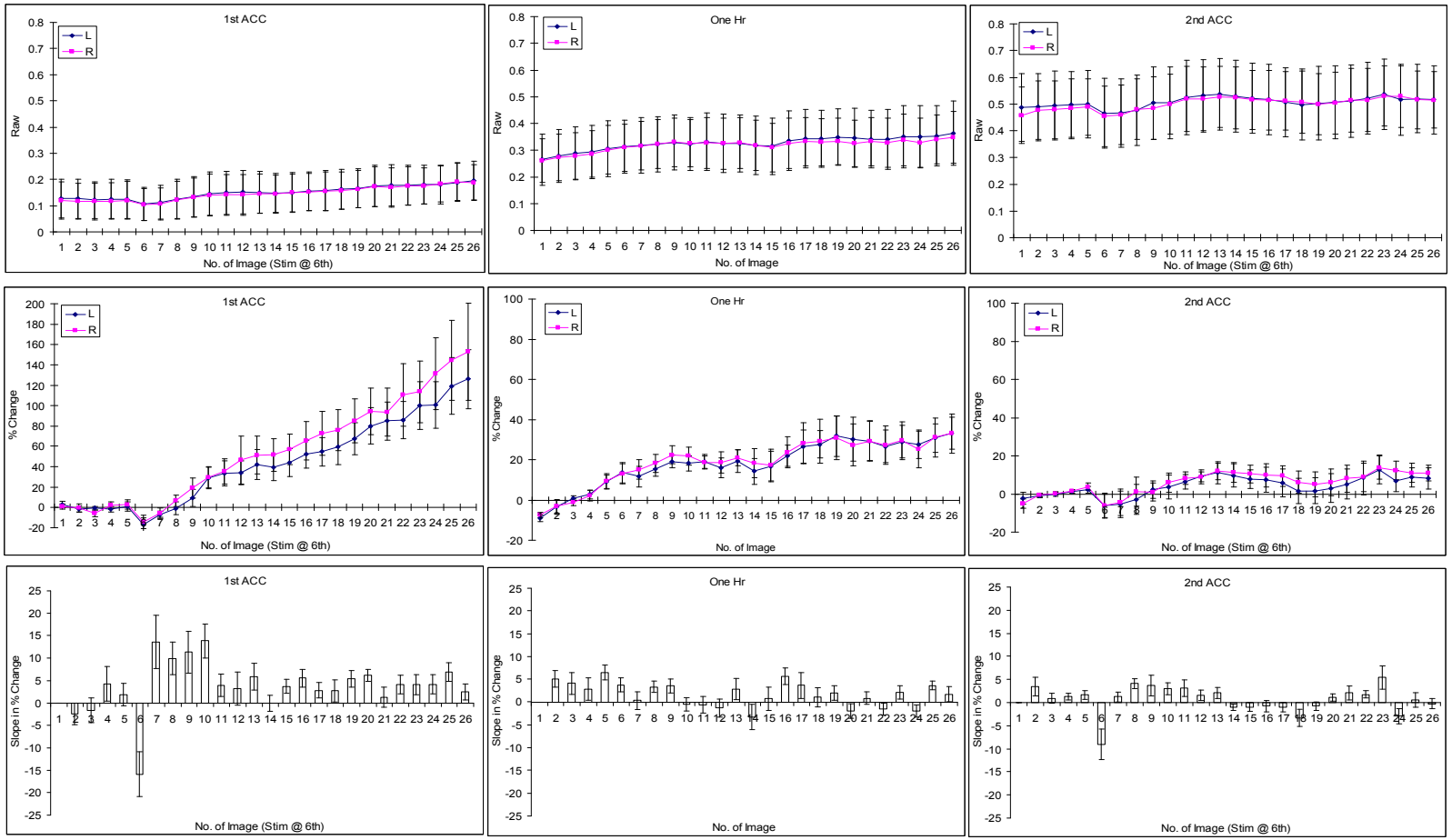

Figure 16. Image data from control group plotted in terms of raw data, percent change of raw data, percent change of slope in three paradigms, 1st ACC stimulation, one hour recording, and 2nd ACC stimulation.

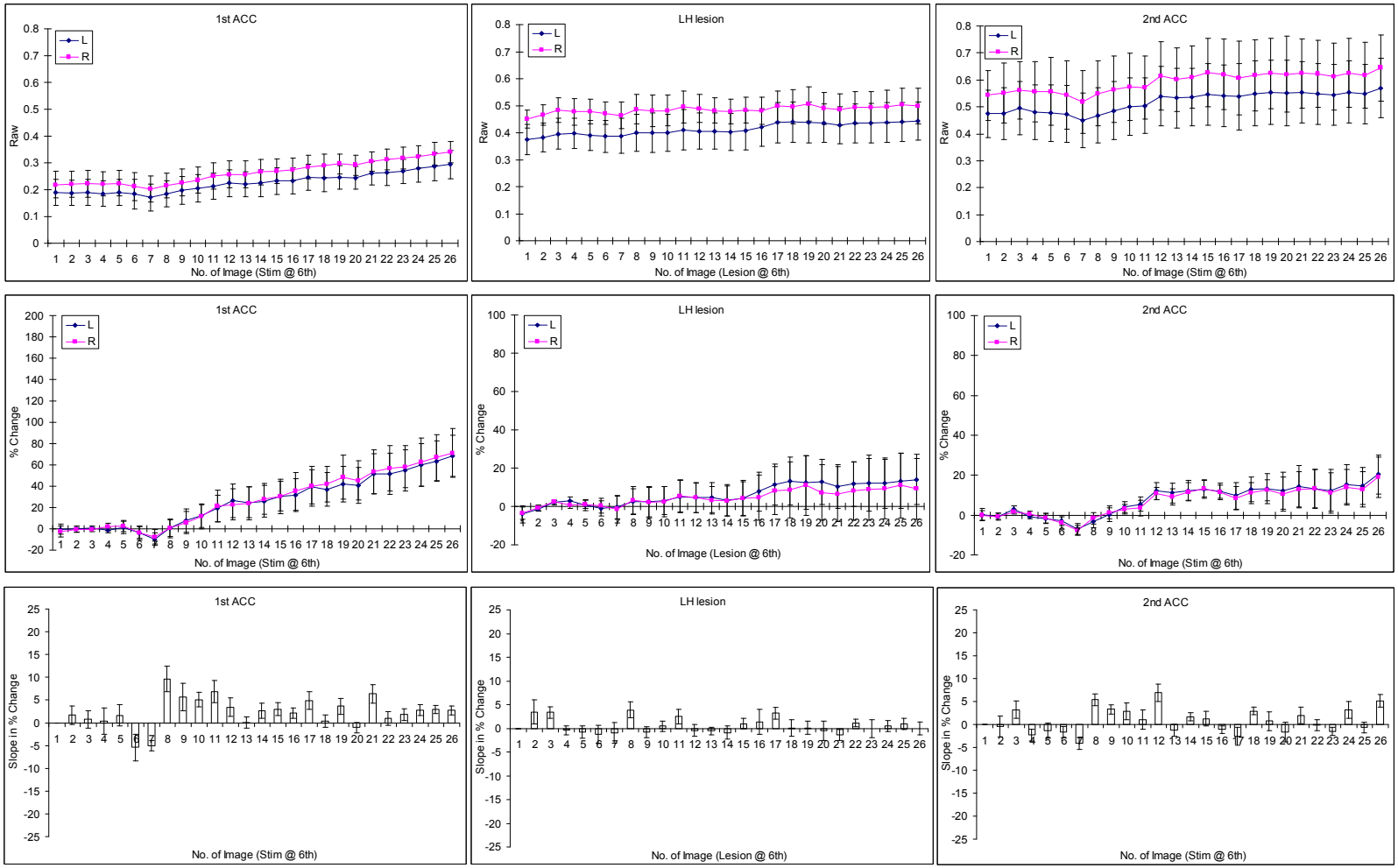

Figure 17. Image data from lesion group plotted in terms of raw data, percent change of raw data, percent change of slope in three paradigms, 1st ACC stimulation, LH lesion, and 2nd ACC stimulation.

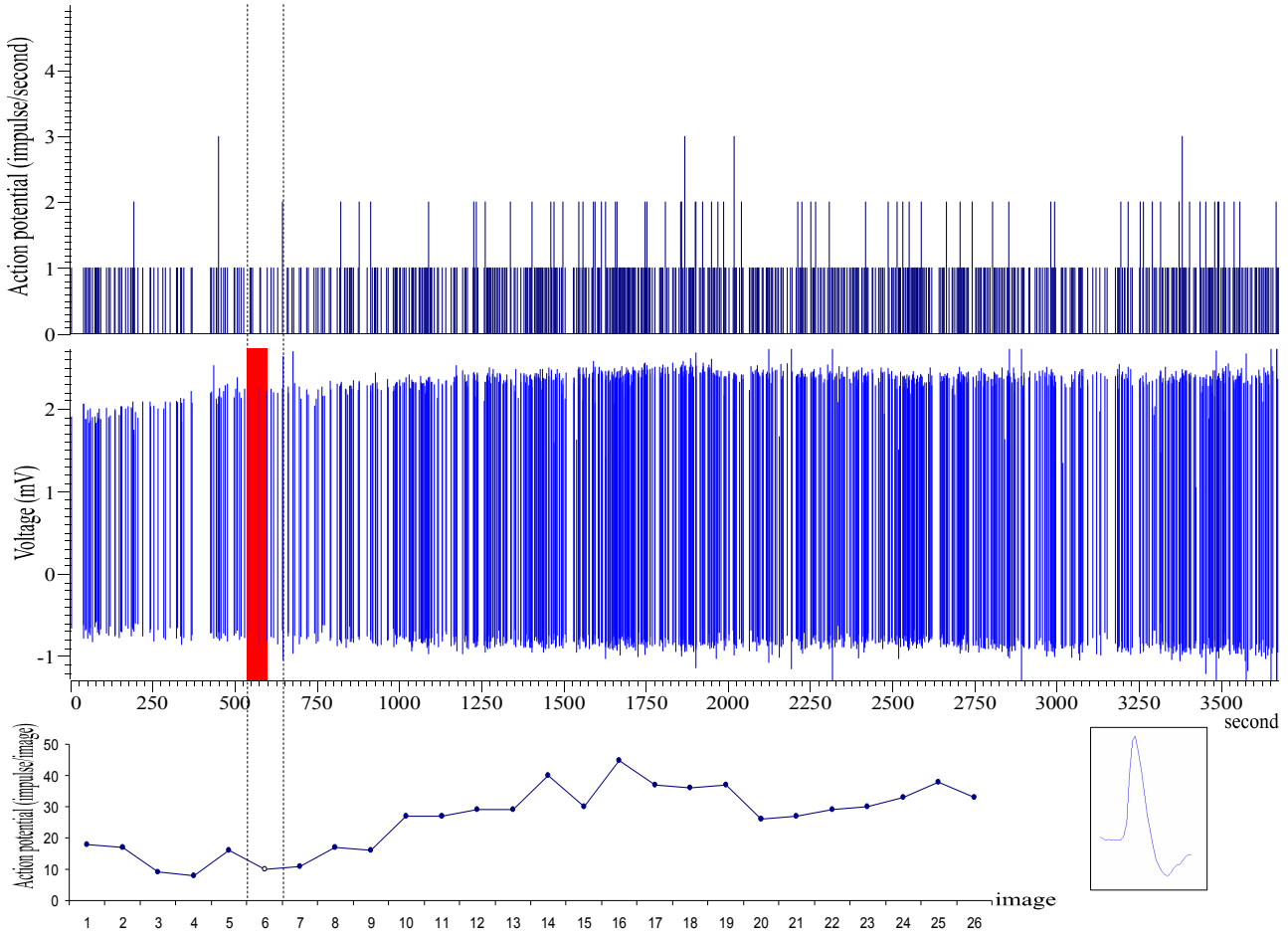

Figure 18. Electrophysiological recording and transformed data into image time course. The upper channel indicates the frequency of DRRs per second, and the bottom channel indicates the frequency of DRRs per image. In the mediam channel, the DRRs are represented by blue bars, and stimulation currents are represented by the red bars.

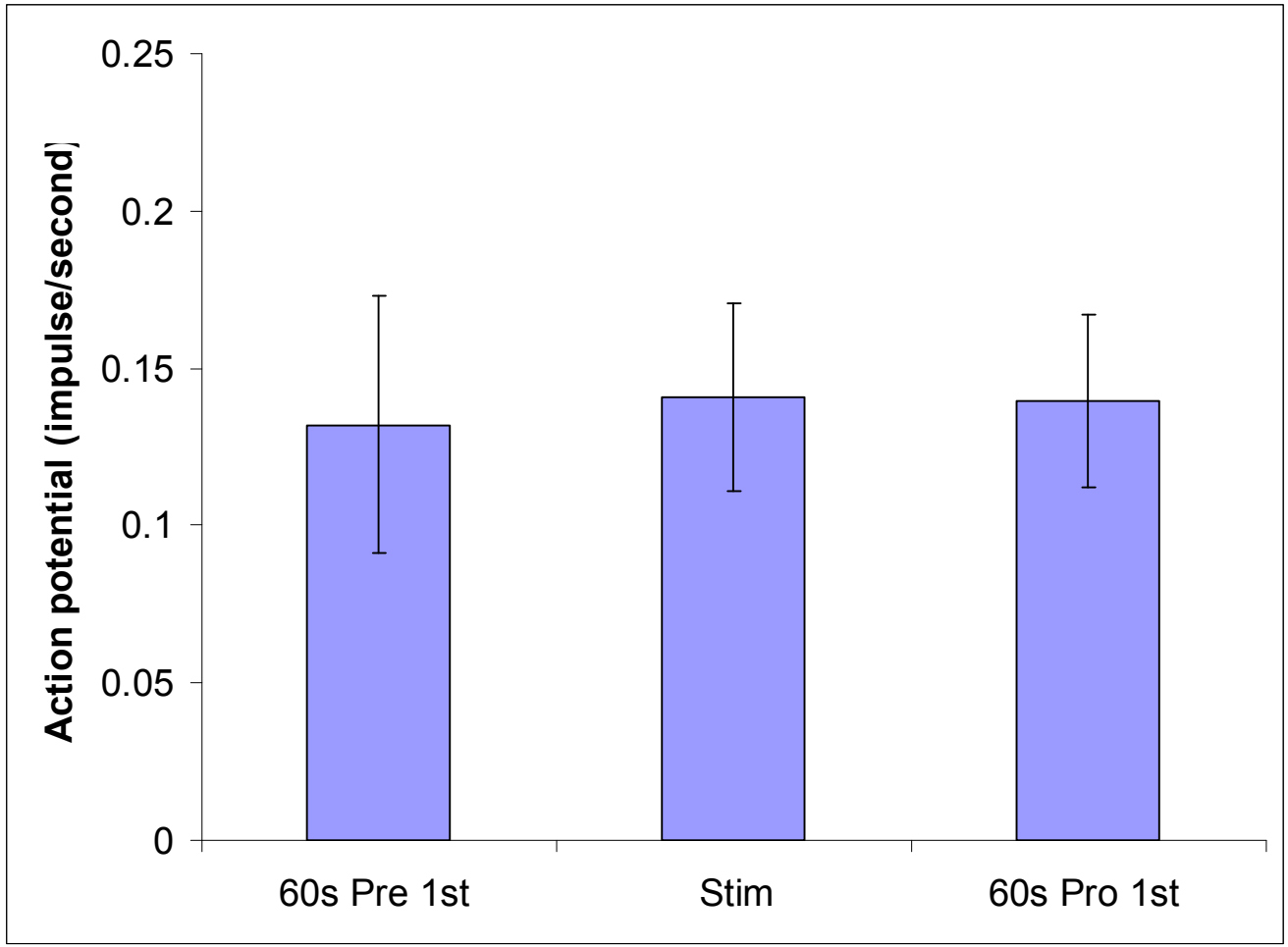

Figure 19. Short term comparison on 1st stimulation versus 60 second before and 60 second after in terms of DRRs. No siginificant difference could be detected at .05 level among three levels.

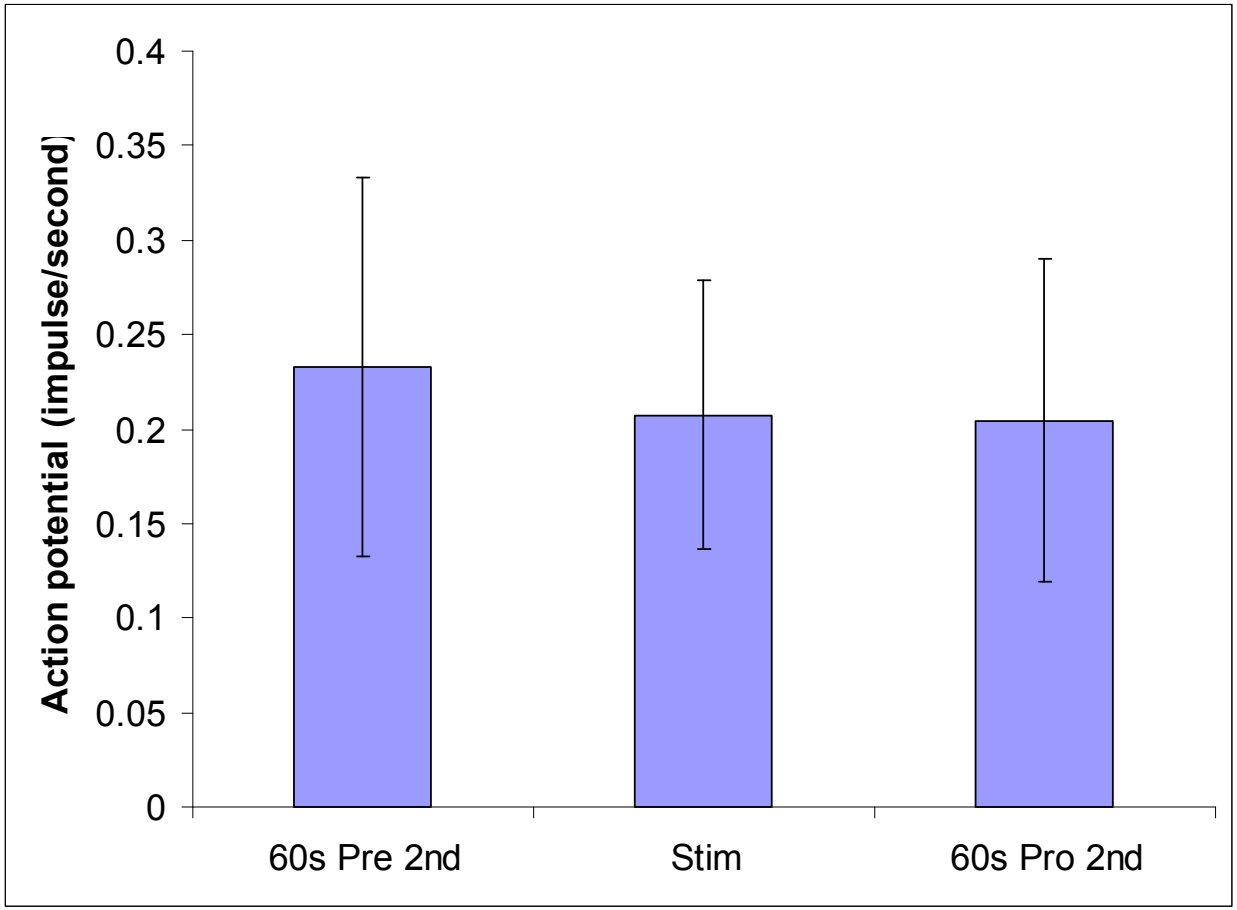

Figure 20. Short term comparison on 2nd stimulation versus 60 second before and 60 second after in terms of DRRs, in control group. No siginificant difference could be detected at .05 level among the three levels.

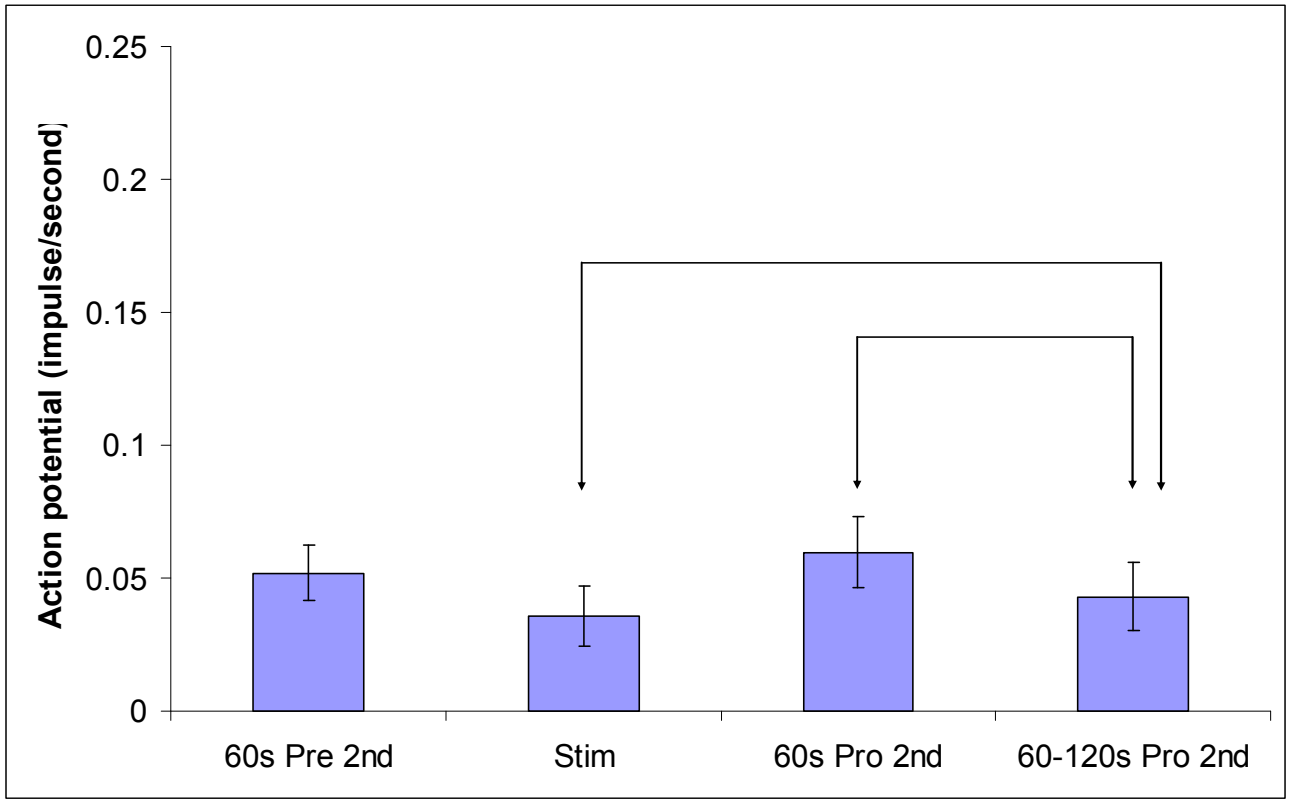

Figure 21. Short term comparison on 2nd stimulation versus 60 second before and 60 second after in terms of DRRs, in lesion group. No siginificant difference could be detected at .05 level among the four levels.

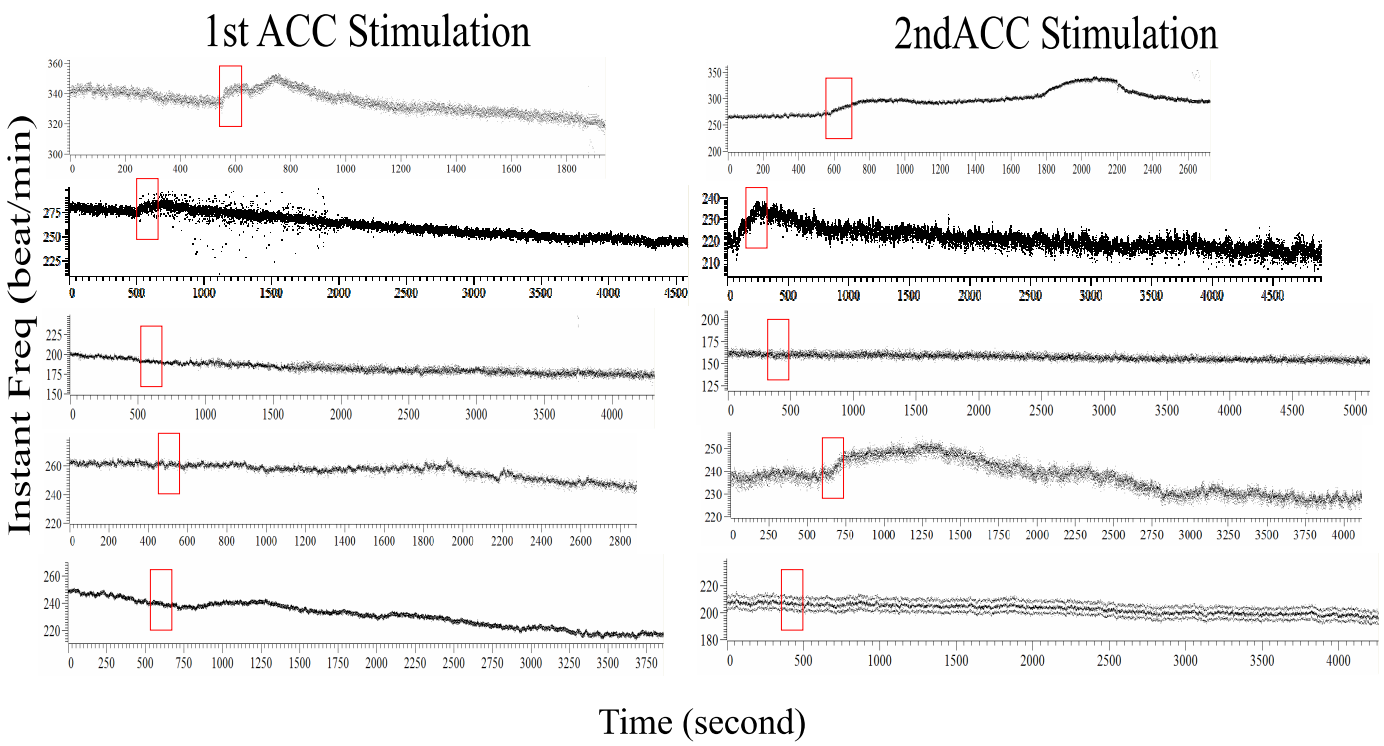

Figure 22. ECG recording and qualitative analyses. The red squares represent the stimulation occurrence. The x axis represents time in second; the y axis represents instant frequency of heart beat in minute.

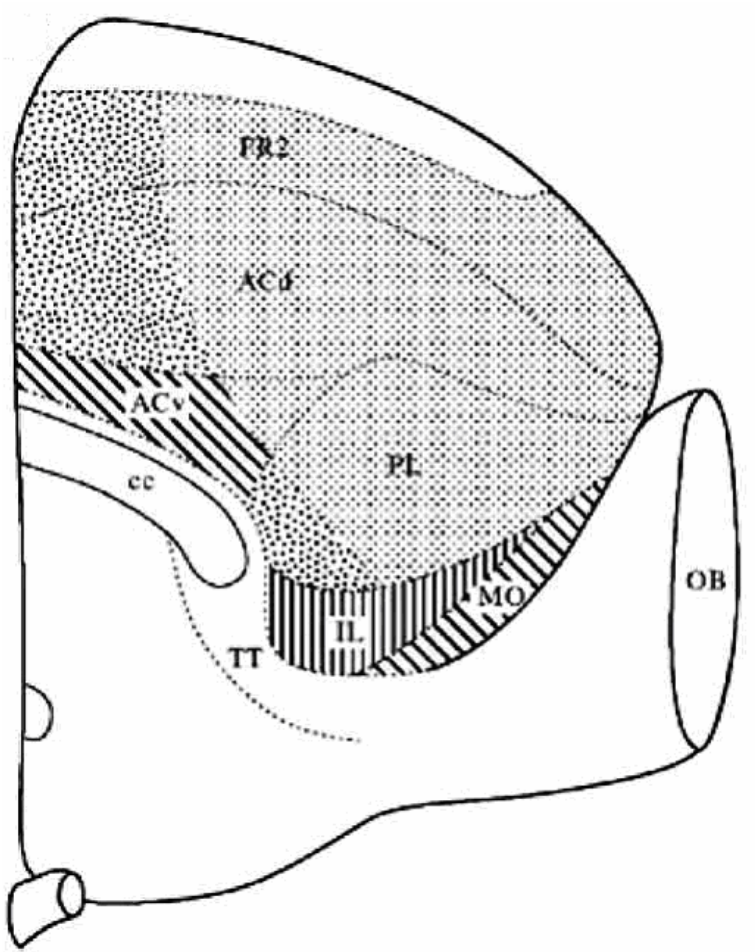

Figure 23. The extent of rat's prefrontal cortex. Cited from Uylings,, Groenewegen, & Kolb, 2003. The sub regions in prefrontal cortex include Fr2, ACd, ACv, PL, IL, and MO.

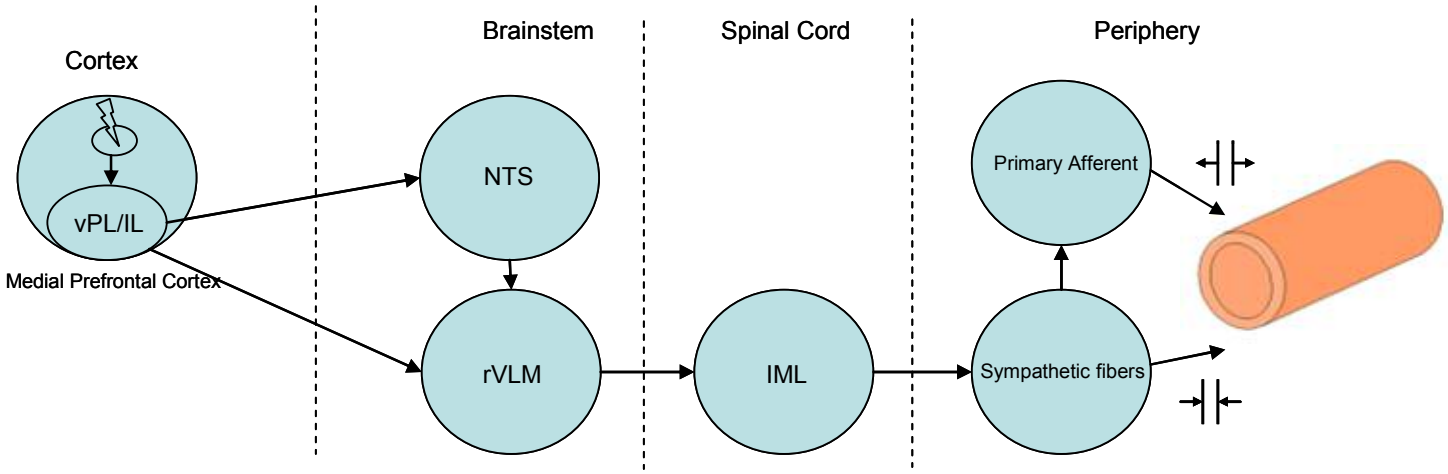

Figure 24. Illustration of mechanism described in discussion. There are four parts, including the cortex level, brainstem level, spinal cord level, and peripheral level.

APPENDIX B

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Appendix B Content

Pathway	Nuclei Connections	Citation
ACC to Hypothalamus	Dorsal ACC to lateral hypothalamus	Gabbott et. al 2005
Hypothalamus to ACC	Tuberal lateral hypothalamus to ACC	Saper 1985
PAG to Hypothalamus	PAG to medial preoptic nucleus (MPO)	Rizvi et. al 1992
	Ventrolateral PAG to ventromedial preoptic nucleus, lateral hypothalamus	Mihaly et. al 2001
Hypothalamus to PAG	Dorsomedial and ventromedial nuclei of the hypothalamus to PAG;	Marchand et. al 1983
	Medial preoptic area (MPO) to dorsomedial PAG;	Rizvi et. al 1992; Simerly et. al 1988

Table 1. The efferent and afferent projections in pairs of ACC and hypothalamus, PAG and hypothalamus.

Rat	No. of Tissues	Thickness	Est. Length
6/19/06	≈14	120 μm	≈1.68 mm
11/2/06	≈11	80 μm	≈ .88 mm
11/4/06	≈20	80 μm	≈1.60 mm
11/18/06	10	80 μm	.80 mm
12/2/06	9	80 μm	.72 mm
12/21/06	>6	80 μm	>.48 mm

Table 2. Summary of lesion size and rough estimate by the calculation, No. of Tissues multiplied by Thickness.

Paradigm		Left vs Time	Right vs Time	Left vs Right Paw	DRRs vs Time
Control	1st ACC Stim.	.746 (7)	.700 (7)	.938 (7)	.086 (22)
	One hr. recording	.565 (7)	.497 (7)	.942 (7)	.057 (21)
	2nd ACC Stim.	.236 (7) .652 (6 of 7)	.337 (7) .631 (6 of 7)	.929 (7)	-.008 (23)
Lesion	1st ACC Stim.	.607 (6)	.597 (6)	.967 (7)	.041 (14)
	LH lesion	.253 (6)	.159 (6)	.967 (7)	.090 (18)
	2nd ACC Stim.	.435 (6) .610 (6)	.435 (6) .667 (6)	.951 (7)	.138 (19)

Table 3. Summary of Pearson correlation coefficients for image data versus time and DRR versus time. The number in bracket represents the sample size. In coloums of Left vs Time, Right vs Time, Left vs Right Paw, the bracketed numbers are the number of subjects, in each of which contains 26-image data points. In column of DRRs vs Time, the bracket number is the number of neurons. The lasting time is similar to 26 images' recording. Specially, for the second ACC stimulation, the lower cell represents 700-second (i.e. 7-image) long recording correlation; the upper cell indicates the overall 26-image correlation.

APPENDIX C

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Appendix C Content

APPENDIX D

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Appendix D Content

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