國立政治大學神經科學研究所

碩士論文

電刺激大鼠側韁核對區辨性低頻操作式制約行為的影響

The effects of electrical stimulation in the lateral habenula on operant behavior maintained by the differential reinforcement of low-rate (DRL) schedule of reinforcement in the rat

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中文摘要

透過神經科學的研究，對於大腦的行為功能已有一定的認識，不同於以往的認識，目前認為神經行為機制不只由單一腦區或單一神經化學系統所調控。深部大腦電刺激經常被用來研究特定腦區的行為功能。但是，深部大腦電刺激的作用機制仍然不清楚。最近幾年臨床研究發現，利用電刺激在側韁核成功的治療憂鬱症患者。然而，目前認為側韁核與多巴胺系統互為負回饋作用，共同參與在動機行為的酬賞反應中。本實驗室先前的研究顯示，破壞韁核造成區辨性低頻操作式制約行為 (簡稱 DRL 行為)學習的障礙，然而，電刺激在側韁核造成 DRL 行為表現的結果還是未知的。所以，本實驗主要以電刺激在側韁核觀察大鼠行為上的改變，探討側韁核在行為上參與的功能。實驗一的結果顯示電刺激在側韁核並不影響自發性運動能力，在不同電流強度的刺激下也不會影響。實驗二的結果顯示電刺激在側韁核造成 DRL 15 秒的行為有類安非他命效果之行為表現，在高頻率電刺激有較顯著類安非他命的效果。實驗三的結果顯示電刺激在側韁核造成 DRL 15 秒的行為之影響，會被多巴胺受體抑制劑所抵消，而單獨注射巴胺受體抑制劑並不影響 DRL 15 秒的行為。實驗四的結果顯示電刺激在側韁核造成 DRL 15 秒的行為之影響，不會被正腎上腺素受體抑制劑所抵消。實驗五的結果顯示電刺激在側韁核造成 DRL 72 秒的行為之影響並不如 DRL 15 秒的行為顯著。實驗六的結果顯示電刺激在側韁核並不會造成大鼠無法區辨酬賞的量。綜合而言，側韁核在動機行為的角色，是透過影響多巴胺系統造成行為的改變。

關鍵詞：深部大腦電刺激，區辨性低頻操作式制約(DRL)行為，側韁核，多巴胺受體抑制劑，正腎上腺素受體抑制劑。

Abstract

Behavioral function of the brain has been studied in neuroscience and progressively accumulated informative data to reveal the neurobehavioral mechanisms. It is now realized that those underlying mechanisms of behaviors is not as such simple as previous thought of limiting only in one locus of the brain or solely by one neurochemical system. The deep brain stimulation is usually used to study the behavioral function of specific brain regions. However, the mechanism of the deep brain stimulation is still unclear. The previous study has shown that electrical stimulation of the lateral habenula (LHb) successfully treated depression symptoms in the patients. It is proposed that an inhibitory role of LHb on the mibrain dopamine (DA) system which mediates the reward-related behavior. A previous study of this lab showed that lesion of habenula impaired the acquisition of differential reinforcement of low-rate responding (DRL) behavior. But, the effect of LHb stimulation on the DRL behavior is still unclear. To determine the functions of LHb involving in the behavior, the electrical stimulation was applied in LHb to observe the behavioral change of rats. The results of Experiment 1 showed that the LHb stimulation had no effect on locomotor activity. In Experiment 2, the LHb stimulation was shown to affect DRL 15-s behavior, which effects were similar to those affected by amphetamine. Experiment 3 showed that the DA receptor antagonists reversed the effects of LHb stimulation, while experiment 4 showed that norepinephrine (NE) receptor antagonists had no reversal effect on DRL 15-s behavior. In Experiment 5, the amphetamine-like behavior induced by LHb stimulation had subtle effects on DRL 72-s behavior. Experiment 6 showed that the LHb stimulation had no effect on a discrimination task. These data suggest that the LHb modulating DRL behavior is DA-dependent.

Key Words: deep brain stimulation, differential reinforcement of low-rate responding behavior, lateral habenula, dopamine receptor antagonists, norepinephrine receptor antagonists

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Introduction

While the issue of behavioral function of the brain has been heavily focused in neuroscience and progressively accumulated informative data to reveal the neurobehavioral mechanisms of various kinds of behavior from the past, it is now realized that those underlying mechanisms for the behavior may not be simply as previous thought of limiting only in one locus of the brain or solely by one neurochemical system. In terms of investigating the brain/behavior interaction, multiple research approaches that use the methods of lesion, stimulation and recording have to be taken in account for providing a better profile in elaborating the underlying neurobehavioral mechanisms. In another words, checking data consistently across different approaches on a specific issue is essential before a more comprehensive conclusion can be made. Such that, also following the idea of neural circuitry for behavioral function, it is important to examine a nucleus interacts with another that has been established (if not completely known) for its function with an acceptable scale. For instance the dopamine (DA) related reward motivation; despite that a great deal of emphasis has been placed on behavior performance driven by the increased DA neuronal activation, an alternative mechanism may have the active removal of an inhibitory control on DA neurons. Accordingly, it is possible that the neural processes of DA related function, from physiological to behavioral level, could be modulated or interacted by other nuclei. A growing body of evidence suggests that the habenula (Hb) plays a modulatory role on the mid brain DA systems (reviews see Hikosaka, 2010; Hikosaka et al., 2008). To further test this proposition, the present study was designed to investigate the role of lateral Hb involved in DA-related behavior by using the brain stimulation approach. The use of brain stimulation approach is set to be complimentary for a previous study using lesion

approach, both examining how the Hb influences the DA-related behavior.

The application of deep brain stimulation in clinical and pre-clinical studies

Deep brain stimulation (DBS) is one of the therapeutic potential of neuromodulatory techniques today. High frequency of DBS had been used in treatment of neurological diseases and movement diseases (Gross et al., 2000; Wichmann et al., 2006). Such as, DBS of the subthalamic nucleus (STN) improved the symptoms of Parkinson's disease (PD) (Perozzo et al., 2001), while DBS of the thalamus was used to treat epilepsy and Tourette's syndrome (Zumsteg et al., 2006; Maciunas et al., 2007). In recent years, DBS had also been used in the treatment of depression patients. Mayberg and associates (2005) demonstrated that DBS of the subgenual cingulate white matter successfully treated depression symptoms in six patients. Several other brain areas have been targeted to test the potential effectiveness of DBS treatment for depressed patients or animal models, including the lateral habenula nucleus (LHb) (Li et al., 2011), the anterior limb of the anterior internal capsule (Gutman et al., 2009), the nucleus accumbens (NAc) (Schlaepfer et al., 2008) and the thalamic peduncle (Jimenez et al., 2005). Despite its clinical application, it is unfortunately that the neural mechanism of DBS is still unknown.

In terms of DBS of STN changed various neurotransmitter systems, previous studies showed that bilateral DBS of STN produced a decreasing effect on the firing rate of 5-hydroxytryptamine (5-HT) neurons (Temel et al., 2007). It was also showed that unilateral DBS of the left STN increased glutamate levels in the STN (Lee et al., 2007). In addition, GABAergic neurons are suggested to play an important role in the high frequency stimulation (Feuerstein et al., 2011). DBS of slices adopted from the striatum was demonstrated to increase extracellular GABA levels *in vitro* (Li et al., 2004), which results are consistent to an *in vivo* study showing DBS of the striatum

enhanced local GABA outflow in freely moving rats (Hiller et al., 2007). Thus, it is likely that the DBS mechanism would cause the changes of neurochemical transmission leading to functional alteration. However, it is still unclear about the neurochemical changes are dependent on the brain area where the DBS is applied. Pharmacological test administered in combining with DBS can be used to encounter this issue.

The lateral habenula

There is a growing body of evidence indicating a strong correlation between the depression state and the hyperactivity in the habenula, especially in the LHb area (Sartorius and Henn, 2007; Hikosaka et al., 2008). In combining with the aforementioned literature addressing DBS induced neurochemical effects, it is reasonable inferred that the habenula is involved in the control of emotion and motivation. The shift of neural activity of habenular may alter the individual's behavioral function and mental state. Thus, to verify the neuroanatomy and neurophysiological function of the habenula may lead a further understanding of the neurobehavioral mechanisms of emotion and motivation which serve as the fundamental processes for the cognitive and higher order function.

The habenula complex was one of the areas of the brain and both hemispheres were symmetrical. The habenula complex formed a part of the cross-talk between limbic forebrain and some important ascending modulatory pathways. The habenula complex situated at the caudal end of the dorsal diencephalon and projected many axons to the brainstem, including the 5-HT neurons and DA neurons. The habenula complex was divided into two distinct nuclei, the medial (MHb) and the lateral (LHb) habenular nucleus. Each nucleus had very different afferent and efferent connections (Geisler et al., 2008). Whereas the MHb contained cholinergic neurons,

and substance P neurons, the LHb contained glutamatergic and only sparse GABAergic neurons (see a review by Lecourtier et al., 2007). The literature review below is focused only in the rodent animals, mainly in considering the rat is the subject used in this study. In addition, the LHb rather than the MHb is attended because the former Hb subarea is of particular interest to be manipulated in this study.

In the rat, the LHb received GABAergic inputs from the entopeduncular nucleus, the lateral hypothalamus and the lateral preoptic area. Also, a cholinergic inputted in the entopeduncular nucleus. A number of studies have shown that the LHb afferent fibers from the medial frontal cortex, the ventrolateral septum, the diagonal band of Broca, the nucleus accumbens, the ventral tegmental area (VTA) and median raphé nucleus. The efferent fibers of LHb projected to the raphé nucleus (medial and dorsal subareas), the hypothalamus (lateral, dorsomedial and posterior nuclei), VTA, the substantia nigra (SN), thalamic nuclei (mediodorsal, central medial, ventromedial subareas), the parafascicular nucleus, the locus coeruleus, the nucleus accumbens, the dorsolateral tegmental nucleus and the supramammillary nucleus (Sutherland, 1982; Geisler et al., 2008). The LHb involved in two neural circuits at least, such as 5-HT and DA systems.

Despites that the LHb had been studied in flourish by the anatomical approach, how the behavioral function of the LHb remains unclear. Using neurophysiological approach, electrical stimulation of the LHb was reported to inhibit firing of almost all the midbrain DA neurons, up to 97% of tested neurons (Ji and Shepard, 2007). Hikosaka and his colleaugues (2008) demonstrated that LHb neurons encoded the negative reward signaling in contrast to the DA neurons mediating the positive reward process. Moreover, LHb neurons activity were excited by no-reward-predicting stimuli, but inhibited by reward-predicting stimuli (Hikosaka et al., 2007). That study also reported that the mesolimbic dopamine neurons exhibited a reverse response

compare with the above phenomenon. In a more recent study, the LHb neurons are indicate to represent mirror-reversed phasic effects with DA neurons in the reward-related behavior (Bromberg-Martin et al., 2010a). Furthermore, it has been further demonstrated that the LHb is involved in reward prediction error and negative reward processes (Bromberg-Martin et al., 2010b). While a progress has been made to delineate the LHb function by neurophysiological approach at cellular level, behavioral (*in vivo*) measurement at the system level is now getting attended by conducting behavioral test the free-moving animals with the manipulation in the LHb. Recently, several studies intended to examine how the LHb would affect the behavior of reward motivation. Electrical stimulation of the LHb was reported to attenuate the positive reward-associated reinforcement (Friedman, et al., 2011). And the electrolytic lesions of the habenula attenuated brain stimulation reward (Morissette et al., 2008). The LHb neurons were activated by no reward responses on reward-oriented eye movement behavior (Bromberg-Martin et al., 2010a). From these data, the role of LHb involved in the reward motivation is still in vague. In considering that the behavioral tasks used to test the reward motivation are mostly established on the basis of operant conditioning paradigm, it is then worthy to challenge a more critical issue regarding to how the LHb affect the operant conditioned behavior.

The LHb and differential reinforcement of low-rate responding behavior

Among different types of operant behaviors maintained by distinctive schedules of reinforcement, the present study particularly employing the differential reinforcement of low-rate responding (DRL) behavior for two reasons described below. First, from a previous study done in this laboratory (Chiang, 2006), it was found that lesion of habenula produced impairment on the acquisition of DRL behavior but not

the fixed-interval (FI) typed behavior. In complimentary to the aforementioned data collected from the subjects under LHb lesion, the present study aimed to test how DRL behavior would be influenced by the LHb stimulation. And, surprisingly, the electrical stimulation of LHb on DRL behavior had not been investigated. Second, the DRL behavior, characterized by self control and timing perception as for its behavioral components (see below), can be used a behavioral task to measuring the motivation and cogitative-like processes simultaneously. This task is widely used to screen clinical drugs treated for the psychiatric disorders, for instance, the DRL 72 sec behavioral task applied in testing antidepressant drugs or treatments (O'Donnell et al., 2005). Thus, using DRL behavior task in the present study was aimed to investigate the role of LHb involved in reward-related motivation or cognitive-like effect by.

The DRL operant behavior was initially developed from an idea to combine the response ratio and time interval schedules (Skinner, 1938). Operant behavior maintained on the DRL schedule of reinforcement had been characterized as exhibiting temporal regulation as well as behavioral inhibition. In other words, the animal subject trained on this schedule of reinforcement are required to inhibit or withhold lever pressing for a minimum specified period of time in order to obtain a reinforcer. Any premature response leads not only to non-reinforcement consequence but also to re-setting of the time requirement to its full interval. This task has been widely used in psychopharmacology to study the relationship between the drug and behavior (reviews see Liao, 2009; Sanger and Jackson, 1989).

Previous studies had shown that acute treatment of amphetamine disrupts DRL behavioral performance by increasing the number of responses and decreasing the number of reinforcers acquired (e.g. Liao and Cheng, 2005; Liao, 2009). A tendency of increasing in burst responding was observed after treatment of amphetamine. Accordingly, amphetamine caused rats to respond more with shorter IRT's leading to

a leftward shift in the IRT frequency distribution. And, amphetamine induced leftward shift is in parallel with that of the saline control, suggesting an internal timing “clock” being speeded up by drug. All these behavioral changes in the DRL task caused by amphetamine have been argued to be mediated by drug produced the enhancement of dopamine release in the brain (Liao, 2009). Moreover, the DRL behavioral alteration induced by amphetamine can be partially reversed by DA receptor antagonists (Cheng & Liao, 2007). Therefore, operant behavior maintained on DRL behavior affected by amphetamine is DA dependent. Also, it is the reinforcement contingency that serves a basic component of reward motivation to lead the subject performs on a DRL behavior. Following this presumption, it is worthy to elucidate the neural mechanisms of reward- or DA-related motivation behavior via the examination of DRL behavior in the subject with experimental manipulation of a specific brain area including the LHb as for a particular interest for this study.

Aims and the rationale of this study

Based on the literature reviewed above, it was hypothesized that the LHb could play an influential role in the DA dependent reward-related behaviors. The DRL behavioral task was used as the major measurement of DA dependent reward-related behaviors. In considering the importance of the interval applied in DRL task, there are two intervals (15 sec and 72 sec) set in the DRL behavioral tasks employed in the stusy, namely a DRL 15-s and a DRL 72-s task. To encounter the inconsistent results caused by different parameters of LHb stimulation applied in the previous reports, the first aim of this study tested by two experiments (Experiment 1 and Experiment 2) was set to verify the parameters of intensity, frequency, and duration for LHb stimulation that could effectively alter the locomotor activity or DRL 15-s task. After building a model of DRL 15-s behavioral changed by LHb stimulation, the

second aim of this study as challenged by Experiment 3 was set to verify whether DA was involved such a behavioral alteration by LHb stimulation. Selective DA D1 and D2 receptor antagonists (SCH23390 and eticlopride, receptively) were administered in combining with LHb stimulation to examine pharmacologically to see if a DA dependent reversal effect is existed. The third aim of this study was set to see if a noradrenergic dependent reversal effect is existed in DRL 15-s behavioral alteration by LHb stimulation. Experiment 4, thus, was conducted by pharmacological treatments of norepinephrine (NE) α1, α2 and β receptor antagonists (prazosin, yohimbine and propranolol, respectively) given in combining with the LHb stimulation on DRL 15-s behavior. The forth aim of this study was set to examine whether the DRL behavior affected by LHb stimulation would be depended by the length of interval applied in its reinforcement schedule. Accordingly, Experiment 5 tested the effects of LHb stimulation on a DRL 72-s behavioral task. Finally, regarding to the fifth aim, the effects of LHb stimulation were tested in discrimination task which required the rat's ability to differentiate the different magnitudes of reward in a two-lever operant chamber (Experiment 6). Together, the results of this study were expected to answer how the LHb is involved in the reward-related motivation as measured by DRL behavior in the subject with the activated LHb by electrical stimulation.

Materials and methods

Subjects

Male (300~350 g) Wistar rats were the subjects obtain from the BioLACO Taiwan Co.,Ltd. All animals were housed individually and allowed *ad libitum* access to food and water. The colony was maintained on a 12-hour light / dark cycle with lights on 08:00 AM, where the room temperature was kept at 22 ± 2 °C. The subjects were treated by a water restriction regimen before the DRL behavioral experiment, and a food restriction regimen before a discrimination behavioral experiment. The water restriction was conducted by gradually reducing the daily access of water to 5 minutes every day. The food restriction was conducted by gradually reduced the daily food intake of 15 g lab chow, which led the subjects body weight remained in about 85 % of free-feeding of body weight. All experiments were conducted following the regulation by the animal use and care committee of National Cheng-Chi University.

Apparatus

DRL operant behavior

Six operant conditioning chambers (Med-Associates, Inc.; St Albans Vermont) were used. Each chamber (30 cm x 20 cm x 25 cm) has one press lever, one house light and a liquid dispenser as controlled by solenoid value. The chamber's floor was formed by 18 stainless steels (5 mm of diameter) separated in 11 mm. The liquid dispenser supplied the water reinforcer based on behavioral contingency set up in each experiment, and each reinforcement supply was 0.04 ml of tab water. Each chamber was placed in a separate wooden soundproof box with a fan to provide ventilation and white noise. All chambers were connected to a microcomputer that controlled the behavioral program and data collection. The raw data were collected

from the inter-responses times (IRT), each generated from a lever press. The raw data were then reduced and calculated into seven variables including total responses, reinforced responses, non-reinforced responses, burst responses, peak rate, peak time and modified response efficiency (MRE). See Cheng and Liao (2007) for additional details.

Locomotor activity

Four black acrylic boxes (45 x 45 x 36 cm each) were assembled and used to measure the locomotor activity. A charge coupled device (CCD) camera was set up 52 cm high from the bottom and located in the center of the four-box assembly. Controlled by a desk-top computer, the CCD simultaneously recorded the distance (mm) that a subject traveled in each of the four boxes.

DRL behavioral training

After manually shaping, the water-deprived rat first learned to press the lever for water reward under a fixed-ratio 1 (FR-1) schedule. When the rat pressed the lever over 40 times in one daily session of 30 min, the DRL schedule of reinforcement was then introduced. In which, a reinforcer was delivered contingent upon a lever press if the time had elapsed since the previous press over the DRL interval set up. Premature responses led to a non-reinforcement contingency and a resetting of the interval delay, as indexed by a non-reinforced response. Each lever press, whether reinforced or not, reset the delay timer. Two intervals, 15 sec and 72 sec, were chosen for the DRL behaviors applied in the present study. A total of five groups of the rats received the DRL behavioral training, in which four groups were trained on the DRL 15-s task and one group was trained on the DRL 72-s task (see details below). For the DRL 15-s behavioral training, after the stabilized performance on FR-1

schedule, the rat was directly subjected to respond for the DRL 15-sec schedule of reinforcement. The subjects reached a steady baseline of DRL 15-s after 25 training sessions (each of 30 min). A criteria to determine the stable baseline level of DRL 15-s behavioral performance was referred by the MRE ratio equal or greater than 0.45 (Cheng et al., 2008). In the DRL 72-s behavioral training, after the initial lever-press training, the subjects were trained on a DRL 18-s schedule for eleven sessions (each of 60 min). Then, the interval of schedule requirement was increased to 72 sec. The subject was run in DRL 72-sec behavioral training for 55 days to reach a stable baseline level. However, to avoid the potential of extinction on operant responding, in the DRL 72-s training, the subject was run with longer daily sessions from the beginning. Such that, within the 55 days of DRL 72-sec behavioral training, the 3 hr training session run for the first 3 days, 2 hr training session run for the next 14 days, and the regular 1 hr training session run for the rest of 38 days. The criterion for the definition of a stable baseline for DRL 72-s task was determined by both the total responses less than 70 and the reinforced responses greater than 12, which performance was also less than 10% variation in the response rate for three consecutive sessions (Zhang et al., 2006). The manipulation of LHb stimulation and pharmacological treatment was only conducted in the rat with a stable performance on either DRL 15-s or DRL 72-s behavioral task.

Discrimination task

A behavioral testing of simple discrimination of different amount of reward was conducted in four operant chambers, each chamber (30.5 x 24 x 21 cm) enclosed in sound-attenuating boxes (Med-Associates, St. Albans, VT., USA). Each box was equipped with a fan to provide ventilation and to mask extraneous noise. Each operant chamber had two retractable levers on the front wall. The holes where the

levers extended were symmetrically 1 cm from the left and right side walls, and 10 cm from the floor. The food reinforcement (45 mg; Bioserv, Frenchtown, NJ) was delivered via a pellet dispenser to a food receptacle (2 x 2.5 cm) set in the middle of two levers and 6.5 cm above the floor. The operant chamber was illuminated by a single 100 mA house light located in the top-center of the wall opposite the levers. The lever choices and reaction latency were recorded by a desk-top computer connected to the chambers via an interface.

Surgery

Before a surgery conducted in a stereotaxic instrument (Stoelting Co.), the rat was anesthetized by intraperitoneal injection (i.p.) of Zoletil 50 (Virbac, Carros, France) in a dose of 1 mg/kg. The subject was implanted with the homemade electrode. The electrode was implanted into the left lateral habenula in coordinated from the bregma, AP = -3.6 mm, ML = +0.7 mm, and DV = -4.8 mm (Paxinos & Watson, 2007). After the electrode placement, the electrode was fixed to the skull with acrylic dental cement to secure its patency. Penicillin (20,000IU) was injected post-surgery with 0.2 ml and intramuscular injection (i.m.) to reduce the potential occurrence of wound infection. The subject was allowed one week to recover from surgery.

Electrode preparation and habenula stimulation

The electrode was a bipolar self-designed stimulating electrode with two stainless steel wires (A-M System, Sequim, WA, USA) in a guide cannula (0.33 mm inner diameter, 0.63 mm outer diameter and 10 mm length). The stainless steel wire was 0.02 mm inter diameter for the stainless steel itself and 0.045 mm outer diameter as measured with the coated insulating materials. The stainless steel wires were extended 1 mm from the one end of guide cannula, and where the other end was

welded into a two channel bases as for connecting the electrical wires from the stimulator.

The electrode was implanted into the lateral habenula of the left hemisphere for all the subjects received the lateral habenula stimulation. The subjects, thus, received habenula stimulation unilaterally. And, the lateral habenula stimulation was given for 15 min each time and conducted right before the behavioral session. The parameters of LHb stimulation current were tested for 0.05 mA and 0.1 mA with a 0.5 ms spike-duration. The stimulation frequencies were manipulated at 10 Hz and 100 Hz. A diagram illustrating these parameters is shown Fig 1C.

Drugs

D-amphetamine sulfate (Sigma-Aldrich, St. Louis, USA), SCH23390 hydrochloride (Tocris, Ellisville, MO, USA), eticlopride hydrochloride (Tocris, Ellisville, MO, USA) and yohimbine hydrochloride (Sigma-Aldrich, St. Louis, USA) were dissolved in 0.9 % saline. Prazosin hydrochloride (Research Biochemicals International, RBI, Natick, MA, USA) and propranolol hydrochloride (Sigma-Aldrich, St. Louis, USA) were dissolved in distilled water. All drugs were administered by intraperitoneal injection in a volume of 1 ml/kg, and the injection was conducted at 20 min before behavioral measurement.

Procedures

Experiment 1: the effects of LHb stimulation on locomotor activity

The rat was gently handled by experimenter for two weeks before receiving the locomotor activity tests and the LHb stimulation. A group of the rats (*n* = 12) was repeatedly used to test the locomotor activity conducted at 0, 10 and 20 min after the end of LHb stimulation. These subjects were further divided into three subgroups (*n*

= 4 each) which received a specific treatment of stimulation current intensity: 0 mA, 0.05 mA or 0.1 mA. These three tests were conducted every other day. The subjects were then remained in the colony for one week, during which a daily 15 min of experimenter's holding was given. Subsequently, a dose effect of amphetamine on locomotor activity was evaluated. For drug dosing treatment, the rat was repeatedly injected amphetamine at doses of 0, 1, and 2 mg/kg under a Latin square design. Three injection days were administered every other day. The locomotor activity was conducted for 15 min after injection. The data of each locomotor test were collected in three 5 min blocks that offered the within-session analysis.

Experiment 2: the effects of LHb stimulation on DRL 15-s behavior

A group of rats (*n* = 12) was initially recruited and used to test the effects of LHb stimulation on DRL 15-s behavior. When the stable maintenance levels were reached, the subject received a surgery of electrode implantation. Following the surgical recovery, the rats were re-trained on DRL 15-s behavior for one week. The rats were then subjected to test the effects of LHb stimulation on DRL 15-s behavior. The parameters for electrical stimulation frequencies (0, 10 and 100 Hz) were manipulated and each test given in a separate day. The aforementioned treatments were conducted in a sequential order of the sham stimulation (sham S), LHb stimulation given at 10 Hz (LHb S 10 Hz) and LHb stimulation given at 100 Hz (LHb S 100 Hz). Behavioral data completely collected after a successful LHb stimulation were obtained by 12 subjects for 0 Hz treatment, 9 subjects for 10 Hz treatment and 9 subjects for 100 Hz treatment. Missing data were due to the incompleted LHb stimulation caused by the electrode implantation being damaged or dropped. The DRL 15-s test session lasted for 15 min. On the day after the LHb stimulation test, the rats were only run in DRL 15-s task without the manipulation of electrical stimulation.

Experiment 3: the effects of DA antagonists on DRL 15-s behavioral alterations induced by LHb stimulation

A group of rats (*n* = 12) was initially recruited and used to test whether DA antagonists would reverse DRL 15-s behavioral alterations induced by LHb stimulation or not. The subjects with the implanted electrode were ensured with a stable performance on DRL 15-s task before the pharmacological tests. Referred by the results from the experiments described above, the parameters of the LHb stimulation conduced in this experiment were set by 900 sec of duration, 100 Hz of frequency, and 0.05 mA of intensity. Doses tested for D1 and D2 receptor antagonists, respectively, were 0, 0.003 and 0.01 mg/kg of SCH23390 and 0, 0.01, and 0.03 mg/kg of eticlopride. Each rat received an injection prior to a stimulation treatment in an experimental day, which was followed by a solely DRL 15-s re-running daily session. Accordingly, under a within-subject design applied in this experiment, the rat was repeatedly tested with six treatments including 1) saline vehicle with sham stimulation initialized as “veh + sham S”, 2) saline vehicle with LHb stimulation initialized as “veh + LHb S”, 3) SCH23390 of 0.003 mg/kg with LHb stimulation initialized as “SCH 0.003 + LHb S”, 4) SCH23390 of 0.01 mg/kg with a LHb stimulation initialized as “SCH 0.01 + LHb S”, 5) eticlopride of 0.01 mg/kg with LHb stimulation initialized as “eti 0.01 + LHb S”, and 6) eticlopride of 0.03 mg/kg with LHb stimulation initialized as “eti 0.03 + LHb S”. Behavioral data completely collected after a successful LHb stimulation were obtained by 12, 8, 8, 6, 8 and 6 rats for the aforementioned treatments, correspondingly. And, the rat was tested DRL 15-s for 15 min immediately after the completion of each of aforementioned treatments.

In order to better elucidating the interaction between DA drugs and LHb stimulation, a drug treatment with sham stimulation should be taken into account with the treatments described above. An additional experiment was conducted, by

recruiting naïve rats (*n* = 12), to examine the dose effects of SCH23390 and eticlopride on DRL 15-s behavior. The experimental protocols of DRL 15-s training and electrode implantation were same as those described above. The rats then received four treatments in sequence: 1) saline vehicle with sham stimulation initialized as “veh + sham S”, 2) saline vehicle with LHb stimulation initialized as “veh + LHb S”, 3) SCH23390 of 0.01 mg/kg with sham stimulation initialized as “SCH 0.01 + sham S”, and 4) eticlopride of 0.03 mg/kg with sham stimulation initialized as “eti 0.03 + sham S”. Behavioral data completely collected after a successful treatments were obtained by 12, 8, 7 and 7 rats for the aforementioned treatments, correspondingly.

Experiment 4: the effects of NE antagonists on DRL 15-s behavioral alterations induced by LHb stimulation

A group of rats (*n* = 12) was initially recruited and used to test the effects of NE antagonists on DRL 15-s behavioral alterations induced by LHb stimulation. The experimental design and protocols applied in this experiment were similar to those described in Experiment 3, except selective NE receptor agonists evaluated in this experiment. The dosing treatments included 0, 0.5, and1 mg/kg for prazosin, 0, 0.5, and1 mg/kg for yohimbine, and 0, 10, 20 mg/kg for propranolol. Thus, the rat was repeatedly tested with eight treatments including 1) vehicle with sham stimulation initialized as “veh + sham S”, 2) vehicle with LHb stimulation initialized as “veh + LHb S”, 3) prazosin of 0.5 mg/kg with LHb stimulation initialized as “pra 0.5 + LHb S”, 4) prazosin of 1 mg/kg with a LHb stimulation initialized as “pra 1.0 + LHb S”, 5) yohimbine of 0.5 mg/kg with LHb stimulation initialized as “yoh 0.5 + LHb S”, 6) yohimbine of 0.1 mg/kg with LHb stimulation initialized as “yoh 1.0 + LHb S”, 7) propranolol of 10 mg/kg with LHb stimulation initialized as “pro 10 + LHb S” and 8) propranolol of 20 mg/kg with LHb stimulation initialized as “pro 20 + LHb S”.

Behavioral data completely collected after a successful LHb stimulation were obtained by 12, 8, 8, 7, 7, 7, 7 and 7 rats for the aforementioned treatments, correspondingly.

Experiment 5: the effects of LHb stimulation on DRL 72-s behavior

A group of rats (*n* = 10) was used to test if LHb stimulation would affect operant behavior maintained on a DRL 72-s schedule. The rats had initially trained in DRL 72-s task and were run through a series of drug dosing tests (SNC80, amphetamine, GBR12909 and fluoxetine) for another project of this lab. To begin this experiment, the subjects were run in DRL 72-s for 48 sessions to ensure their stable performance on this behavioral task. Subsequently, the rat was implanted with the electrode aimed to the left LHb via the stereotaxic surgery. The subject was re-run in DRL 72-s task for 48 sessions before the LHb stimulation manipulated. The protocols set for LHb stimulation was the same as those described above.

Experiment 6: the effects of LHb stimulation on discrimination task

A group of rats (*n* = 4) was used to test whether the LHb stimulation could affect the rat's ability to discriminate different magnitudes of reward in a discrimination task run in a two-lever operant chamber. The procedure of discrimination task consisted of four blocks of 12 trials, two forced choice trials and ten free choice trials for each block. In which, a single response made on one of the two levers immediately led to a delivery of four pellets, whereas a press of the other lever caused a delivery of one pellet. The subject ran through this discrimination procedure in about 60 min daily. The subject reached the baseline of discriminate task by making 85 % of choices of large reward after one week training. Subsequently, the rats were received LHb stimulation with different current (0.05 mA and 0.1 mA) and tested by discrimination task in every other day.

Histology

At the conclusion of the experiments, the rat was anesthetized and transcardially perfused with PBS followed by 24 % paraformaldehyde. The brain was removed and immersed in preservation solution (100 ml 24 % paraformaldehyde, 165 ml distilled water, 12.2 g sucrose, 1 tablet of phosphate buffered saline) for 48 hours. The brain was then frozen on dry ice and slices (40 μm sections) using a cryostat microtome. Sections were mounted on glass slides (coated with 0.5 % gelatin), stained with Cresyl violet and subsequently examined under a microscope to verify the placement of the electrode.

DRL behavioral data

The DRL behavioral data were based on the IRT's collected from each session for each rat. In addition to categorizing the IRT's into the total responses, reinforced responses, and non-reinforced responses, a plot of IRT distribution curve was made for both qualitative and quantitative analyses. The IRT data of DRL 15-s behavior were plotted into a distribution with response frequencies for 30 consecutive 1 sec time bins, while the IRT data of DRL 72-s behavior would be plotted in to a distribution with response frequencies for 144 consecutive 1 sec time bins. A bimodal IRT distribution was reliably shown at the baseline stage as well as the control condition. Quantitative analyses of the IRT distribution included burst responses, peak time, peak rate, and MRE ratio. The burst responses were the summed frequencies of IRT less than 2 sec. The peak rate and peak time were calculated from the de-burst IRT's, in which a moving average based on four consecutive 1 sec bins was applied to smooth the distribution. With the maximum frequencies of a 4 sec epoch identified, the peak time was the mean value of the IRT's that fell within those four bins. The peak rate was calculated by the summed frequencies of those four bins divided by four. The MRE ratio, indexed the response efficiency, was calculated from

reinforced responses divided by de-burst responses: [number of reinforced responses ÷ (number of total responses – number of burst responses)].

Statistical analyses

The data for each dependent variable were subjected to analyses of variance (ANOVA) following the experimental design used. All the results are presented as the mean with the standard error (mean ± sem). Locomotor activity data of Experiment 1 were analyzed by a three-way ANOVA for the factors of stimulation current, blocks and time. In which, the stimulation current was on a between-subject design, while the blocks and time were the within-subject repeated measures. DRL behavioral data collected from Experiment 2 to Experiment 5 were analyzed by a one-way repeated ANOVA on each of dependent variables including the total responses, reinforced responses, non-reinforced responses, burst responses, peak rate, peak time, and MRE ratio. The data of discrimination behavior in Experiment 7 were first analyzed by a one-way repeated ANOVA on the stimulation frequency. And, a two-way repeated ANOVA was further conduced for a within-session analysis on the factors of stimulation frequency and block. Statistical significance was determined by $p < 0.05$. When the main effect was significantly yielded from ANOVA, the post hoc test was run by the use of *Scheffe's* method. Simple main effect comparisons were conducted when a significant interaction was revealed. Statistical analyses were conducted using commercial software (Statistica version 5.5, Statsoft, Tulsa, OK, USA).

Results

Experiment 1: the effects of LHb stimulation on locomotor activity

Figure 3. shows the effects of electrical stimulation in LHb on locomotor activity. The results of a three-way ANOVA only revealed a significant main effects of block, $F_{(2, 81)} = 94.5$, $p < 0.001$. Post hoc tests following the block main effect confirmed that traveling distance in first block was significantly longer than that of the second and third blocks (both $p < 0.001$). And, a significant difference was detected in between the second and third blocks ($p < 0.001$).

Figure 4. shows the dose effects of amphetamine on the distance of locomotor activity. The results of a two-way ANOVA revealed significant main effects of dose ($F_{(2, 99)} = 15.98$, $p < 0.001$) and block ($F_{(2, 99)} = 54.14$, $p < 0.001$), but no significant interaction. Post hoc tests following the dose main effect revealed that traveling distance was significantly increased by given at 1 mg/kg and 2 mg/kg (both $p < 0.001$) treatments, but no significant difference in between treatments of 1 mg/kg and 2 mg/kg. Post hoc tests following the block main effect confirmed that traveling distance in first block was significantly longer than that of the second and third blocks (both $p < 0.001$), but no significant difference in between the second and third blocks.

Experiment 2: the effects of LHb stimulation on DRL 15-s behavior

Figure 5. presents the effects of LHb stimulation on DRL 15-s behavior, as measured by total responses, reinforced responses, non-reinforced responses, burst responses, peak time and peak rate. LHb stimulation increased the total responses only in a marginal significance, $F_{(2, 27)} = 3.26$, $p = 0.053$ (Fig. 5*A*). LHb stimulation significantly decreased the reinforced responses, $F_{(2, 27)} = 5.98$, $p < 0.01$ (Fig. 5*B*). Post hoc tests revealed the significant decreases of reinforced responses by low

frequency ($p < 0.05$) and high frequency ($p < 0.01$) treatments. LHb stimulation significantly increased the non-reinforced responses, $F_{(2, 27)} = 5.41$, $p < 0.01$ (Fig. 5*C*). Post hoc tests showed that the non-reinforced responses were significantly increased by low frequency ($p < 0.05$) and high frequency ($p < 0.001$) treatments. LHb stimulation produced a marginally significant increment on the burst responses, $F_{(2, 27)} = 3.20$, $p = 0.056$ (Fig. 5*D*). LHb stimulation significantly decreased the peak time, $F_{(2, 27)} = 6.11$, $p < 0.01$ (Fig. 5*E*). Post hoc tests showed that the peak time was significantly decreased by either low frequency ($p < 0.05$) or high frequency ($p < 0.01$) treatment. In contrast to the five dependent variables described above, the peak rate was not significantly affected by LHb stimulation (Fig. 5*F*).

Figure 6. presents the effects of LHb stimulation on the MRE ratio of DRL-15 behavior, and the IRT distribution curves are plotted. LHb stimulation significantly decreased the MRE ratio, $F_{(2, 27)} = 6.71$, $p < 0.01$ (Fig. 6*A*). Post hoc tests showed that MRE ratio was significantly decreased by either low frequency ($p < 0.05$) or high frequency ($p < 0.001$) treatment. The electrical stimulation of LHb produced a leftward shift on the IRT distribution (Fig. 6*B*).

Experiment 3: the effects of DA antagonists on DRL 15-s behavioral alterations induced by LHb stimulation

Figure 7. presents the effects of SCH23390 and eticlopride on the alteration of DRL 15-s behavior induced by LHb stimulation, as measured by the six dependent variables. The results of ANOVA revealed that co-adminstration drugs with LHb stimulation significantly affected the total responses, $F_{(5, 41)} = 4.22$, $p < 0.01$ (Fig. 7*A*), non-reinforced responses, $F_{(5, 41)} = 3.67$, $p < 0.01$ (Fig. 7*C*) and burst responses, $F_{(5, 41)} = 4.27$, $p < 0.01$ (Fig. 7*D*). In comparing to the control, post hoc tests on total responses revealed a significant increment by LHb stimulation ($p < 0.01$). Such an

effect was significantly reversed by the higher dose of SCH23390 or eticlopride, whereas no reversal effect was detected for the lower dose of either drug. The results of post hoc comparisons on non-reinforced responses and burst responses were similar to those of total responses described above. In contrast to the three dependent variables described above, the reinforced responses, peak time and peak rate were not significantly affected by co-adminstration drugs with LHb stimulation.

Figure 8. presents the effects of SCH23390 and eticlopride on the alteration of DRL 15-s behavior induced by LHb stimulation as measured by MRE ratio, and the IRT distribution curves are plotted. The results of ANOVA revealed that co-adminstration drugs with LHb stimulation significantly affected the MRE ratio, $F_{(5, 41)} = 2.68$, $p < 0.05$ (Fig. 8*A*). In comparing to the control, post hoc tests revealed a significant decrement on the MRE ratios by LHb stimulation ($p < 0.05$). Such an effect was significantly reversed by the higher dose of SCH23390 or eticlopride, whereas no reversal effect was detected for the lower dose of either drug.

Figure 9. presents the effects of SCH23390 and eticlopride on DRL 15-s behavior, as measured by the six dependent variables. The results of ANOVA revealed that experimental treatments of drug administrations and LHb stimulation significantly affected the total responses, $F_{(3, 28)} = 6.45$, $p < 0.01$ (Fig.9*A*), non-reinforced responses, $F_{(3, 28)} = 6.77$, $p < 0.01$ (Fig.9*C*), burst responses, $F_{(3, 28)} = 7.07$, $p < 0.01$ (Fig.9*D*) and peak rate, $F_{(3, 28)} = 3.52$, $p < 0.05$ (Fig.9*F*). In comparing to the control, post hoc tests on total responses revealed a significant increment by LHb stimulation ($p < 0.01$). Such an effect was not significantly detected for the dose treatment of SCH23390 or eticlopride. The results of post hoc comparisons on non-reinforced responses and burst responses were similar to those of total responses described above. Post hoc tests on peak rate revealed a significant increment by SCH23390 ($p < 0.05$), and no such an effect for LHb stimulation or eticlopride. In contrast to those

four dependent variables described above, the measures of reinforced responses and peak time were not significantly affected by drug treatments or LHb stimulation (Fig.9*B* and 9*E*).

Figure 10. presents the effects of SCH23390 and eticlopride on DRL 15-s behavior as measured by MRE ratio, and IRT distribution curves are plotted. The results of ANOVA revealed a significant treatment effect on the MRE ratio, $F_{(3, 28)} = 4.44$, $p < 0.05$ (Fig.10*A*). Post hoc tests revealed only a significant decrement by LHb stimulation ($p < 0.05$).

Experiment 4: the effects of NE antagonists on DRL 15-s behavioral alterations induced by LHb stimulation

Figure 11. presents the effects of prazosin, yohimbine and propranolol on the alteration of DRL 15-s behavior induced by LHb stimulation, as measured by the six dependent variables. The results of ANOVA revealed that co-adminstration drugs with LHb stimulation significantly affected the total responses, $F_{(7, 55)} = 7.63$, $p < 0.001$ (Fig.11*A*), reinforced responses, $F_{(7, 55)} = 2.75$, $p < 0.05$ (Fig.11*B*), non-reinforced responses, $F_{(7, 55)} = 6.13$, $p < 0.001$ (Fig.11*C*), burst responses, $F_{(7, 55)} = 5.94$, $p < 0.001$ (Fig.11*D*) and peak rate, $F_{(7, 55)} = 4.50$, $p < 0.001$ (Fig.11*F*). In comparing to the control, post hoc tests on total responses revealed a significant increment by LHb stimulation ($p < 0.01$). Such an effect was significantly reversed by prazosin and propranolol given at the higher dose of, but not for the lower dose of either drug. No significant reversal effect was produced by yohimbine. The results of post hoc comparisons on non-reinforced responses were similar to those of total responses described above. Regarding to the reinforced responses, post hoc tests revealed only a significant decrement by the both doses of yohimbine given in combining with LHb stimulation. Post hoc tests on burst responses showed a significant increment

by LHb stimulation ($p < 0.001$). Such an effect was reversed by prazosin and propranolol, but not by yohimbine. Post hoc tests on peak rate revealed a significant increment by the both doses of prazosin ($p < 0.05$) and yohimbine ($p < 0.001$) with LHb stimulation.

Figure 12. presents the effects of prazosin, yohimbine and propranolol on the alteration of DRL15-s behavior induced by LHb stimulation as measured by MRE ratio, and IRT plots are shwon. The results of ANOVA revealed a significant treatment effect on the MRE ratio, $F_{(7, 55)} = 5.26$, $p < 0.001$ (Fig.12*A*). Post hoc tests on the MRE ratio revealed a significant increment by LHb stimulation ($p < 0.05$). Such an effect was not significantly reversed by any drug treatment back to the control level.

Experiment 5: the effects of LHb stimulation on DRL 72-s behavior

The effects of LHb stimulation on the DRL 72-s behavior as measured by the six dependent variables is presented in Table 1. Paired-samples *t*-test on the burst responses revealed a significant increment by LHb stimulation ($p < 0.05$). On peak time revealed a significant decrement by LHb stimulation ($p < 0.05$). None of other behavioral measurements was significantly affected by LHb stimulation (Table 1).

Experiment 6: the effects of LHb stimulation on a reward discrimination task

Figure 13. presents the effects of electrical stimulation in LHb on a reward discrimination task, as measured by choice of large reward, omission rate and response latency. The results of ANOVA revealed that LHb stimulation significantly affected the choice of large reward, $F_{(2, 45)} = 6.90$, $p < 0.01$ (Fig.13*A*), omission rate, $F_{(2, 45)} = 3.20$, $p < 0.05$ (Fig.13*B*) and response latency, $F_{(2, 458)} = 4.44$, $p < 0.05$ (Fig.13*C*). In comparing to the control, post hoc tests that the higher, but not the low, frequency of LHb stimulation significantly decreased the choice of large reward and

increased the omission rate and response latency (all $p < 0.05$).

Within-session analyses of the effects of lateral habenula (LHb) stimulation on a reward discrimination task are presented in Table 2. Regarding to the choice of large reward, the results of a two-way ANOVA revealed a significant main effect of frequency, $F_{(2, 36)} = 9.52$, $p < 0.01$, and a significant frequency-by-block interaction, $F_{(6, 36)} = 2.49$, $p < 0.05$. Post hoc tests following the frequency main effect revealed only a significantly decrement by the high frequency treatment ($p < 0.05$). From simple main effect comparisons, the trend of a lower choice of large reward rate under the 100 Hz LHb stimulation was not significantly confirmed over all four blocks in comparing to the corresponding blocks of the control ($p > 0.05$). Regarding the omission rate, the results of a two-way ANOVA significantly yielded main effects of frequency ($F_{(2, 36)} = 8.6$, $p < 0.05$) and block ($F_{(3, 36)} = 10.4$, $p < 0.01$) and its interaction, $F_{(6, 36)} = 7.43$, $p < 0.001$. Post hoc tests following the frequency main effect were resulted by the significant increment by high frequency treatment ($p < 0.05$). Post hoc tests following the block main effect confirmed that the omission rate in the first block was significantly higher than those in the second ($p < 0.001$), the third ($p < 0.05$) and the fourth ($p < 0.01$) blocks. Simple main effect comparisons revealed a significant increment in the first block under high frequency treatment ($p < 0.05$), but no such an increment occurred in the other three blocks. In terms of the response latency, the results of a two-way ANOVA revealed significant main effects of the frequency, $F_{(2, 449)} = 11.49$, $p < 0.05$, and the block, $F_{(3, 449)} = 14.93$, $p < 0.001$. And the frequency-by-block interaction was significantly confirmed, $F_{(6, 449)} = 7.43$, $p < 0.001$. In comparing to the control, post hoc tests on the frequency factor revealed a significantly increment by the high frequency treatment ($p < 0.05$). Post hoc tests following the block main effect confirmed that the response latency in the first block was significantly more than the second ($p < 0.001$), the third ($p < 0.001$) and the fourth

($p < 0.01$) blocks, In comparing to the control, simple main effect on response latency revealed a significant increment by the first block in high frequency treatment ($p < 0.001$). In addition to the first block in high frequency treatment, there was no any significantly affected between frequencies and blocks. (The results of post hoc tests and simple main effect comparisons for the measure of response latency revealed a similar pattern to those described on the omission rate.)

Discussion

The present study mainly investigated whether the LHb stimulation would affect operant behavior maintained on DRL schedule of reinforcement. Experiment 1 showed that the LHb stimulation as manipulated by two currents (mA) and tested at three different time points after stimulation did not affect locomotor activity. The lower current of LHb stimulation given immediately before behavioral test was then set for the follow-up experiments. Two frequencies (Hz) of LHb stimulation was tested on DRL 15-s behavior in Experiment 2, which found a significant frequency-dependent effect of LHb stimulation on DRL 15-s behavior. Such a behavioral change was pharmacologically reversed by SCH23390 and eticlopride as revealed by the results of Experiment 3. In Experiment 4, prazosin or propranolol was shown to produce a similar, but subtle, reversal effect on LHb stimulation induced DRL 15-s behavioral changes. In Experiment 5, the LHb stimulation was demonstrated to affect a DRL 72-s behavior, but with less degree of influence (on two out of seven behavior measurements) as compared to that of DRL 15-s task. Experiment 6 confirmed that the LHb stimulation could affect the ability of discrimination, which effectiveness was significant only in the first block (out of four).

No effects on locomotor activity by LHb stimulation

In the first part of Experiment 1, the LHb stimulation did not affect on locomotor activity. This part of results was in consistent to a previous report by Friedman and associates (2010) showing that the traveling distance produced by LHb stimulation rat was not different from that by sham-operated rat. However, there was a report showing the locomotion was affected by LHb stimulation. The locomotor activity was increased in an animal model of depression by LHb stimulation given in chronic, 30

min every day for 28 days (Meng et al., 2011). In that study, the rat treated by that repeatedly exposed to a set of chronic mild stressors for 4 consecutive weeks was then subjected under a animal model of depression. In addition, the LHb stimulation was conducted in a long-term fashion before behavioral testing. Thus, the difference between these two studies and in comparing the present study, in terms of the effects of LHb stimulation on locomotion, could be attributed to different experimental protocols used among these studies. A question may be raised in concerning the negative results of LHb stimulation on locomotion in this study. That is, it might be due to the subjects were insensitive to any experimental treatment that is related to brain DA. Accordingly, the same rats were tested in the second part of Experiment 1, which results clearly showed those subjects were significantly affected by amphetamine. The acute injection of amphetamine increased the locomotor activity is a well established model to test the general motor function modulated by brain DA (e.g. Cole, 1978). Combining the results from the first and second parts of Experiment 1, it is indicated that the subjects could be sensitively affected by drug treatment agonizing brain DA systems to increase locomotion. Thus, the negative results of LHb stimulation applied in this experiment implied that the intensities of LHb stimulation applied in this study would not affect the general motor function. However, whether the DA level was altered by the LHb stimulation or not has not been examined in this study, which is essential before making a conclusive remark.

DRL 15-s behavior affected by LHb stimulation

Experiment 2 showed that the LHb stimulation significantly affected DRL 15-s behavior in a frequency-dependent manner. Furthermore, these behavioral changes by LHb stimulation are similar to those induced amphetamine on DRL behavior with interval set in a range of 10 to 20 sec (Liao, 2009). A previous study of this lab

showed that amphetamine increased the total responses, non-reinforced responses and burst responses, but decreased the reinforced responses and peak time (Cheng and Liao, 2007). With the similarity of DRL 15-s behavioral effects produced by the LHb stimulation and amphetamine treatment, there might be a common mechanism was shared for these two treatments. In terms of neural substrates, with amphetamine pharmacologically acting as a DA agonist, it was then inferred that the behavioral alterations induced by LHb stimulation was modulated by DA related mechanisms. This was a rationale to carry out Experiment 3 and Experiment 4 (see the relevant discussion below).

The unilateral, but not bilateral, LHb stimulation was applied in the present and induced significant behavioral changes on DRL 15-s behavior. A question may be asked: how did the unilateral LHb stimulation on one side of the brain adequately affect the Hb and then change the behavior? A recent study addressed a strong connectivity between the Hb of left and right hemispheres causes a reliable influence each other via its connecting commissure in terms of anatomy (Kim, 2009). A few recent studies demonstrated significant behavioral effects also induced by the application of unilateral stimulation (Friedman et al., 2010, 2011; Li et al., 2011; Meng et al., 2011), which may support the aforementioned anatomical argument. So far, most (if not all) studies conducting with LHb stimulation may be due to a technical consideration. That is, the distance between the left and right hemispheres of habenula was about 1.4 mm. This distance was not allowed to make the bilateral implantation of two electrodes in the Hb. Even by the manipulation of unilateral stimulation in the LHb, behavioral changes on DRL 15-s behavior were significantly and reliably observed throughout this study.

The results of DRL 15–s behavioral alterations produced by the present LHb stimulation are worthy to discuss. According to a series of studies done by Hikosaka

and his associates (2008), low current stimulation in the LHb has been argued to induce an inhibitory effect in DA neurons. Consistent with this hypothesis, Friedman and associates (2011) showed that LHb stimulation attenuated the positive reward-associated reinforcement as measured by the self-administration of sucrose solution. Also, the DA release was reduced in the subject given that the LHb was activated by electrical stimulation (Lecourtier et al., 2008). These results were not compatible with what Experiment 2 found in this study, given that DA is positively correlated with reward-related processes of behavior or neurochemistry. The difference might be due to stimulation intensity parameters set and electrode used across these studies. The results from a pilot test conducted along with Experiment 1 showed a seizure-like effect was appeared in rats receiving LHb stimulation with the current given over 0.2 mA. Accordingly, the current used for present LHb stimulation was set less than 0.2 mA, where 0.2 mA (or higher amplitude) was applied to the other studies (Friedman et al., 2011; Li et al., 2011). Furthermore, Friedman and associates (2011) used two stainless steel electrodes and 1 mm between cathode and anode. In this study, the stainless steel wire was the same, but the distance between cathode and anode was almost close together. Thus, the electrical stimulation of the sphere of influence may also contribute to lead a different result in terms of the neuronal effectiveness. It is then possible that the results of Experiment 2 were derived from the LHb neurons suppressed by the present electrical stimulation. If so, it might cause the DA neurons to be activated and then induced the amphetamine-like behavior on DRL 15-s behavior. Two possibilities can be addressed to elaborate the aforementioned results. First, LHb stimulation may directly activate the soma of dopaminergic neurons located in the VTA and enhance DA release in the striatal areas. Li and associates (2011) administered a retrograde tracer (Alexa Fluor 488) in VTA to target the afferent inputs sent from the LHb.

Glutamatergic, rather than GABAergic, neurons were further characterized as the LHb efferents projecting to the VTA. Thus, LHb stimulation may directly activate the mesolimbic DA system. Second, if the present LHb stimulation still provokes the LHb neuronal activity, the efferent projection from LHb may have a double-synapse connection of inhibitory interneuron (e.g. GABA) before connecting to DA neurons. For instance, LHb efferents connecting to GABAergic neurons located in the rostromedial tegmentum (RMTg) were demonstrated by Hong and associates (2011), which may synapse to some ultra short GABAergic (inter)neurons within the VTA. Given this neuroanatomical presumption, the present LHb stimulation could produce a disinhibition on DA neuron that leads to a DA agonism effect. Further study conducted in a more systemic examination manner is needed for testify these possibilities.

The LHb has been demonstrated as one of brain sites that can be applied by the intracranial self-stimulation (ICSS; Vachon and Miliaressis, 1992). Accordingly, the LHb stimulation can generally produce a rewarding effect. It is, then, likely that the DRL behavior changes produced by the present LHb stimulation are similar to those affected by amphetamine or stimulant drugs. Systemic injection of stimulant drugs is known to affect behavior via drug action with pharmacological property of rewarding.

Does the LHb directly involve in the cores of DRL behavior? One of the key components required for individual to perform on DRL behavior is the timing capability (Skinner, 1938). A previous study showed that the LHb neurons encoded the multiple time scales of memory (Bromberg-Martin et al., 2010a). It means that the LHb neurons could encode behavioral outcomes of 6 trials prior to the testing trial in the primate subject. In that study, each trial (including its prior inter-trial interval) took approximately 8 sec to complete. Accordingly, in case the LHb neuronal firings to predict a reward and make an operant response, such a behavior may be depended

on the memory of episodes that occurred in the second-scaled timing. Thus, the LHb might be involved in the function of timing throughout those 6 trials described above. Therefore, the LHb stimulation might affect the timing process where any behavioral performance relies.

Despite an accumulating data in supporting an inhibitory role of the LHb over the midbrain DA neurons (Christoph et al., 1986; Matsumoto and Hikosaka, 2007; Ji and Shepard, 2007; Reisine et al., 1982), some controversial results were reported for DA related behaviors as measured when the LHb had been manipulated by either lesion or stimulation. Wang and associates (2009) reported a negative result of electrolytic lesion on heroin self-administration. Using ICSS model, the reward effectiveness of the brain stimulation in the lateral hypothalamus, VTA, or dorsal raphe nucleus was attenuated by electrolytic stimulation (Morissette and Boye. 2008). More recently, Gifuni and associates (2012) showed that LHb lesion by ibotenate enhanced the locomotion response to amphetamine, but did not alter the reward-potentiating effect of amphetamine on ICSS in the medial forebrain bundle (MFB) or the posterior mesencephalon. All these data indicate the involvement of LHb in modulating the DA related behavior may be more complex at behavioral or system level than it was thought on the basis of in vitro tests. Further, how the LHb affect the DA related behavior may be task dependent. More work is needed before a conclusion can be made for this issue.

DA antagonists reversed the effects of LHb stimulation on DRL 15-s behavior

Behavioral alterations induced by LHb stimulation on DRL 15-s were reliably seen in different experiments conducted in this study. Even though it is still not clear about the inhibitory role of LHb stimulation can be play on DA system, one way to help delineating this issue could be approached by pharmacological antagonism tests.

Thus, with the assumption that behavioral alterations induced by LHb stimulation on DRL 15-s were DA dependent, it is then expected to observe that DA antagonist blocks those behavioral changes by LHb stimulation. These data collected from Experiment 3 indicated that the LHb stimulation effects on DRL 15-s behavior were reversed by DA antagonists. These data first replicated the result of Experiment 2 showing the DRL behavioral changes by LHb stimulation. Further, the SCH23390 and eticlopride treatment administered with the LHb stimulation produced behavioral outcomes returning to the control level. Such an effect indicates the DA antagonist could reverse the behavioral effects produced by LHb stimulation.

Could the results in Experiment 3 be influenced by drug alone? A previous study of this lab showed that SCH23390 in 0.02 mg/kg decreased the total responses, non-reinforced responses, burst responses and peak rate on DRL 10-s behavior (Liao and Cheng, 2007). Compared with Experiment 3, SCH23390 in 0.01 mg/kg was no effect on DRL 15-s behavior. Therefore, the lower dose of DA antagonists could just reverse the effects of LHb stimulation but not affected the DRL behavior.

What is the relationship between the LHb and DA system? The previous studies showed that LHb efferent fibers projected to the VTA and SN. And, in return, the LHb receives afferent fibers from the VTA (Lecourtier et al., 2007, Geisler et al., 2008). This circuit has been argued to be involved in reward-related behavior (Bromberg-Martin et al., 2010a). Taking the results of Experiment 3 into account by following this argument, the DA circuit may participate in modulating the effects of LHb stimulation on DRL 15-s behavior. If the LHb stimulation affect behavioral performance was in along with increasing the level of DA release, the DA antagonists blocking the DA receptors could attenuate the DA-related changes. This inference is confirmed by the effects of LHb stimulation were reversed by DA antagonists as reported here.

Mixed results of NE antagonists reversing behavioral changes by LHb stimulation on DRL 15-s behavior

These data collected from Experiment 4 indicated that the LHb stimulation effects on DRL 15-s behavior were reversed by propranolol, but not by prozosin or yohimbine. The LHb stimulation may not rely on the NE system as much as the DA system to affect DRL 15-s behavior. What is the relationship between the LHb and NE system? The previous study indicated that LHb stimulation in depression model enhanced level of DA, NE and 5-HT in the brain tissue and the blood serum (Meng et al., 2011). However, the DRL behavior was more like to be as a kind of DA-related behavior (Liao, 2009). It is still possible that the NE level might be increased in the brain tissue and the blood serum by LHb stimulation, but the pharmacological reversal effects couldn't be detected when these effects were measured by the DRL behavior paradigm. Further tests are needed to verify this issue.

The results of Experiment 4 should be cautiously interpreted if taking the side effects induced by NE antagonists into consideration. Previous studies showed that yohimbine and propranolol, injected via peripheral route, significantly induced the side effect of lowering blood pressure (Okamoto et al., 2012, Richardson et al., 1968). Thus, the reversal effect of propranolol observed in Experiment 4 might be a result of side effect by this drug. For this reason, the intracerebroventricular (i.c.v.) injection is suggested to overcome this potential confounding effect induced by propranolol given by i.p. injection.

Less degree of influence on DRL 72-s behavior by LHb stimulation

In comparing with DRL 15-s behavior, Experiment 5 tested the effect of LHb stimulation on DRL 72-s behavior. The degree of behavioral changes by LHb

stimulation on DRL 72-s behavior was less than that observed on DRL 15-s behavior. Compare with DRL 15-s behavior, the waiting time for the subject to obtain reinforcer was increased to 72 s in DRL 72-s behavioral task. Even though the session time was increased to 60 min (in contrast to 15 min for DRL 15-s task), the response rate in DRL 72-s behavior was lower than that of DRL 15-s behavior. This may be a reason for observing the less effect by LHb stimulation on DRL 72-s behavior, namely the behavioral response was not sensitive. Could the effect of LHb stimulation on DRL 72-s behavior be magnified? Following the idea from a study using the animal model of depression to test LHb stimulation delivered in chronic (Meng et al., 2011), the less degree of LHb stimulation effect as tested on DRL 72-s behavioral task in Experiment 5 may be due to the acute (but not chronic) delivery of stimulation conducted in this study. If the duration time of stimulation would increase to 30 min and conducted over more daily sessions, it might a more significant change effect on DRL 72-s behavior.

The LHb stimulation for 15 min was always conducted right before the behavioral measure. It is, then, more accurate to describe what the present study investigated was engaged in examining the post-stimulation effects of DRL behavior and other behavioral tests. The session time of behavioral test after LHb stimulation might influence the test outcomes (e.g. DRL 15-s vs. DRL 72-s) as described above. Similarly, a few recent studies demonstrated significant behavioral effects as tested by the after effect of stimulation (Friedman et al., 2010, 2011; Li et al., 2011; Meng et al., 2011). Notice that most of the in vitro studies applied the LHb stimulation almost (if not all) at the same period of time when the neurophysiological or neurochemical test was conducted, which experimental protocols were different from what described above for the behavioral measures.

No effect of LHb stimulation on discriminability

To verify whether the LHb stimulation would change the ability of discriminating, Experiment 6 was tested on discrimination task. These data of Experiment 6 indicate that the choice of large reward, the omission rate and response latency altered by high frequency of LHb stimulation. But, from statistic tests of simple main effect, it is indicated that the significant change was only seen in the first block out of the four-block consisting test session on the measures of omission rate and response latency. The data of this part was surprising. The previous study showed that flupenthixol decreased choice of the large reward during the last two blocks (St. Onge et al., 2010). Compared with Experiment 6, the effects of LHb stimulation was only in the first block and the choice rate of large reward was 83.6% in high frequency stimulation. If the LHb stimulation would affect the discrimination task, the effects must be apparent more than just affecting in one block. In the second to the forth block, the three behavior measurements were back to the level of the control treatment. It is generally believed that the present LHb stimulation did not impair the discrimination ability in the rat.

The application of LHb stimulation on the clinical

One of the clinical applications of electrical stimulation is used to treat the symptoms of depression. The electrical stimulation of the subgenual cingulate white matter successfully treated depression symptoms in six patients for the first time (Mayberg et al., 2005). Following this study, the electrical stimulation in the treatment of depression has been attended. For example, the treatment of LHb stimulation successfully treated depression symptoms in human patients (Sartorius et al., 2007). Compatible with the animal model, Li and associates (2011) demonstrated that the LHb stimulation reduced the firing of LHb neurons, and acutely

reversed helpless behavior in rats. Therefore, with more studies from basic research in tackling the neurobiological mechanisms of LHb stimulation, it will be more promising to use LHb stimulation as for a clinical treatment of depression and the other psychiatric disorders may as well.

The roles of LHb on different stages of behavior processing

As mentioned above, this study examined the post (LHb) stimulation effects on certain behavioral tests. In this study, the LHb stimulation was always conduced in the subject who performed reliably in a specific behavioral task. Since different neural mechanisms are involved in distinctive stage of behavior from acquisition to expression. It is interested to learn if any behavior effect would be changed by the LHb stimulation given to the subject during the acquisition stage as comparing to the performance of a learned behavior. For instance, to conditioning the subject with a contextual box by brain stimulation, the subjects were stimulated in LHb during the acquisition on a place conditioning task (Friedman et al., 2011), which results showed LHb stimulation produced a conditioned place aversion. Furthermore, in a case of memory processing being involved, the strategy of manipulating the LHb stimulation immediate after the behavioral session or given at the end of learning trial(s). It is possible that the LHb stimulation given right after the behavioral session may change the memory consolidation processing of a specific behavioral task. In order to study the any brain site involved in memory consolidation processing, the striatal stimulation was given for 4 hours right after a behavioral learning session and tested the effect of consolidation in next day (Schumacher et al., 2011). Shumake and associates (2010) reported LHb stimulation given right after the gerbil made a correct avoidance response significantly impaired the acquisition of an avoidance learning, whereas VTA stimulation could enhance it. To understand the functions of the LHb during the

different stages of behavior, it is necessary to proceed LHb stimulation conducted with the behavioral task at different stages or timing points.

Conclusion and suggestion for future work

The present study applied the LHb stimulation to test DRL behavior. Significant behavioral alterations by LHb stimulation were revealed in DRL 15-s task, where a less degree of LHb stimulation effect was observed on DRL 72-s task. The former behavioral changes were similar to those induced an amphetamine on DRL behavior. And, DRL behavioral alterations induced by LHb stimulation were pharmacologically reversed by DA antagonists. These findings of DA dependent behavior change under LHb stimulation were independent to locomotor activity or the discrimination ability changed by the LHb stimulation.

In order to increase the time of electrical stimulation, it will be important for future studies to improve the electrode and the device. Anatomical relationship between the LHb and the raphé nucleus, the 5-HT antagonists are needed to test on DRL behavior. For the purpose of verify the effects of LHb stimulation, measurement of the extracellular neurotransmitters are demanded.

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Table 1. The effects of lateral habenula (LHb) stimulation on DRL 72-s behavior (n = 10, within-subject design).

LHb stimulation	sham control	LHb stimulation
total respones	86.4 ± 6.5	102.2 ± 8.0
reinforced responses	7.0 ± 1.7	6.9 ± 1.2
non-reinforced responses	79.4 ± 8.3	95.3 ± 9.3
burst responses	7.5 ± 2.6	9.2 ± 2.3 *
peak time (sec)	45.8 ± 3.4	31.0 ± 3.6 *
peak rate	5.6 ± 0.4	6.0 ± 0.5
MRE ratio	0.09 ± 0.02	0.08 ± 0.02

data presented by mean ± SEM

* different from the sham control $p < 0.05$ (paired *t*-test)

Table 2. Within-session analyses of the effects of lateral habenula (LHb) stimulation on a reward discrimination task (n = 4, within-subject design).

LHb stimulation	Blocks	Choice of LR (%)	Omission rate (%)	Latency (sec)
0 Hz	1	100 ± 0.00	0.00 ± 0.00	1.10 ± 0.20
	2	98 ± 3.00	0.00 ± 0.00	0.70 ± 0.10
	3	92 ± 5.00	5.00 ± 3.00	0.90 ± 0.20
	4	93 ± 3.00	25.00 ± 25.00	1.00 ± 0.10
10 Hz	1	100 ± 0.00	0.00 ± 0.00	1.10 ± 0.20
	2	100 ± 0.00	0.00 ± 0.00	0.80 ± 0.20
	3	100 ± 0.00	25.00 ± 25.00	0.70 ± 0.10
	4	100 ± 0.00	0.00 ± 0.00	1.10 ± 0.20
100 Hz	1	65 ± 17.00	35.00 ± 10.00 *	3.10 ± 0.60 ***
	2	80 ± 9.00	0.00 ± 0.00	1.20 ± 0.20
	3	95 ± 3.00	0.00 ± 0.00	0.60 ± 0.10
	4	95 ± 3.00	25.00 ± 25.00	1.00 ± 0.20

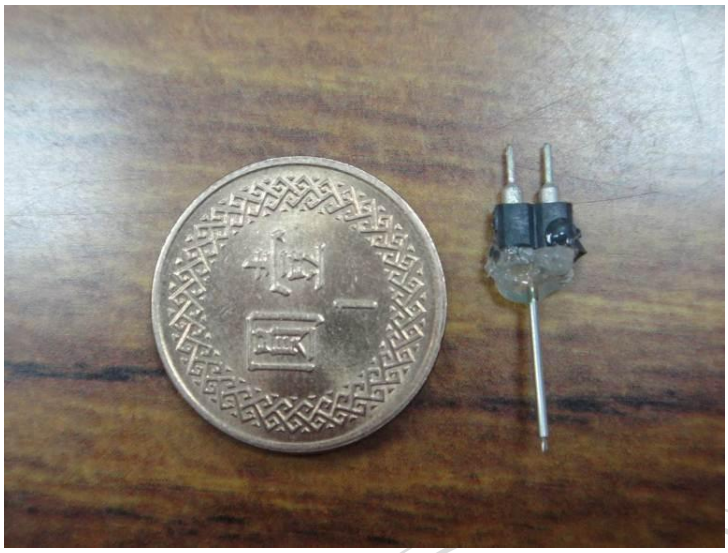
LR, large reward

*$p < 0.05$, ***$p < 0.001$ compared to the corresponding block of the control (0 Hz).

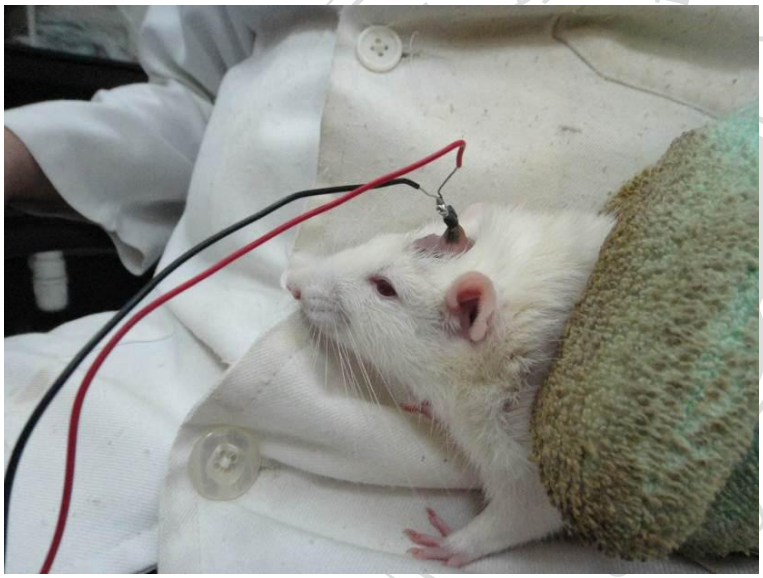
Table 3. Number of choices made in each 10-trial block on discrimination task.

LHb stimulation	subject no.	block 1	block 2	block 3	block 4	average
0 Hz	1	10	10	10	10	10
	2	10	10	9	9	9.5
	3	10	10	10	10	10
	4	10	10	9	10	9.75
10 Hz	1	10	10	10	10	10
	2	10	10	10	10	10
	3	10	10	10	10	10
	4	10	10	9	10	9.75
100 Hz	1	5	10	10	10	8.75
	2	7	10	10	10	9.25
	3	9	10	10	10	9.75
	4	5	10	10	9	8.5

A



B



C

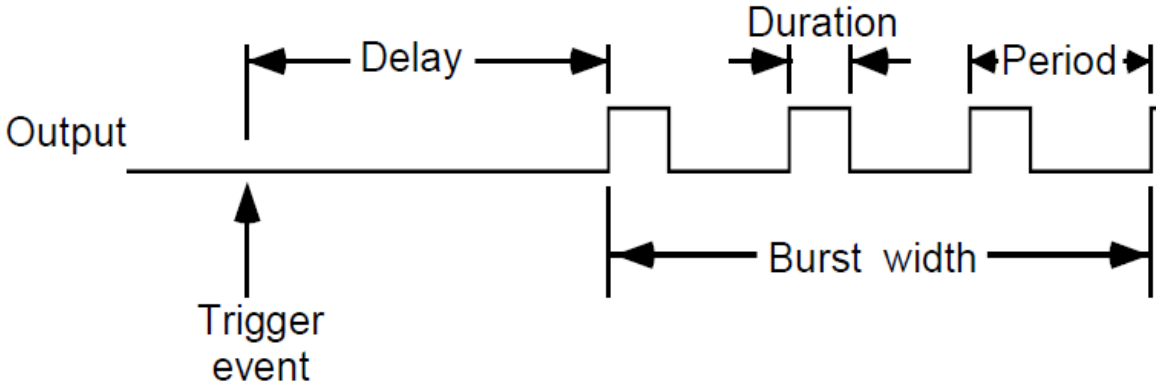
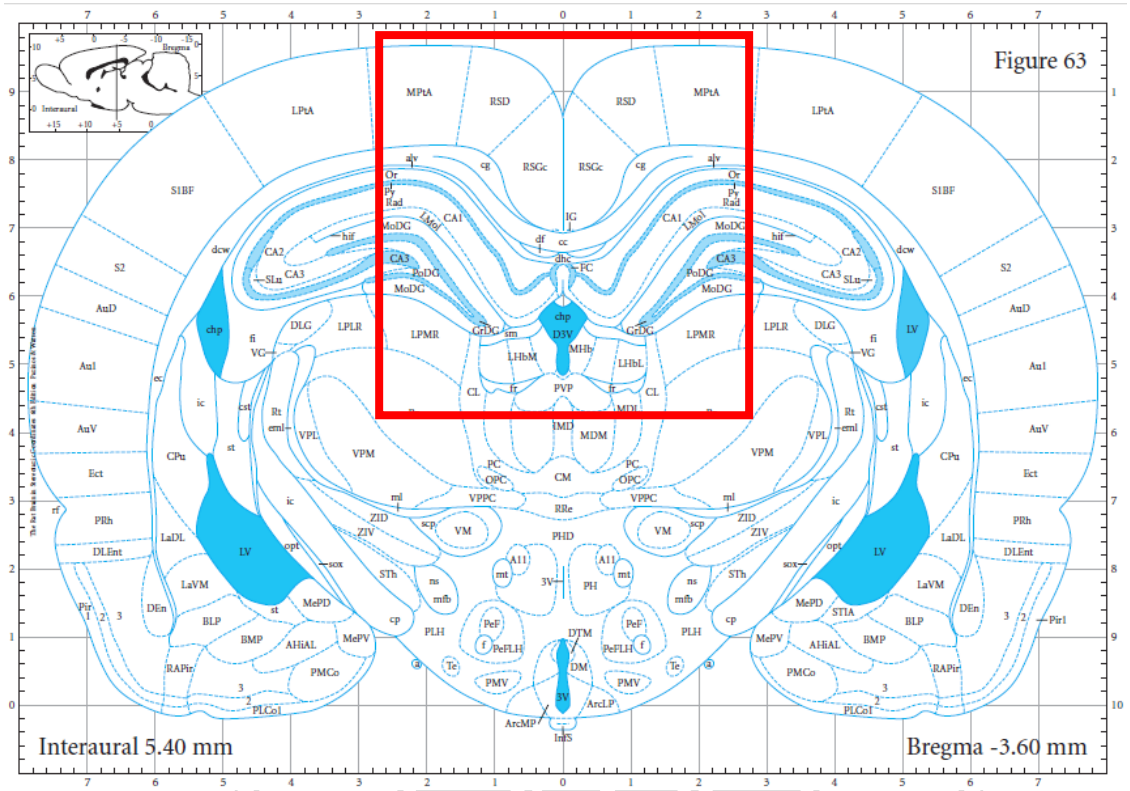


Figure. 1 The electrode (A), brain stimulation in the rat (B) and the square wave of electric stimulation (C).

A



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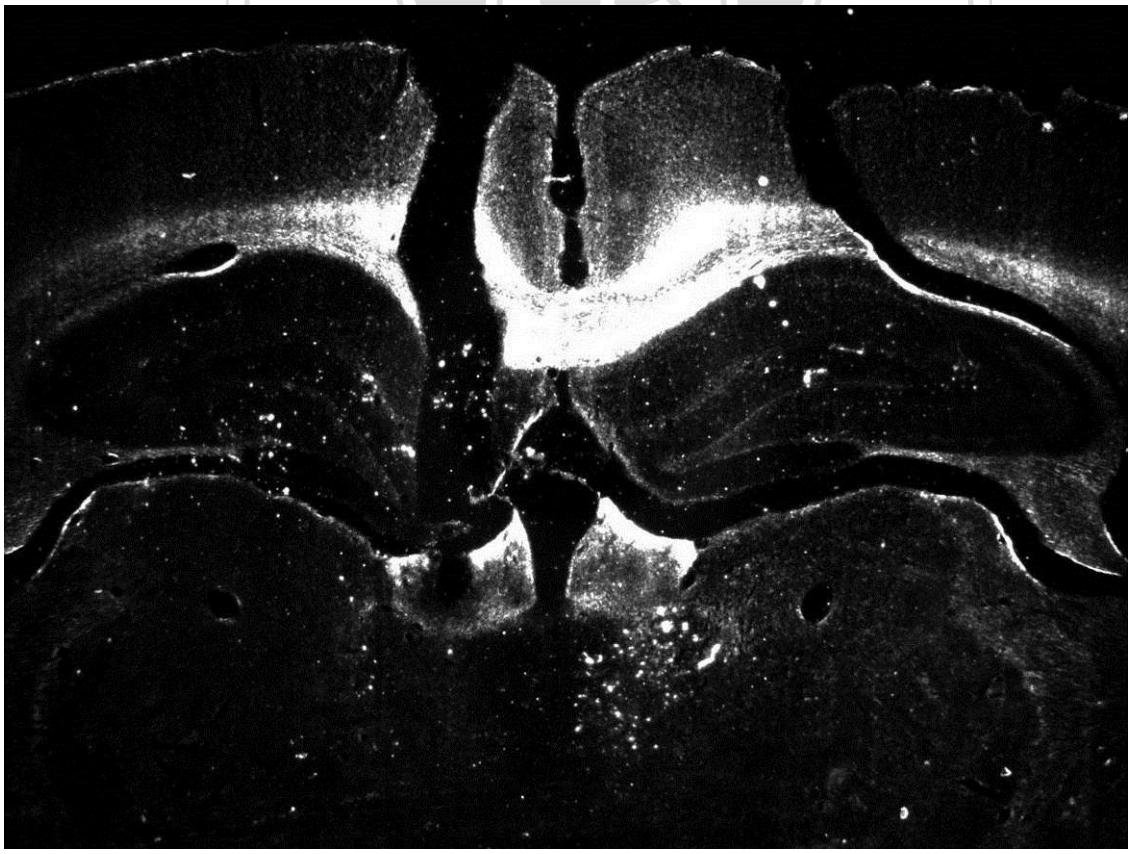

Figure. 2 Histology of brain section with the LHb.

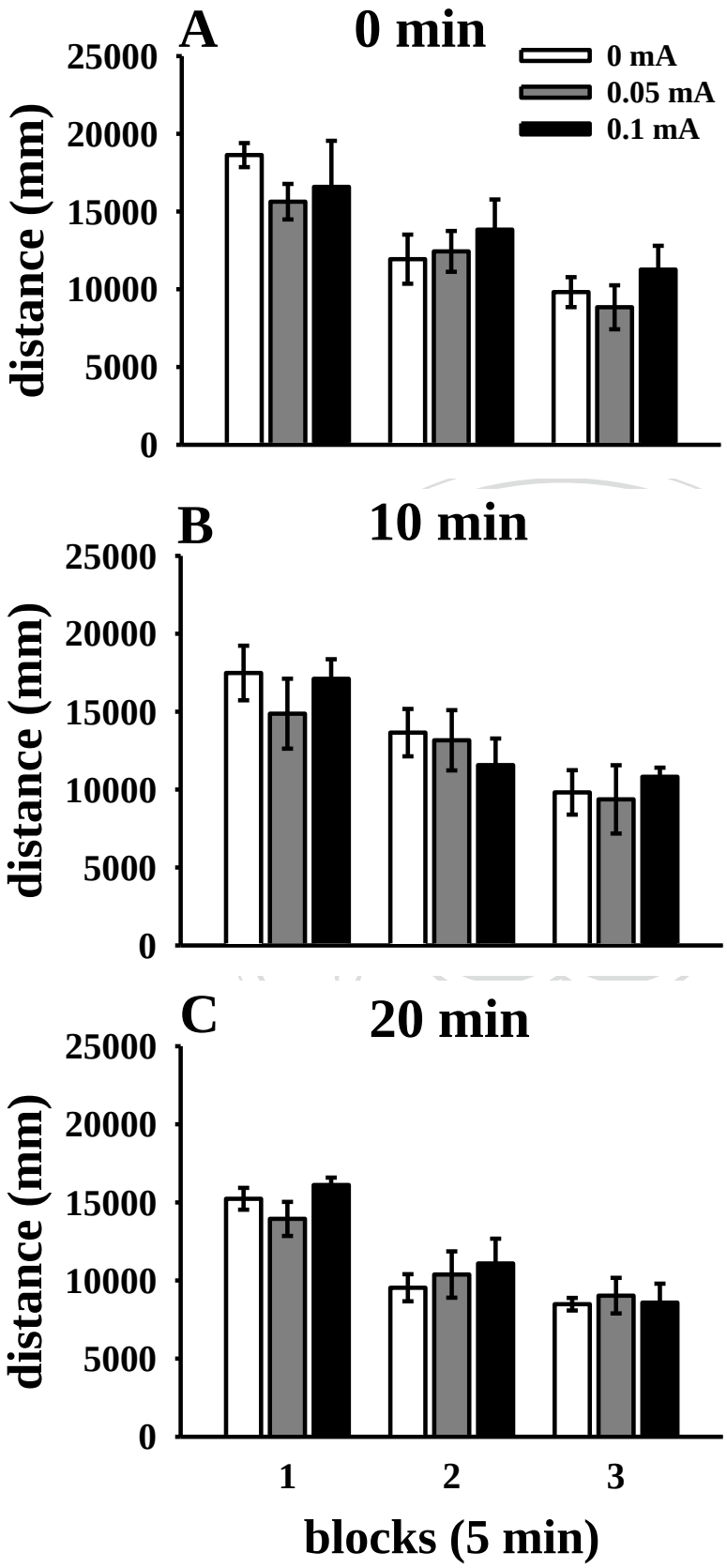

Figure. 3 The effects of electrical stimulation in LHb on locomotor activity (Experiment 1; n = 4 for each group).

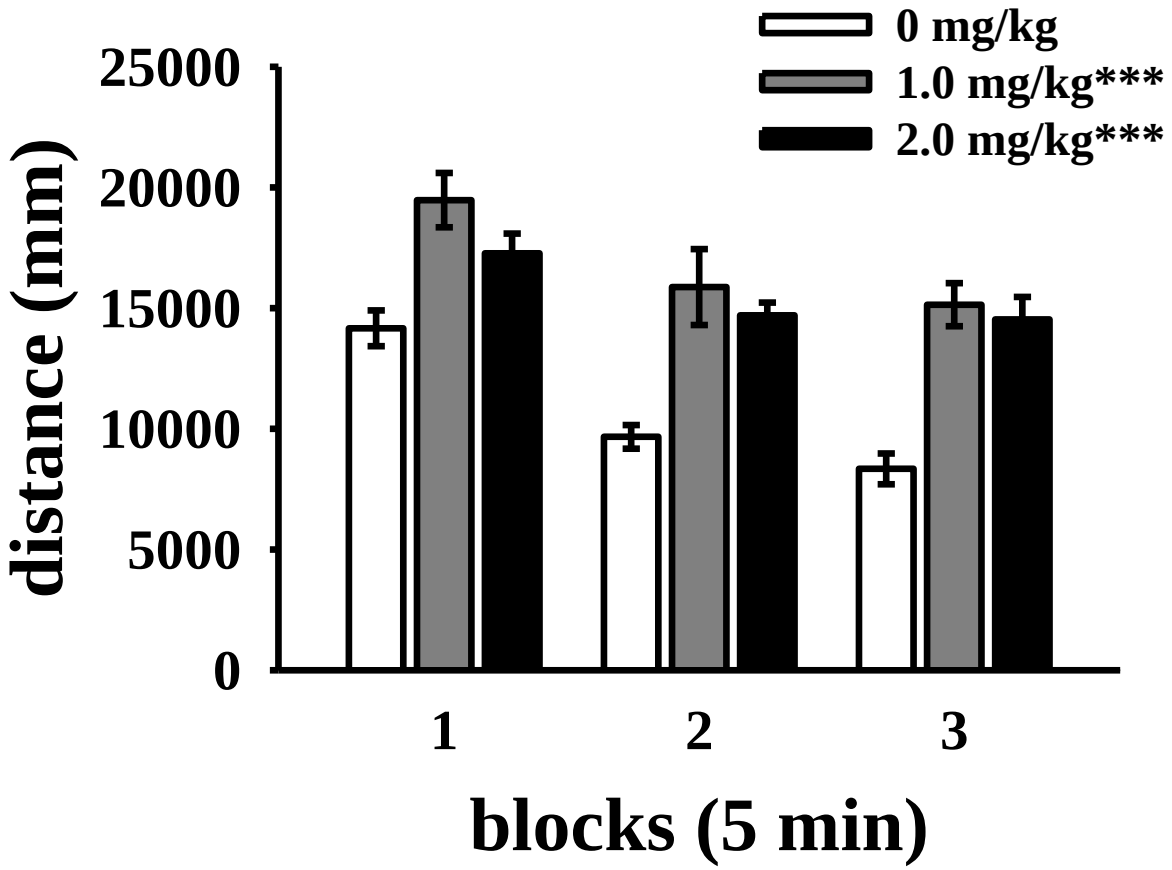

Figure. 4 The dose effects of amphetamine on the distance of locomotor activity (Experiment 1; n = 12 for each treatment).

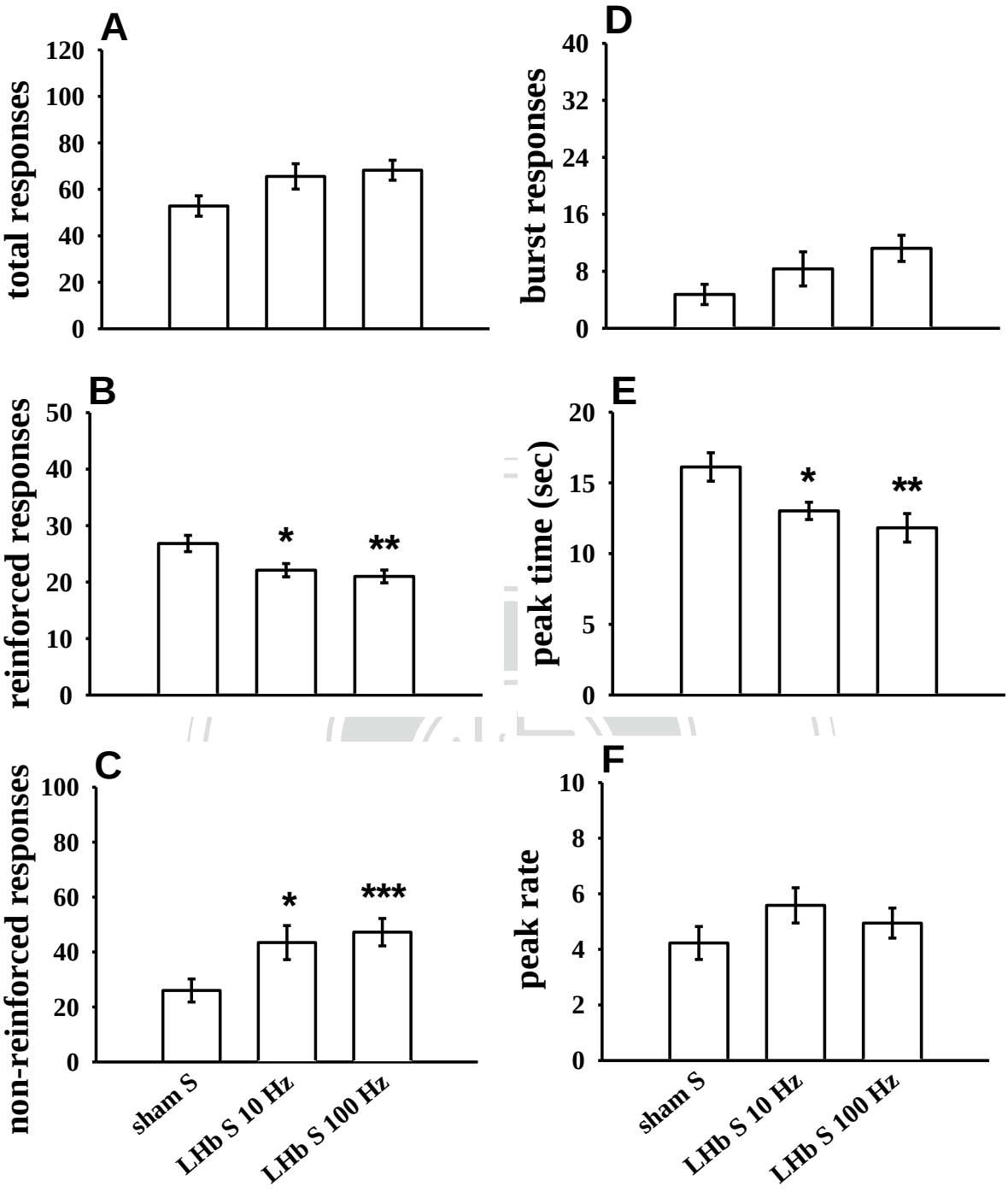

Figure. 5 The effects of LHb stimulation on DRL 15-s behavior as measured by the six dependent variables (Experiment 2). A within-subject design was applied in this experiment, the numbers of subjects completing the test of treatment were12 for sham S, 9 for LHb S 10 Hz and 9 for LHb S 100 Hz.

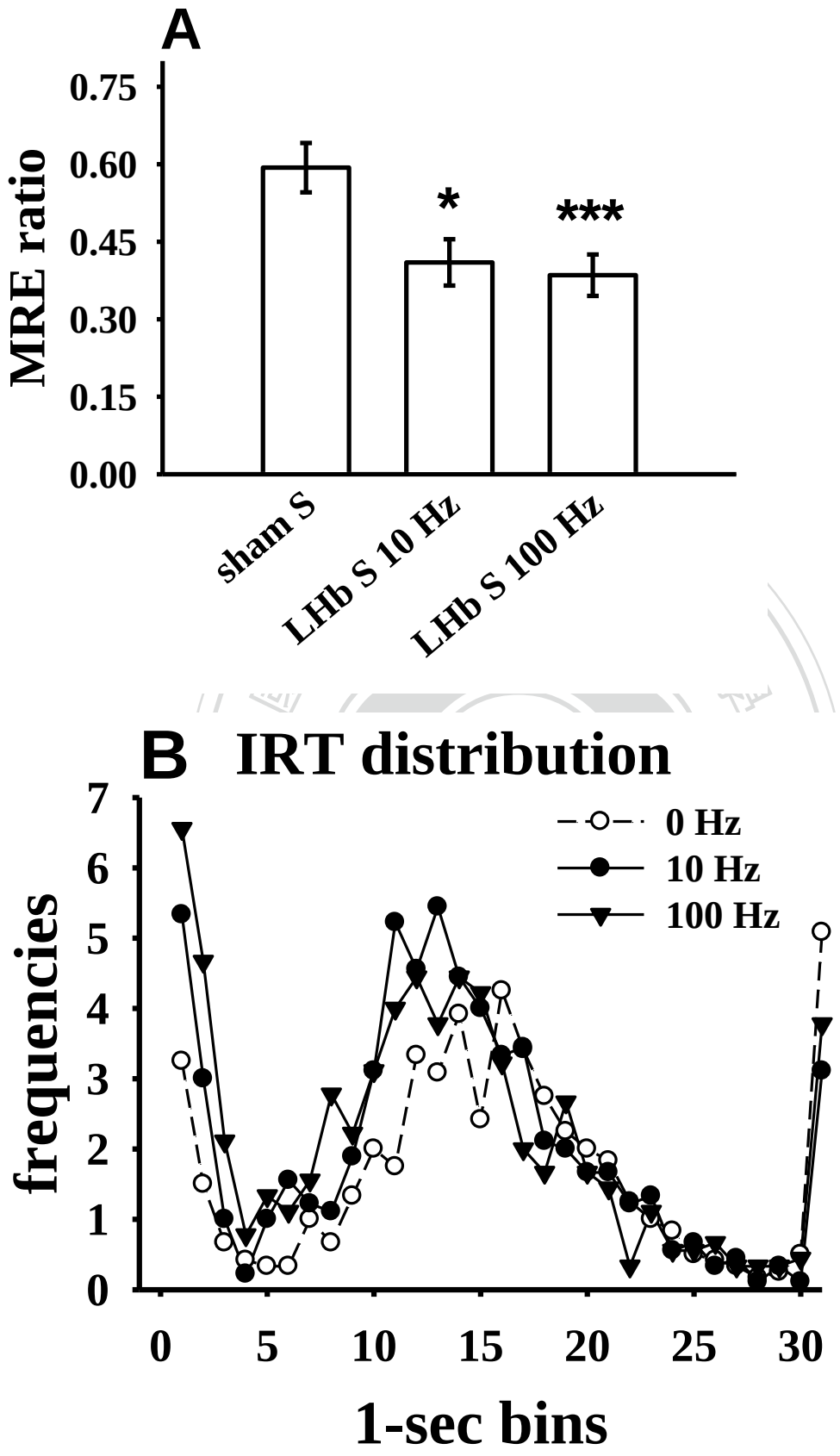

Figure. 6 The effects of LHb stimulation on the MRE ratio of DRL-15 behavior (Experiment 2).

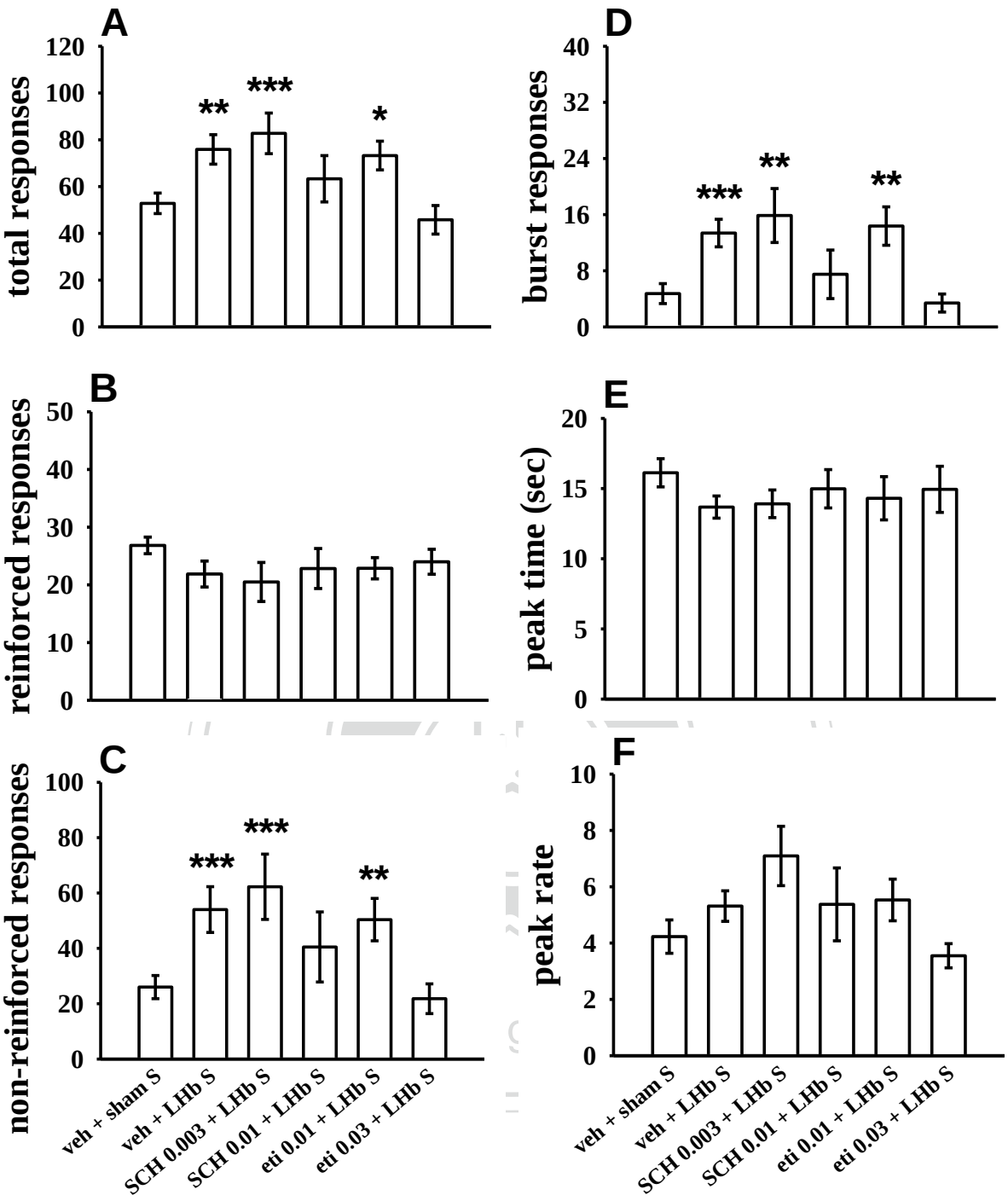

Figure. 7 The effects of SCH23390 and eticlopride on the alteration of DRL 15-s behavior induced by LHb stimulation as measured by the six dependent variables (Experiment 3). A within-subject design was applied in this experiment, the numbers of subjects completing the test of treatment were12 for veh + sham S, 8 for veh + LHb S, 8 for SCH 0.003 + LHb S, 6 for SCH 0.01 + LHb S, 8 for eti 0.01 + LHb S and 6 for eti 0.03 + LHb S.

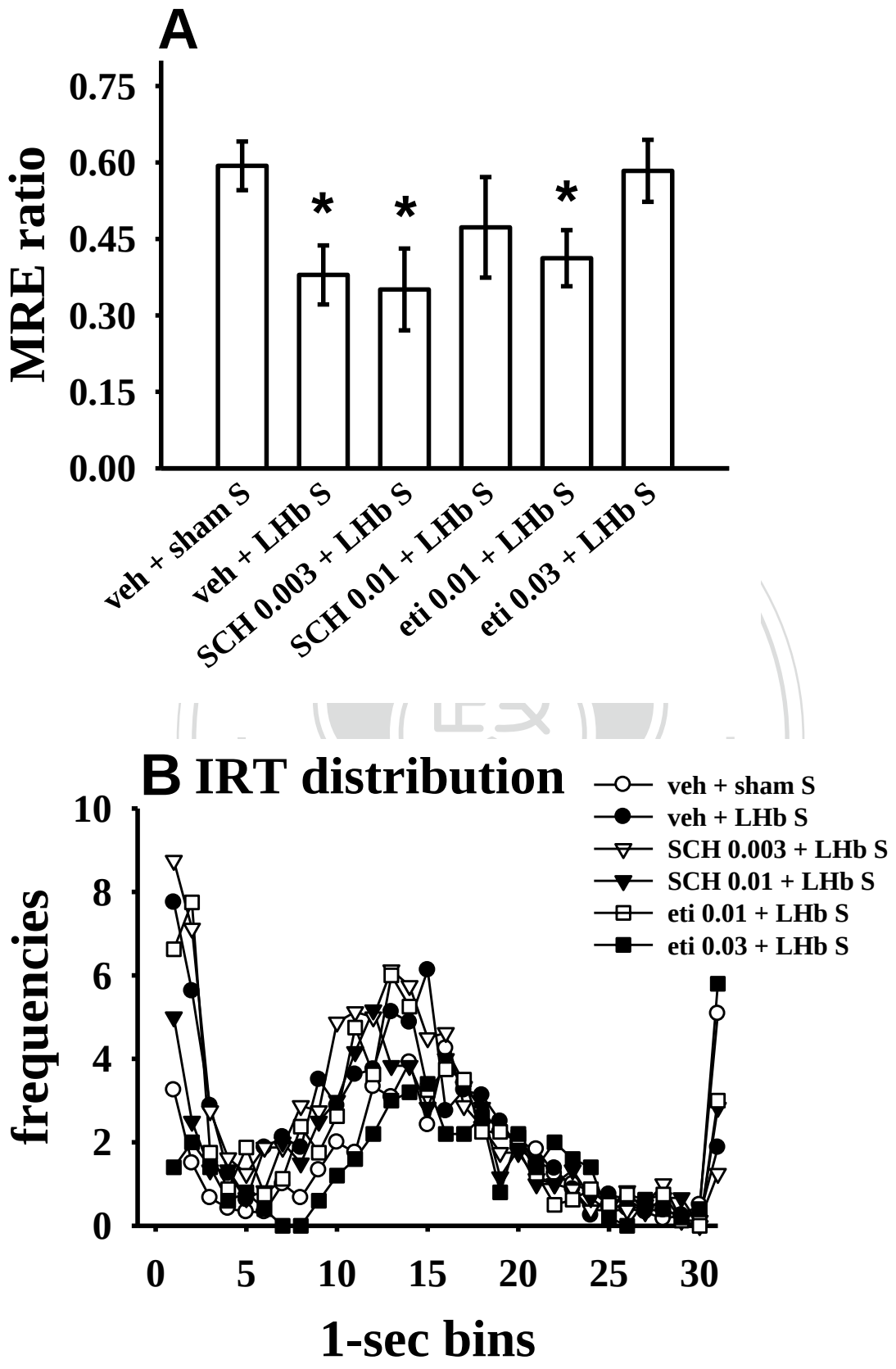

Figure. 8 The effects of SCH23390 and eticlopride on the alteration of DRL 15-s behavior induced by LHb stimulation as measured by MRE ratio (Experiment 3).

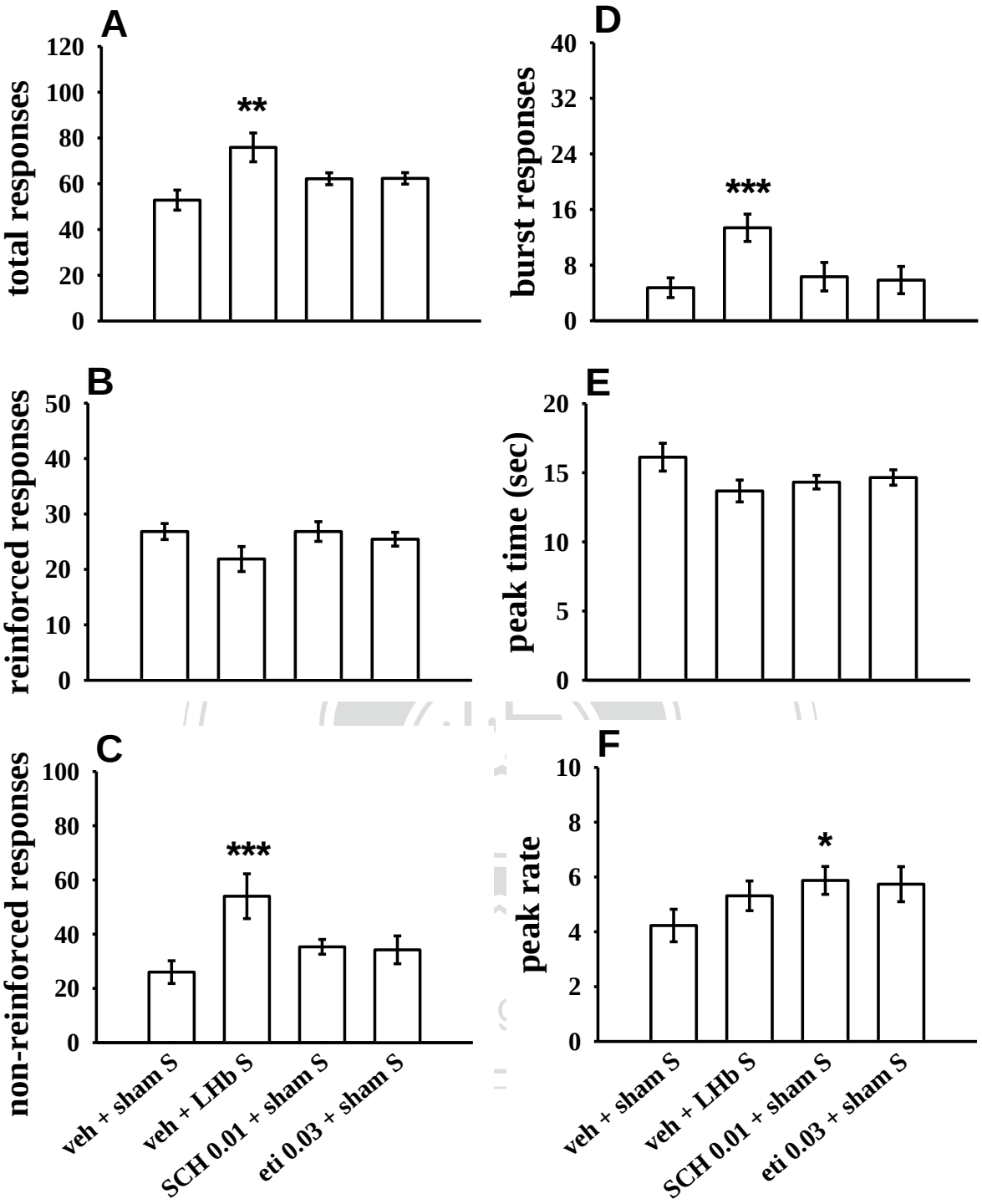

Figure. 9 The effects of SCH23390 and eticlopride on DRL 15-s behavior, as measured by the six dependent variables (Experiment 3). A within-subject design was applied in this experiment, the numbers of subjects completing the test of treatment were12 for veh + sham S, 8 for veh + LHb S, 7 for SCH 0.01 + LHb S and 7 for eti 0.03 + LHb S.

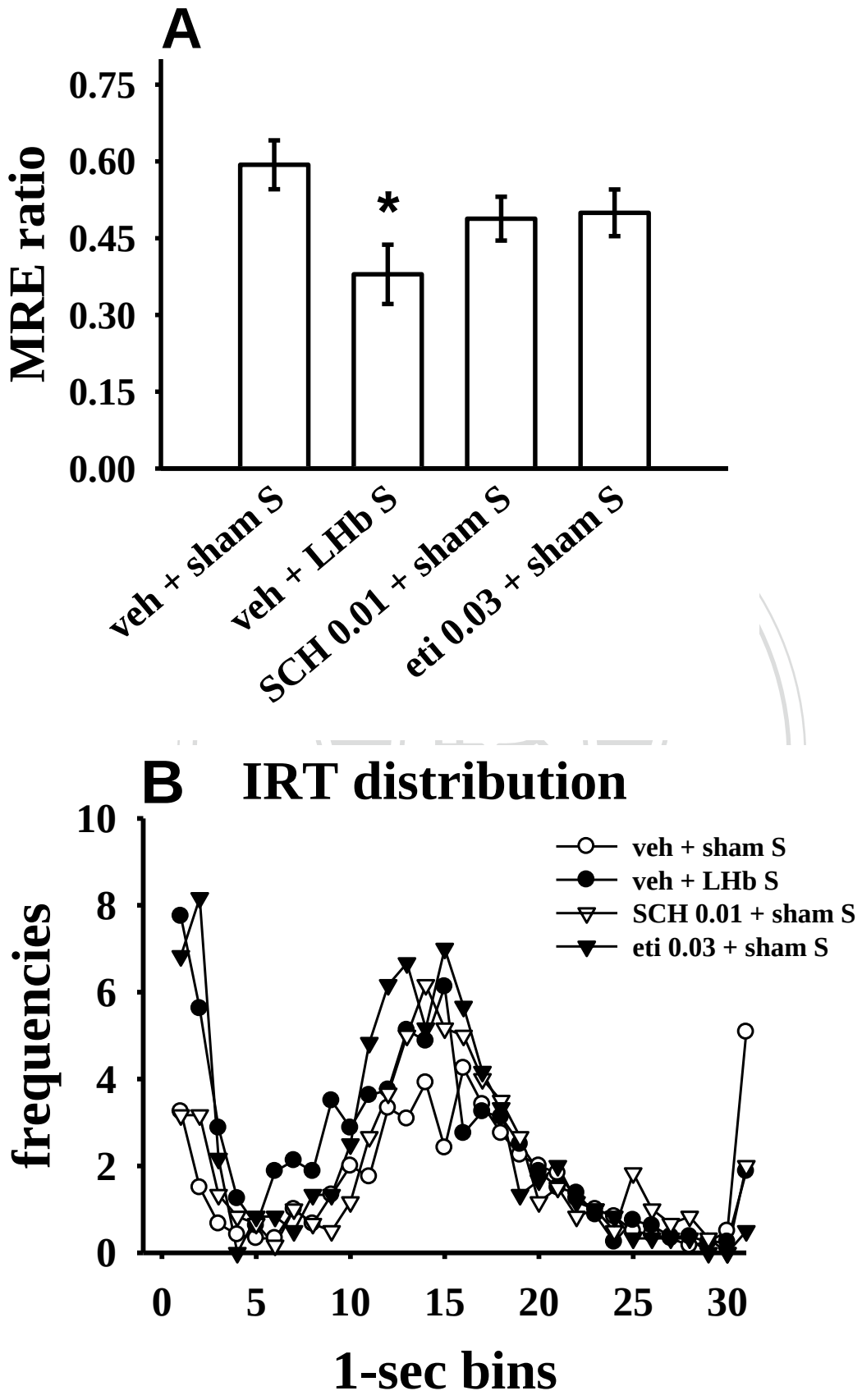

Figure. 10 The effects of SCH23390 and eticlopride on DRL 15-s behavior as measured by MRE ratio (Experiment 3).

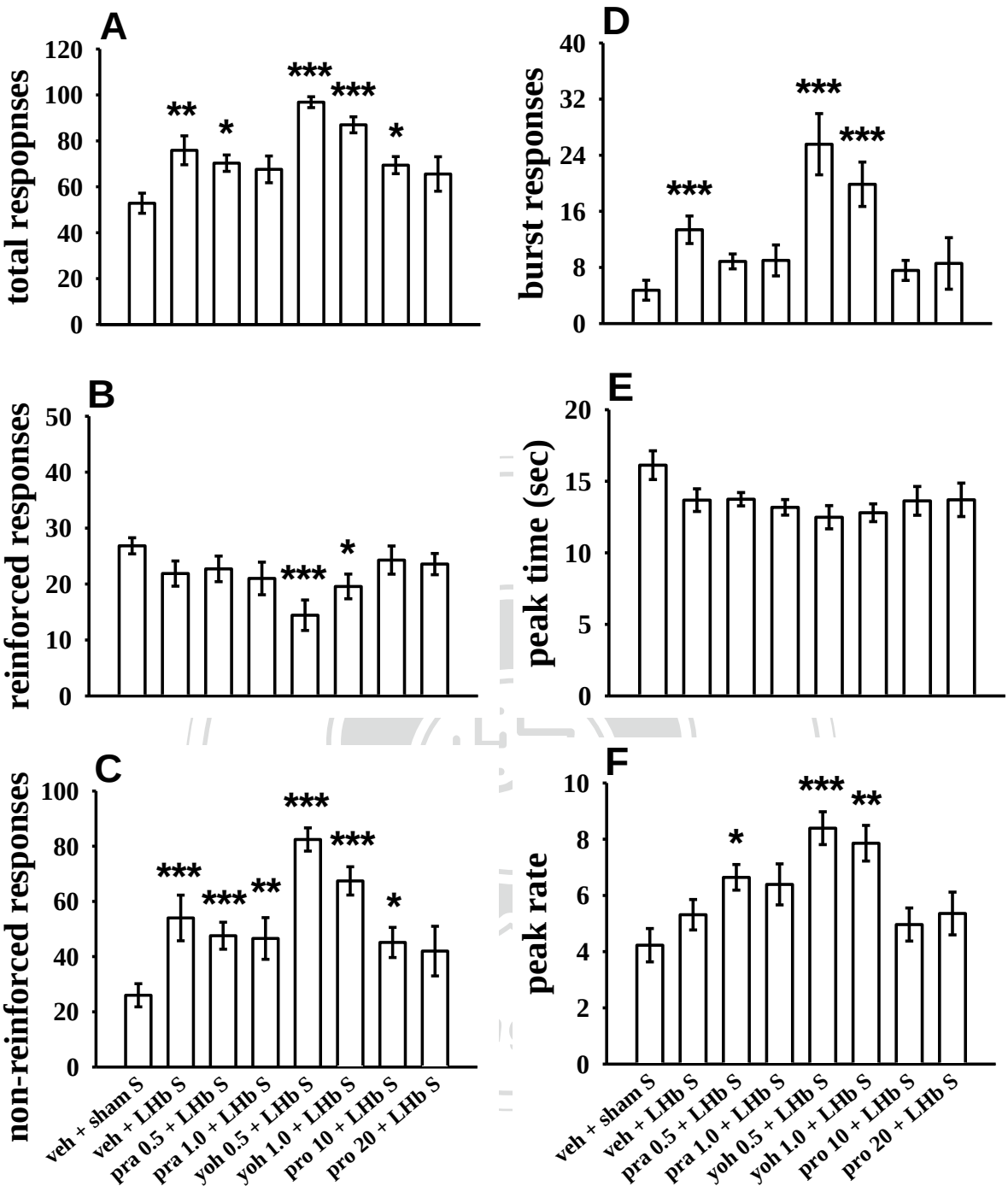

Figure. 11 The effects of prazosin, yohimbine and propranolol on the alteration of DRL 15-s behavior induced by LHb stimulation, as measured by the six dependent variables (Experiment 4). A within-subject design was applied in this experiment, the numbers of subjects completing the test of treatment were12 for veh + sham S, 8 for veh + LHb S, 8 for pra 0.05 + LHb S, 7 for pra 1.0 + LHb S, 7 for yoh 0.5 + LHb S, 7 for yoh 1.0 + LHb S, 7 for pro 10 + LHb S and 7 for pro 20 + LHb S.

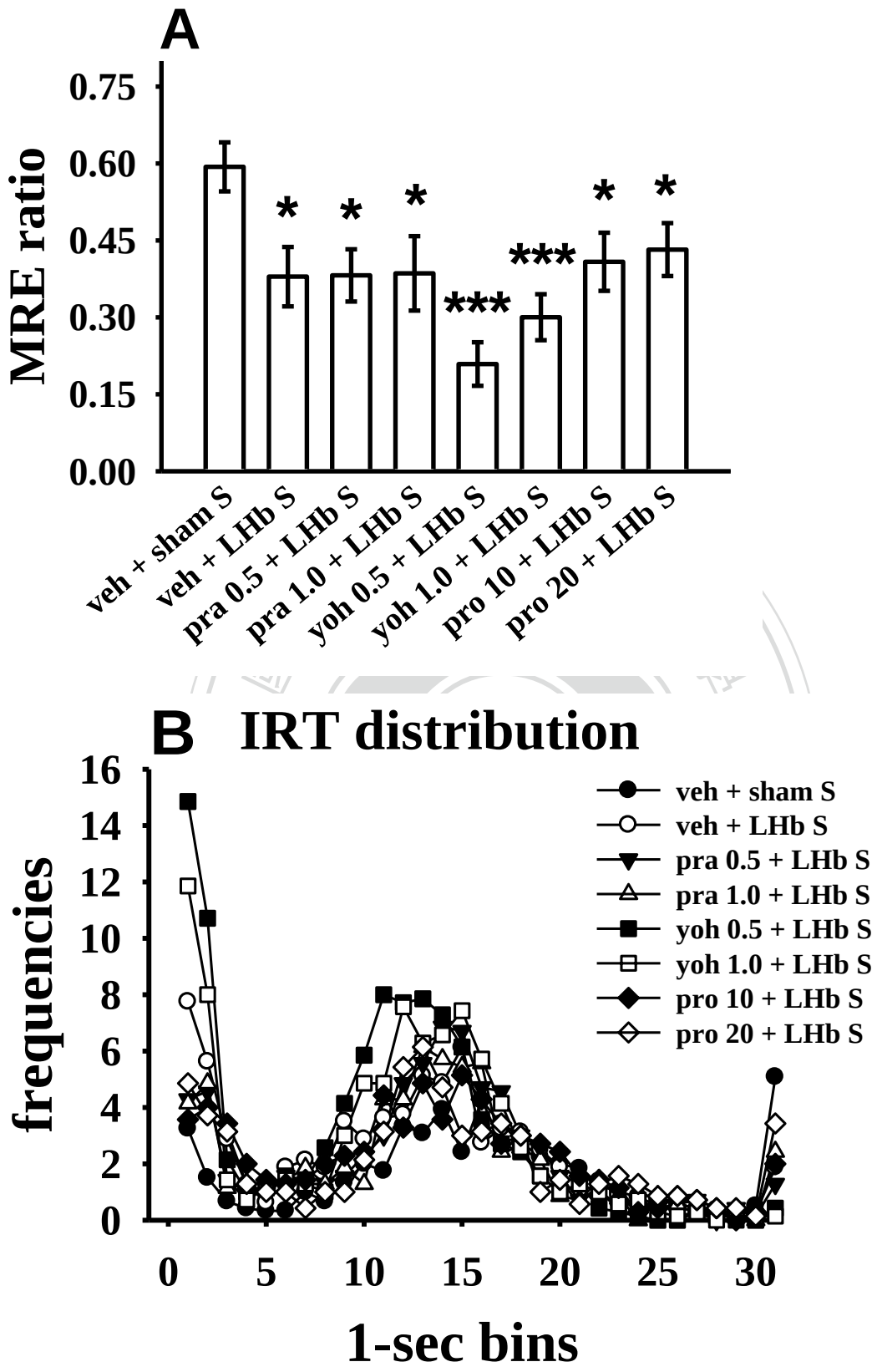

Figure. 12 The effects of prazosin, yohimbine and propranolol on the alteration of DRL15-s behavior induced by LHb stimulation as measured by MRE ratio (Experiment 4).

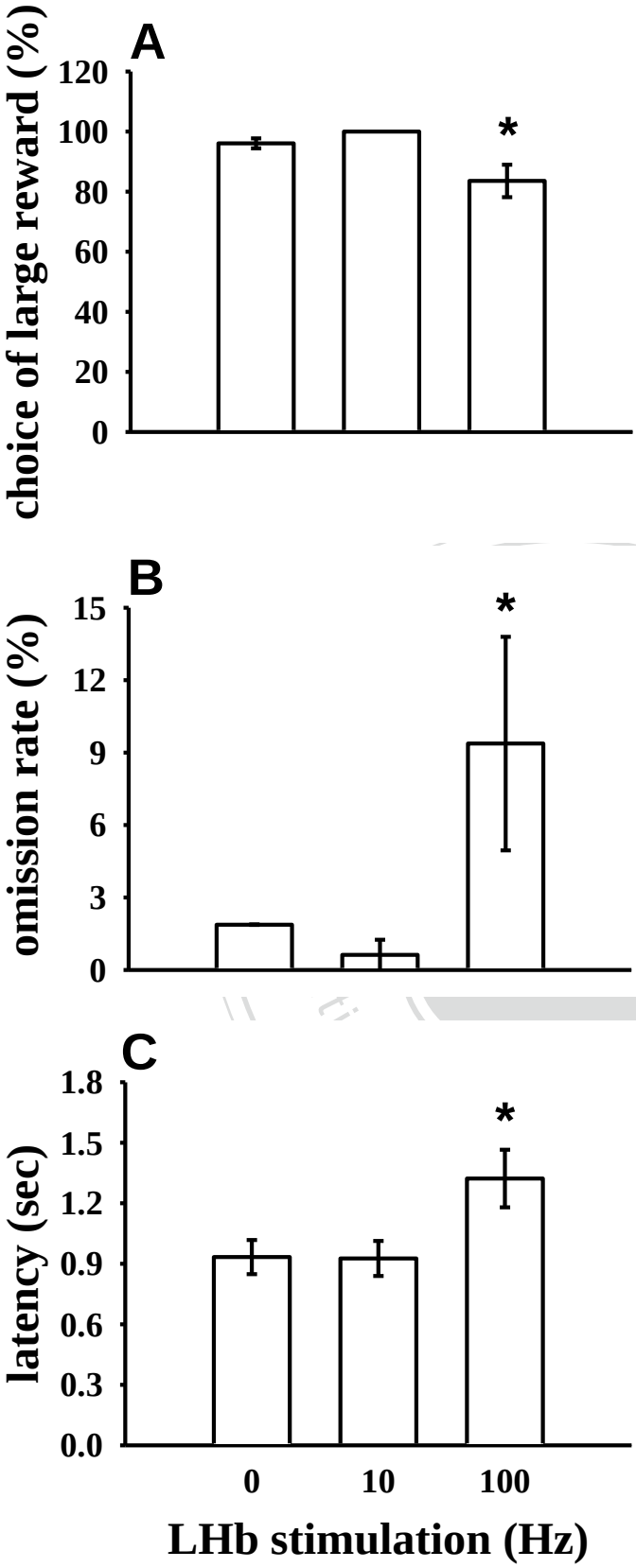

Figure. 13 The effects of electrical stimulation in LHb on a reward discrimination task, as measured by choice of large reward, omission rate and response latency (Experiment 6; n = 4 for each treatment).