A Role for the Lateral Habenula in a Delay Based Decision Making Test

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Abstract

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The lateral habenula (LHb) is thought to provide an aversive or anti-reward signal in the mammalian brain under Pavlovian conditions. However, recent evidence suggests that the LHb also controls more complex decision-making during appetitive behaviors, since LHb disruption impairs hippocampal (HPC)-dependent memory. This study tested whether LHb's role in appetitive behaviors is at least in part due to its integration of internal state information and limbic cortical information to enable flexible responding that reflects the most beneficial choice under changing task conditions. Since delay-discounting performance relies on an analysis of subjective values of reward (i.e. a cost-benefit interpretation) to determine choice preferences as task conditions change, the current study tested rats' performance on a maze-based delay-discounting task. Male and female Long-Evans rats were trained to make choices between two

options on an elevated T-Maze: a short delay (3 sec) followed by delivery of a small reward (1 sugar pellet), or longer delays (10, 20, or 40 sec) followed by a large reward (4 sugar pellets). Rats showed the typical delay-discounting function that illustrated their strong preference for the large reward option after the 10 sec delay, but a significantly reduced preference for the large reward option after a 20 or 40 sec delay. Muscimol, (MUS, a GABAA receptor agonist) microinfusion into the LHb eliminated delay-discounting behavior such that LHb inactivated rats showed no preference for the large reward at any delay interval ($< .01$). There were no sex differences in the LHb effect, and whether the rat received left or right arm as the large reward arm were counterbalanced across animals. Similar elimination of delay-discounting behavior was found after hippocampal inactivation. The employed reward magnitude discrimination task further indicated that, MUS inactivation did not impair animal's ability to distinguish between a small reward and a large reward with equal delay (3 sec). Confirming that LHb is functioning in a higher order flexible responding process, our results reveal that the LHb plays a necessary role in discounting behaviors since rats exhibited chance performance when choosing between a sooner-smaller reward and a later-larger reward regardless of the delay between choice and reward acquisition. Further, these data show that the LHb is critically important in a hippocampal-dependent appetitive task. Although there are no known anatomical connections between LHb and hippocampus, these results are consistent with our hypothesis that these brain areas are functionally connected to enable animals to flexibly respond in adaptive ways.

1 INTRODUCTION

In the learning and decision-making process, human beings and other species share a common behavior called behavioral adaptation, which is the process to rapidly switch learned cognitive and behavioral strategies when a subjective aim or objective framework alters (Mizumori et al., 2004; White et al., 2013; Hasson et al., 2015). The switching process is fast and dynamic, indicating this mechanism is not simply jumping from one memory system to another, rather, it is the simultaneous cooperative progress implemented by different memory systems (Mizumori & Phillip, 2017). Multiple decades of research have led to an understanding to the system that provide this lively behavioral adaptation progress when the consequence of a previous choice based on experience is no longer desirable. The flexible response ability following changes in both internal cues and external contexts is a capacity that primarily ascribed to PFC (Dalley et al., 2004; Ragozzino, 2007; Baker & Ragozzino, 2014). While it is evident, that PFC controls particular functions that enable these flexible strategic behaviors including consequence evaluation and response selection, species without a well-defined PFC (such as zebrafish), can also perform general behavioral adaptations in appetitive or aversive responses (Randlett et al., 2015). Therefore, in the current study, we are working to understand a non-cortical mechanism in the brain that establishes behavioral adaptation when results of a prior act or choice are no longer anticipated based on the changing environment.

From previous researches, one possible explanation of the reason why these animals without a PFC can still make choices in adjustable manners is that an evolutionarily conserved brain area, the habenula nucleus, is playing a role in this decision making process (Parker et al., 2012). Electrophysiological studies in zebrafish revealed that the ventral habenula (a homolog of the

mammalian lateral habenula) plays a central role in regulating behavioral and cognitive selections by influencing the monoaminergic system (dopamine, DA, and serotonin, 5-HT; Amo et al., 2009).

The DA system has been involved in multiple aspects of behavioral adaptation from outcome analysis to choice implementation (Barnéoud et al., 2000; Lee et al., 2007; Kehagia et al., 2010). For instance, dopaminergic lesions in marmoset monkeys produces an intentional disregard, a deficiency in determination. In other words, this DA impairment in the nigrostriatal bundle resulted in a defect in the "top-down" motor control mechanisms (Ridley et al., 2006). Moreover, an in vivo voltammetry and in vitro patch-clamp electrophysiology study showed that midbrain dopamine neurons could be excited by reward-predicting cues in the environment. Further, enhanced synaptic transmission between dopaminergic neurons could also be observed in a reward-predicting learning process (Stuber et al., 2008). In addition, looking closely into the PFC dopaminergic neural system, DA (and noradrenaline) display efflux in medial PFC (mPFC) when rats performing serial reversals tasks. The efflux from baseline is mainly noticed in acquisition phase of the task but not for later reversals. Confirming that DA neurons in the mPFC is critically involved in response flexibility (van der Meulen et al., 2006).

Moving away from the dopaminergic system, another monoaminergic element that also contributes to the behavioral adaptation mechanism is the serotonergic system. Unlike the DA system, 5-HT system supports the decision-making process in a subordinate way, as it monitors the ongoing behaviors recording imminent results. For instance, in dorsal raphe (DR), one of the key nodes in the 5-HT system, neurons code progress of behaviors including schedule onset,

reward expectation and reward outcome (Inaba et al., 2013, Luo et al., 2015). In addition, on the one hand, reduced activities at 5-HT receptors with selective serotonin reuptake inhibitors (SSRIs) enhances reversal learning, and on the other hand, disturbance of serotonin transmission results in task learning deficits (Nilsson et al., 2012; Wallace et al., 2014). It's worth pointing out that, when the evaluation process for decision of future outcomes involve reward-risk analysis, both 5-HT and DA networks are working together to realize the optimal consequence (Kim et al., 2014; Balasubramani et al., 2015).

As mentioned above, LHb has a pivotal role in the monoaminergic system. There is growing interest in understanding how the dopaminergic and serotonergic system work together to coordinate the flexible response process. In this case, it is crucial to have a full investigation into the afferent and efferent connections of this key structure.

The habenula (Hb) is a well-preserved ancient nucleus that locates along the midline of the train, in front of the pineal body, and above the thalamus (Braitenberg & Kemali, 1970; Kemali & Guglielmotti, 1977). In amphibians and fishes, the Hb displays an asymmetry feature, while in birds, reptiles and mammals, Hb exhibits bilateral symmetry (Kemali et al., 1980; Aizawa et al., 2005; Amo et al., 2009). The mammalian Hb, as a part of the epithalamus, comprises of medial and lateral divisions. It can be distinguished clearly by connectivity and gene expression (Cajal, 1911; Herkenham et al., 1979; Aizawa et al., 2012; Proulx et al., 2014; Ichijo & Toyama, 2014). The medial habenula (MHb)'s neuronal connectivity is relatively homogeneous, as its input and output pathways are quite unitary, with its afferent information mainly from one structure, the supracommissural septum, and most of its efferent pathways go through another structure, the

interpeduncular nucleus (IPN; Sutherland, 1982; Lecoutier & Kelly, 2007; Bianco & Wilson, 2009; Viswanath et al., 2014; Zahm & Root, 2017).

Unlike MHb's highly preserved connectivity pattern across species, LHb's afferent and efferent connections are more widespread and varied across different parts of the brain. Moreover, there is increasing elaboration to the LHb functional and anatomical connections as one moves to a higher level of amniotes brain (Ahumada-Galleguilos et al., 2016). Considering the convoluted pathways through LHb, within this structure, it has been divided into two divisions medial LHb (mLHb) and lateral LHb (lLHb) based on its specializations in connections (Andres et al., 1999; Hikosaka, 2010). Looking closer into these two parts, they are further defined as 10 distinct subnuclei with specialization in connections (Wagner et al., 2014; Zahm & Root, 2017). Decades of retrograde labeling studies have identified multiple afferent nodes for LHb throughout the central nervous system, such as, vertical limb of the diagonal band of broca (vDBB), ventral pallidum (VP), lateral preoptic area (LPO), suprachiasmatic nucleus (SCN), endopedencular nuclues (EPN, or globus pallidus in primates), and the structure mentioned above, mPFC (Nauta, 1974; Herkenham & Nauta 1977; Parent et al., 1981; Li et al., 1993; Felton et al., 1999; Kowski et al., 2008; Kim & Lee 2012; Yetnikoff et al., 2015).

As indicated before, a crucial and urgent task is to dissect the neural mechanism that lead to the dynamic and rapid switches when the context information changes in the behavioral adaptation process. The important role of LHb in this progress could be analyzed through its afferent pathways originate throughout the decision-making brain circuits (Lecourtier & Kelly, 2007). Generally, functional anatomical evidence suggests that LHb integrate previous response results,

internal state and motivational information, sensory registration, and other behavioral economic factors to achieve optimal future outcome.

Firstly, LHb receives projections from the infralimbic and prelimbic area of the mPFC, and the terminals from this pathway are concentrated to the medial portion of LHb (Kim & Lee, 2012). The mPFC is known to be involved in the planning and choice selection process in various flexible response tasks (Delatour & Gisquet-Verrier, 2000; Dalley et al., 2004; Boulougouris et al., 2007; Ragozzino, 2007). Modulation of neural function in the mPFC by the DA system is necessary for tasks that require higher cognitive process related to working memory, directing attention, and planning of future (Phillips et al., 2004). Thus, considering LHb's broder role in the monoaminergic system, the afferent information from mPFC about task demands, previous outcomes, and choice selection can be integrated with other inputs at LHb to direct behavioral changes (Lecourtier et al., 2008).

Secondly, the main input from the voluntary movement control center, basal ganglia, to LHb arises from EPN (Filion & Harnois, 1978; Shabel et al., 2012). In primates, globus pallidus relays prominent reward-related signals to the LHb (Hong & Hikosaka, 2008). As previously described, in rodent model, EPN is equivalent to the primate globus pallidus; and within the broader basal ganglia nuclei, it contributes to providing dynamic gating mechanism for controlling ongoing behavioral and cognitive process (Parent et al., 1980; Atallah et al., 2004; O'Reilly & Frank, 2006). It is worth noting that neurons in the EPN-LHb pathway co-release both glutamate and GABA (Shabel et al., 2014; Meye et al., 2016). Within LHb, in addition to the peptides, LHb neurons are mainly glutamatergic, expressing vesicular glutamate transporter 2

or 3, VGluT2 or VGluT3 (Geisler et al., 2007; Brinschwitz et al., 2010). Another small group of neurons is GABAergic, prominently expressing GAD-67 (Brinschwitz et al., 2010). In this EPN to LHb GABA/glutamate pathway, neurons increased firing when the animals encountering a positive event (receiving water reward), and decreased firing when the animals confront a negative stimuli (air puffs to face; Stephenson-Jones et al., 2016). Therefore, the results suggest that the EPN-LHb neurons are encoding valence of appetitive and aversive learning, further making evaluations and predictions in the flexible response progress for behavioral outcome.

In addition, anterograde axonal tracing and retrograde labeling studies indicate that in the preoptic region and rostral hypothalamus, LPOA and LH (or LPO-LH continuum) projects to LHb (Troiano & Siegel, 1975; Swanson, 1976; Parent et al., 1981; Kowski et al., 2008; Yetnikoff et al., 2015). Both areas are crucial in emotional arousal, motivation, associative learning, and food consumption (Ono et al., 1986; Saad et al., 1996; Stratford & Wirtshafter, 2012; Sohn et al., 2013; Cole et al., 2015). Given all the prominent collective afferent connections related to internal state and contextual information to the LHb, it is apparent that this area is the integrative center in emotional regulation and stress in behavioral adaptation and sensory processing (Heldt & Ressler, 2006; Neumann et al., 2014; Mathis et al., 2015; Jacinto et al., 2017).

In all, looking at the different afferent connections projected to the LHb, this previously undermined structure may generally monitor information to implement behavioral adaptation, which includes emotional and motivational information, as well as contextual and salient cues. With the growing complexity of mammalian LHb as one moves to a higher level in the

evolutionary gradation, the information LHb can process become more and more complex and specific based on the changing goal and environment.

As indicated earlier, both the DA system and 5-HT system have significant roles in processing information related to reward-related decision-making. Hence, more and more literatures looking into adaptive behaviors have directed their attention onto the integration center, LHb, as it passes information to both monoaminergic systems. As one of the most prominent areas in the dopaminergic system, VTA (along with RMTg) receives bi-directional connection to the LHb to direct appetitive and aversive learning behaviors (Sutherland, 1982; Swanson, 1982; Skagerberg et al., 1984; Gruber et al., 2007; Hikosaka, 2010; Baker et al., 2016; Ichijo et al., 2017). Particularly, within this pathway, literature suggested that RMTg neurons receive excitatory input from the LHb first, exhibiting reward-prediction errors, then send the projections along the axonal output to VTA, inhibiting DA neurons, thus transmit negative reward predictions cues to aid aversive learning (Hong et al., 2011). In addition, studies indicate that the LHb detects and represents immediate outcome changes in the ongoing trial (Baker et al. 2015; Kawai, T. et al., 2015). Namely, the LHb is monitoring the current internal and external states, combining past choice experience, to achieve the optimal outcome in the behavioral adaptation process.

Similarly to the DA system, growing interest has been drawn to the role of the 5-HT system in behavioral flexibility; and the LHb has direct connections to the two main serotonergic nuclei in the brain, the DRN and MRN (Aghajanian & Wang, 1977; Pasquier et al., 1977; Reisine et al., 1982; Behzadi et al., 1990; Nagao et al., 1993; Lavezzi et al., 2011). Specifically, single-unit recording studies in the DRN, while monkeys performed saccade tasks, revealed that the neurons

were modulated tonically by the expected reward size (Nakamura et al., 2008; Bromberg-Martin et al., 2010). In other words, DRN neurons are actively encoding expected and received rewards, further signaling the outcome value associated with the ongoing behavior.

Looking into the other major serotonergic nucleus, early tracing studies discovered strong bilateral projections from the MRN to the LHb (Conrad & Pfaff, 1976; Vertes and Martin, 1988). Because of its prominent role in mood regulation, a large number of studies devoted to this area were focused on depression and fear learning, but not the decision-making process (Płażnik et al., 1980; Borelli et al., 2005; Bach-Mizrachi et al., 2006; Lanzenberger et al., 2012). Early electrophysiological studies looking at the MRN indicated that, inactivation the MRN caused reversal-learning deficits, and stimulation of this area resulted in behavioral inhibition (Graeff & Filho, 1978; Wirtshafter & Asin, 1986). Further, there is a robust efferent projection from the MRN to the HPC (Azmitia & Segal, 1978; Vertes et al., 1999). Looking deeper into the functional connection, impairments of the MRN generated hippocampal low-frequency theta activity, while activation of the area caused the desynchronization of hippocampal electroencephalographic activity (Maru et al., 1979; Leranth & Vertes, 1999).

Though there are no known anatomical connections between the LHb and the HPC, more and more interests have been drawn to understand the functional connections between the two areas (Goutagny et al., 2013; Mathis et al., 2015). The LHb not only involves in behavioral tasks that are known to be hippocampal dependent (such as, delay discounting, water-maze memory task), but also exhibits theta coherence with the HPC in a spatial recognition test (Lecourtier et al, 2004; Goutagny et al., 2013; Mathis et al., 2015; Mathis et al., 2016). However, the fact that the LHb

can influence hippocampal theta waves is dependent on a functional MRN (the intermediary center for the two structures; Lecourtier & Kelly, 2007; Quina et al., 2014; Aizawa et al., 2015). Though evidence supports that the LHb and HPC are interacted in multiple behavioral paradigms, the mechanism and capacity of this functional link is not well explored. In order to understand the role of this connection in the behavioral adaptation process, additional studies need to be carried out to look at the 2 structures in a more concrete setting.

Previous publications in our lab provided corroborative evidences where LHb contributes in integrating internal and external cues in performing flexible response behaviors, and we have proposed a working hypothesis toward understanding the role of LHb, HPC and mPFC together as a crucial mechanism that enables animals to behave in adaptive ways in high demanding tasks (Baker et al., 2015; Baker et al., 2016; Baker et al., 2017; Baker & Mizumori, 2017; Mizumori & Baker, 2017). To further investigate the role of LHb in the decision-making process, the current study adopts a delay counting task on an elevated maze. This type of spatially extended environment paradigm employs the HPC, as well as the reinforcement learning system (Chapman & Kaelbling 1991; Cardinal, 2006; Bett et al., 2015). As the LHb impairments not only hinders spatial memory, but also signals choice flexibility in freely moving rats, it's not surprising that the HPC and LHb connections are most strong when observe through a task that requires both spatial capacity as well as flexible choice ability (Baker et al., 2015; Mathis et al., 2015). In summary, the present study facilitates to understand the essential role of LHb in the limbic-habenular circuit that ensures the fast and strategic choices based on the changeable internal state condition, behavioral outcomes, and contextual information.

2 MATERIALS AND METHODS

2.1 Subjects

Four experimentally naïve male Long–Evans rats (320-400g, 60-70 days old, Charles River) and five female Long–Evans rats (220-270g, 60-70 days old, Charles River) were individually housed in a temperature-controlled laboratory (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care). The room in which they were housed maintained a 12 h light/dark cycle (lights on at 7:00 A.M.). Animals were allowed to free feed for a week, and then were placed on a standard food restriction schedule to gradually reach and maintain 85% of their original free-feed weight to ensure motivation for food during the behavioral task. Rats were fed in their home cages and fed daily, and were trained and tested between 9:00 a.m. and 5:00 p.m., in accordance with the University of Washington's Institutional Animal Care and Use Committee guidelines.

2.2 Behavioral apparatus (Figure 2A)

An elevated T-maze (79 cm from the floor) was used in the current study. The maze was made of black Plexiglas and was composed of one start arm and two goal arms (58 × 5.5 cm each). There was a metal food cup at the end of each reward arm. Wooden barriers were put in front of each food cup, which contained the reward. Upon entering a reward arm, a second block was placed behind the rat so that it could not exit the arm. Once the specified delay time has been complete, the block in front of the food was lifted; when the animal finished eating, the second barrier behind the animal was lifted so that it could return to the starting arm. Each maze arm was hinged such that its proximal end closest to the maze center could be raised and lowered by remote control. During forced trials, only one of the two arms was available to the animal.

During free choice trials, both arms were available to the animal. The maze was encircled by black curtains that were decorated with spatial cues.

2.3 Habituation and Pre-surgical training

Over the course of 3-5 days, animals underwent a habituation phase. Wherein all rats were allowed to freely forage for sucrose pellets that were randomly scattered on four maze arms (1 start arm and 2 goal arms). Then, they were shaped to collect a shortly delayed reward (3 second, 1 pellet) only from the goal arms. Specifically, each rat was placed on the start arm in a given trial and was encouraged to choose one of the goal arms. Upon arrival at the block, the animals had to wait for 3 s before acquiring reward. The elapsed time was measured by an experimenter using a digital stopwatch. As the block was removed by the experimenter at the termination of the delay, the rat could approach and consume the reward. After replacing the barrier and then re-baiting the food cup, the experimenter gently guided the animal to the start arm for the next trial. Once the rat was able to finish 16 trials within 20 min, it underwent the surgical implantation of bilateral cannulae.

2.4 Surgery

Under anesthesia with isoflurane (4% mix with oxygen at a flow rate of 1L/min), rats were mounted on a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). Subsequently the isoflurane concentration was reduced to 1-3%. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. All animals were implanted with two 25-gauge cannulae bilaterally in the LHb (anteroposterior = −3.5 mm; mediolateral = ±0.8 mm; dorsoventral = −4.5 mm, top of skull). The double cannulae were secured in place with

anchoring screws and dental cement. Following implantation, 33-gauge double dummy cannulae were inserted to prevent clogging, and fitting caps were added to keep dummy cannulae in place. After surgery, all rats were given 5-7 days of surgical recovery and daily handling before postoperative training began. Rats in the current experiment were then retrained in the delay-discounting task until they reached stable, consistent discounting behavior.

2.5 Behavioral Task

2.5.1 Delay-discounting Task (Figure 2B)

After a week of recovery, all rats were put back on a food-restricted diet. The 9 animals were trained in a delay-discounting task in which they could choose between a sooner smaller (SS) reward and a later larger (LL) reward. To assess choice performance as a function of delay to LL reward, three different lengths of delay (10, 20, and 40 s) prior to LL reward (3 pellets) were tested in separate blocks of trials. However, the delay to SS reward (1 pellet) remained constant at 3 s throughout the experiments.

In a daily testing session, the three delays before LL reward were randomly assigned to different blocks of trials and only one delay was used in a given block. Since the animals did not initially know how long they needed to wait for an LL reward, each block began with 10 forced-choice trials followed by 6 or 8 free-choice trials. During the forced-choice trials, 5 SS and 5 LL reward trials were presented and only one goal arm was made available in each trial by lowering the other goal arm. Both goal arms were present during the free-choice trials in which animals' choice preference for LL reward was analyzed. The three testing blocks were separated by an inter-block interval of 5 minutes during which the animals were placed on a holding area

adjacent to the maze. The location of SS and LL rewards in the goal arms remained constant within each rat but was counterbalanced across rats.

2.5.2 Reward Discrimination Task

A following task after the delay-discounting procedure was a reward discrimination task in which rats had to discriminate two goal arms associated with a smaller(1 pellet) and a larger (4 pellets) reward. On the last day of muscimol injection, after the animals underwent 3 blocks of 16 trials, they ran an extra block with 16 trials (10 forced trials, and 6 free choice trials). While the two reward options remained the same (smaller 1 pellet, and larger 4 pellets), there were no delays between animals reaching the block and removal of the barrier.

2.6 Microinjection Procedure (Figure 2C)

A day before microinjection, the injection cannula (Plastics One, Roanoke, VA, USA), which extended 1 mm beyond the guide was inserted into the guide cannula and left in place for 1 min. This was done to control for any initial mechanical damage done by the injector. On a test day, rats were injected with drug (muscimol, Sigma) in 0.9% saline, or vehicle. Both injections used a volume of 0.2 μL (50 ng/0.2 μL drug) and a 0.15 μL/min infusion rate. This volume and rate similar to those used in other LHb inactivation studies that used baclofen and muscimol (Stopper and Floresco, 2014; Baker et al., 2015). The injection cannula was connected to a 10 μl syringe (Hamilton) via polyethylene tubing (PE 20) using an infusion pump (KD Scientific).

2.7 Histology (Figure 3B)

After completion of all experiments, animals were perfused transcardially with physiological

saline followed by 10% formalin. Their brains were extracted and stored in a 10% formalin-30% sucrose solution at 4°C for 72 h. The brains were cut in coronal sections (40 μm) on a freezing microtome. The serial sections were stained with cresyl violet to stress cannula placements.

2.8 Statistical Analyses

Data were analyzed with two-way ANOVA, one-way ANOVA, and paired sample t-test. Two-tailed p values <0.05 were considered statistically significant. All data are expressed as mean ± SEM.

3 RESULTS

3.1 Abolished delay-discounting behavior following LHb inactivation (Figure 3C)

Nine animals (5 male and 4 female rats) were trained to choose between SS and LL rewards in a delay based decision making task on an elevated T-maze. To investigate the animal's choice preference as a function of delay to LL reward, three different delay (10s, 20s and 40s) were used to deliver of the LL reward in separate blocks of trials. On the other hand, the delay to the SS reward was kept consistent at 3s.

During baseline training and SAL control days, rats displayed a strong preference for LL reward over SS reward (choosing LL reward more than 50% of all choices) when the delay to rewards was short (10s). As delays became longer, they showed a stronger preference for SS rewards. Taking all 3 delay blocks into consideration, Figure 3C displays a typical delay-discounting curve, indicating that rats readily discounted the value of rewards with longer wait times.

By contrast, a repeated measure ANOVA found significant interaction between delay and drug (bilateral MUS injection; $F(2, 16) = 34.725$, $P < 0.01$; no single out drug effect, $F(1, 8) = 3.510$, $p = 0.098$; significant delay effect, $F(2,16) = 15.561$, $p<0.01$), such that rats no longer demonstrated a preference for either reward arm regardless of the delay condition. MUS injections resulted in rats choosing both reward arms roughly equally at both the 10s 20s, and 40s delays. While SAL injections resulted in normal discounting performance, which animals significantly prefer LL reward at 10s (paired sample t, $t = 7.498$, $p < 0.01$) and SS reward at 40s (paired sample t, $t = 5.062$, $p < 0.01$). This result was extends the previous finding that the LHb impairs delayed discounting when tested in an operant chamber (Stopper and Floresco, 2014).

As the wooden blocks needed to be manually added and removed, an experimenter was required to be present in the maze room throughout the session. This raises the question of whether the experimenter's movement could have guided the animal's choice. This is unlikely as the experimenter always stood in a neutral position near the start arm, equal distance to both reward arm. Furthermore, after the animal finished a trial, the experimenter would bait both arms so as not to bias the rat's next choice.

3.2 No sex differences in delay-discounting behavior (Figure 3D)

Male and female rats showed similar discounting functions during both baseline and SAL control days. Also, drug injections did not reveal sex differences in their response to LHb inactivation (no interaction effect under MUS infusion: $F (2, 14) = 0.206$, $p = 0.816$, and no significant sex effect: $F (1,7) = 0.012$, $p = 0.916$; no interaction effect under SAL infusion: $F (2, 14) = 0.371$, $p = 0.697$, and no significant sex effect, $F (1,7) = 0.177$, $p = 0.687$). These observations indicate

that there were no effects of sex when performing this maze-based flexible response task with or without a normal functioning LHb.

3.3 No spatial bias for delay-discounting behavior (Figure 3E)

In order to exclude the possibility that animals made choices based on the spatial position of the LL or SS arm (as opposed to the subjective evaluation of delay and reward), 9 animals (across sex) were randomly assigned to conditions where the right arm always contained the larger reward arm or the left arm contained the larger reward. The choice behavior for the two groups were not different from each other (no interaction effect under MUS infusion: $F(2,14) = 0.127$, $p = 0.882$, and no spatial effect: $F(1,7) = 0.057$, $p = 0.819$; no interaction effect under SAL infusion: $F(2, 14) = 2.268$, $p = 0.140$, and no spatial effect: $F(1,7) = 2.356$, $p = 0.169$). This result reveals that the location of the large reward arms did not inadvertently bias the rat's responses. Rather, their evaluation of the positive value of the reward, and the negative value of waiting, determined their choices.

3.4 Intact reward magnitude discrimination after LHb inactivation (Figure 3F)

Following the last MUS injection day, after the 3 delay-discounting blocks, rats were trained to discriminate between two goal arms associated with a small (1 pellet) and a large (4 pellet) reward with equal 3s delays. The results showed that reward magnitude discrimination ability remained intact after LHb inactivation, as rats preferred the larger reward significantly more often (above 50%) when the delay was constantly short (paired sample t test, $t = 10.513$, $p<0.01$). This result shows that LHb inactivation did not ablate rat's ability to understand reward value.

Rather, it appeared to have disrupted rats' ability to flexibly respond when task conditions changed.

4 DISCUSSION

4.1 Summary of current findings and caveats

The hippocampal-dependent, maze-based delay discounting task tested LHb's involvement in a flexible responding task (Bett et al., 2015; Davis & Mizumori, 2017). LHb inactivation eliminated delay-discounting behavior on our maze. Additionally, supplemental tests indicated that the impaired discounting preferences could not be accounted by changes in reward magnitude discrimination, absolute reward locations, or sex of the animal. The behavior patterns displayed under LHb inactivation (abolished discounting curve, and intact reward discrimination ability) are consistent with bilateral HPC inactivation under the same paradigm (Davis & Mizumori, 2017; Figure 3A). Hence, the current study provides further evidence that LHb plays an important role in HPC-dependent spatial and memory tasks to enable flexible and rapid decision-making.

4.2 Complementary functions between HPC and LHb

Looking further in to the LHb-HPC functional association, deficits in the habenular region resulted in impairments in various hippocampal dependent tasks, and our results are consistent with these findings (Thornton and Davies, 1991; Lecourtier et al., 2004). Previous inactivation study looking at the role LHb in a probability-based discounting behavior and a delay-based discounting behavior in an operant chamber indicated that LHb impairments suppressed choice biases, making animals indifferent in choosing between rewards correlated to various subjective

value (Stopper & Floresco, 2014). Expanding this preceding finding, our experiment adopted a delay-discounting task on an elevated T-maze, adding a spatial component to the economic behavioral paradigm. Prior to the current LHb inactivation study, we also conducted an HPC inactivation study under the exact setting. Our findings suggested that drug infusion to the dHPC area led to abolished discounting function comparing to vehicle infusion (Davis & Mizumori, 2017). Combining with the result from our study, we observed same behavioral pattern with LHb inactivation and HPC inactivation, which animals entering a guessing mode and unable to make alternative choices based on the changing situations. In all, current study further elucidated the role of LHb in behavioral flexible based on the changing internal subjective value and dynamic external environment. Future research directions should include multisite recording involving both LHb and HPC in complex decision-making tasks to analyze exact phase coherence between the two structures.

4.3 The behavioral implementation of HPC and PFC memory processing

Previously, studies related to LHb have mostly been focused on depression, fear learning and substance use (Płażnik et al., 1980; Borelli et al., 2005; Bach-Mizrachi et al., 2006; Lanzenberger et al., 2012; Maroteaux & Mameli, 2012; Batalla et al., 2017). In the present study, we have revealed a previously undermined functional connection between the LHb and HPC in complex flexible response tasks. Our results suggested that LHb may serve as an important integration center for the midbrain monoaminergic system in complex decision-making process. Anatomical studies confirmed that LHb has direct connections between the prominent dopaminergic areas (VTA and RMTg), and serotoninergic areas (DRM and MRN), which greatly contributes to various perspectives of behavioral adaptation, including outcome analysis, choice

implementation, reward-prediction error perception, reward-risk analysis, reversal learning, monitoring ongoing task process (Conrad & Pfaff, 1976; Aghajanian & Wang, 1977; Pasquier et al., 1977; Graeff & Filho, 1978; Reisine et al., 1982; Sutherland, 1982; Swanson, 1982; Skagerberg et al., 1984; Wirtshafter & Asin, 1986; Vertes & Martin, 1988; Behzadi et al., 1990; Nagao et al., 1993; Barnéoud et al., 2000; Kim et al., 2014; Baker et al. 2015; Balasubramani et al., 2015). It's worth pointing out that there is a robust efferent projection from the MRN to the HPC (Azmitia & Segal, 1978; Vertes et al., 1999). Impairments of the MRN generated HPC low-frequency theta activity, while activation of the area caused the desynchronization of HPC electroencephalographic activity (Maru et al., 1979; Leranth & Vertes, 1999). Though there are no known direct connections between the LHb and HPC at this moment, the two areas are only one step away via this crucial node.

LHb has long been identified as a source of negative reward processing center, while HPC and PFC are crucial in complex goal-directed decision-making process (Matsumoto & Hikosaka, 2007; Hong et al., 2011; Viard et al., 2011; Euston et al., 2012). Consistent with the working hypothesis that involve the LHb, HPC and PFC in integrating together to enable animal's ability to perform in adaptive ways in well learned and highly demanding tasks (Baker & Mizumori, 2017; Mizumori & Baker, 2017). According this model, HPC distributes information related to updated behavioral decisions during periods of theta comodulating or sharp wave ripple events. Information on expected and actual response consequence related to the lately choices are transformed to PFC via striatum and orbitofrontal cortex. The PFC integrated the multiple pieces of information to determine whether the current strategy or responses should continue or adapt to reach the expected outcome. Following this process, PFC transfers signals to efferent targets

including the LHb, as to whether the current choices should be implemented or adjusted to optimize the upcoming outcome. LHb accommodates internal state information and sensory inputs from LH, SCN and EPN to conclude whether the response decision from PFC is still relevant to the current situation. If internal information indicate that the current response is the optimal to seek the goal, a 'match' signal would be transferred to the efferent targets (dopaminergic and serotonergic systems) of LHb to enable continuation of the response. If internal information indicates otherwise, a 'mismatch' signal would be sent out to stop or adjust current response resulting in a different behavioral sequence. After the implementation of the choice, LHb could feed back to HPC on the most recent sequence to enhance neural plasticity. As a result, within the HPC, spatially and temporally sequenced memory information can be modified to reflect the developing experience-dependent trajectories.

In summary, current study reveals new insights on the LHb in enabling flexible responding behavior in complex tasks. Moreover, as there is growing evidence that LHb abnormality is related to psychiatric disorders which resulted in a person's inability to flexibly switch responding strategies in aversive learning conditions (Proulx et al., 2014; Admon & Pizzagalli, 2015). Further replenish of the current model will contribute to future therapies for behavioral and cognitive disorders that resulted in insufficient control of behaviors in the changing environment.

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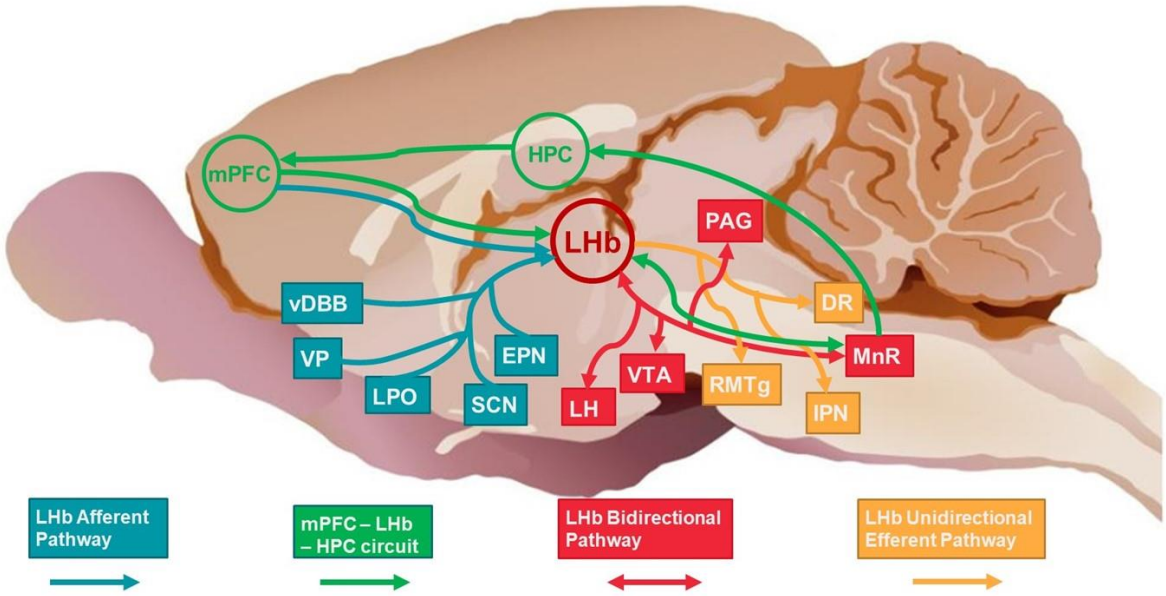

Figure 1. Schematic of selected afferent, bidirectional and efferent pathways of LHb (red circle) and HPC-mPFC-LHb circuit.

Afferent structures/pathways are shown in blue, bidirectional structures/pathways are shown in red, efferent structures/pathways are shown in yellow. The mPFC-LHb-HPC circuit structures/pathways are shown in green.

LHb, lateral habenula; HPC, hippocampus; mPfc, medial prefrontal cortex; vDBB, vertical diagonal band of Broca; VP, ventral pallidum; LPO, lateral preoptic area; SCN, suprachiasmatic nucleus; EPN, entopeduncular nucleus; LH, lateral hypothalamus; VTA, ventral tegmental area; RMTg, rostromedial tegmental nucleus; IPN, interpeduncular nucleus; MnR, median raphe; DR, dorsal raphe; PAG, periaqueductal gray.

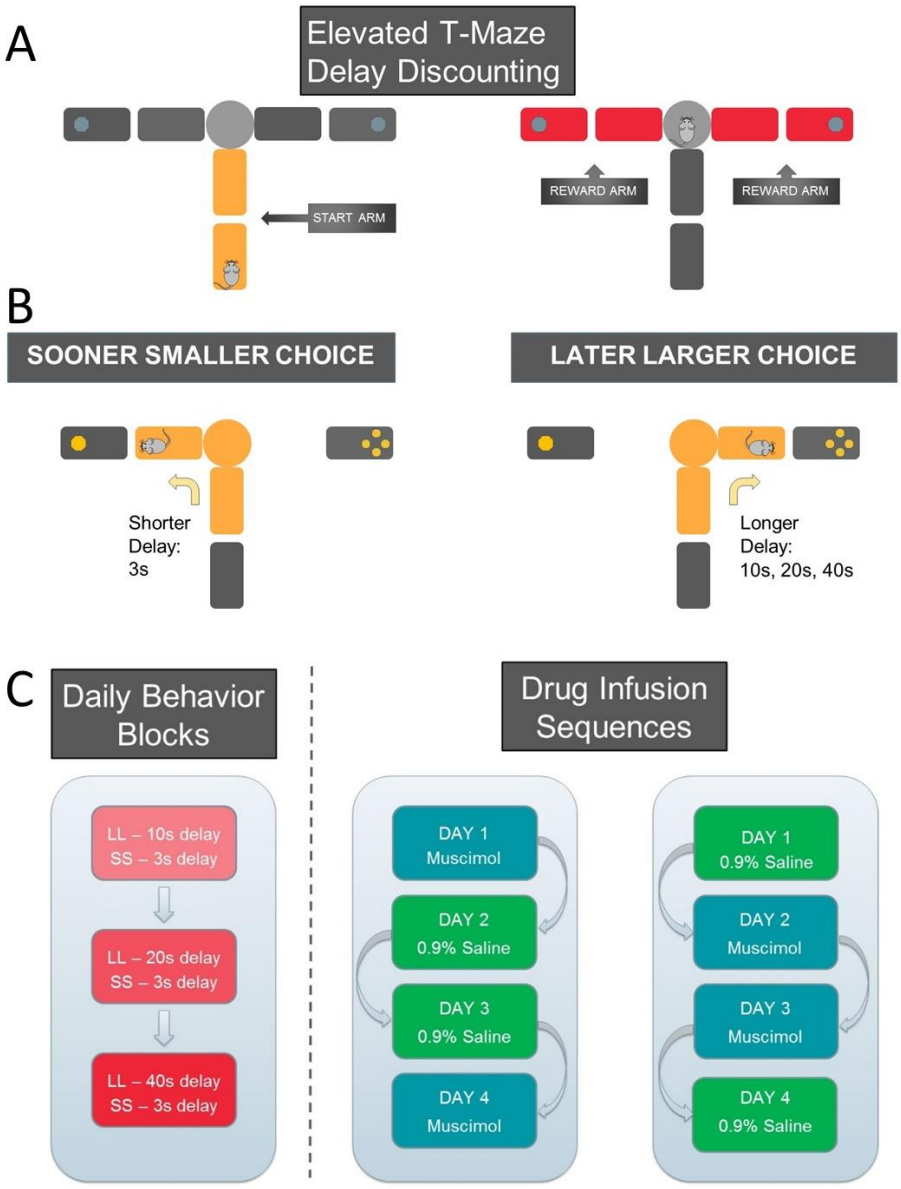

Figure 2. Schematic of Delay-Discounting Task.

(A) Reward arm (marked in yellow) and two start arms (marked in red) on the elevated T-maze.

(B) Sooner smaller choice: the animal would wait for 3s and obtain 1 sugar pellet. Later larger choice: the animal would wait for 10s and obtain 4 sugar pellets. Once the animal entered a certain reward arm, the other reward arm or start arm were blocked by wooden barriers to confine animal at the chosen arm for the entire delay period.

(C) Daily behavior blocks of an infusion day consist of 3 blocks with different length of LL delay (10s, 20s, and 40s) and consistent SS delay (3s). The blocks were separated by an inter block interval of 5 minutes. Drug infusion sequences follow an ABBA pattern. On day 1 and day 4, a certain animal would receive one type of infusion (0.9% saline or muscimol), and day 2 and day 3 of the alternative type of infusion.

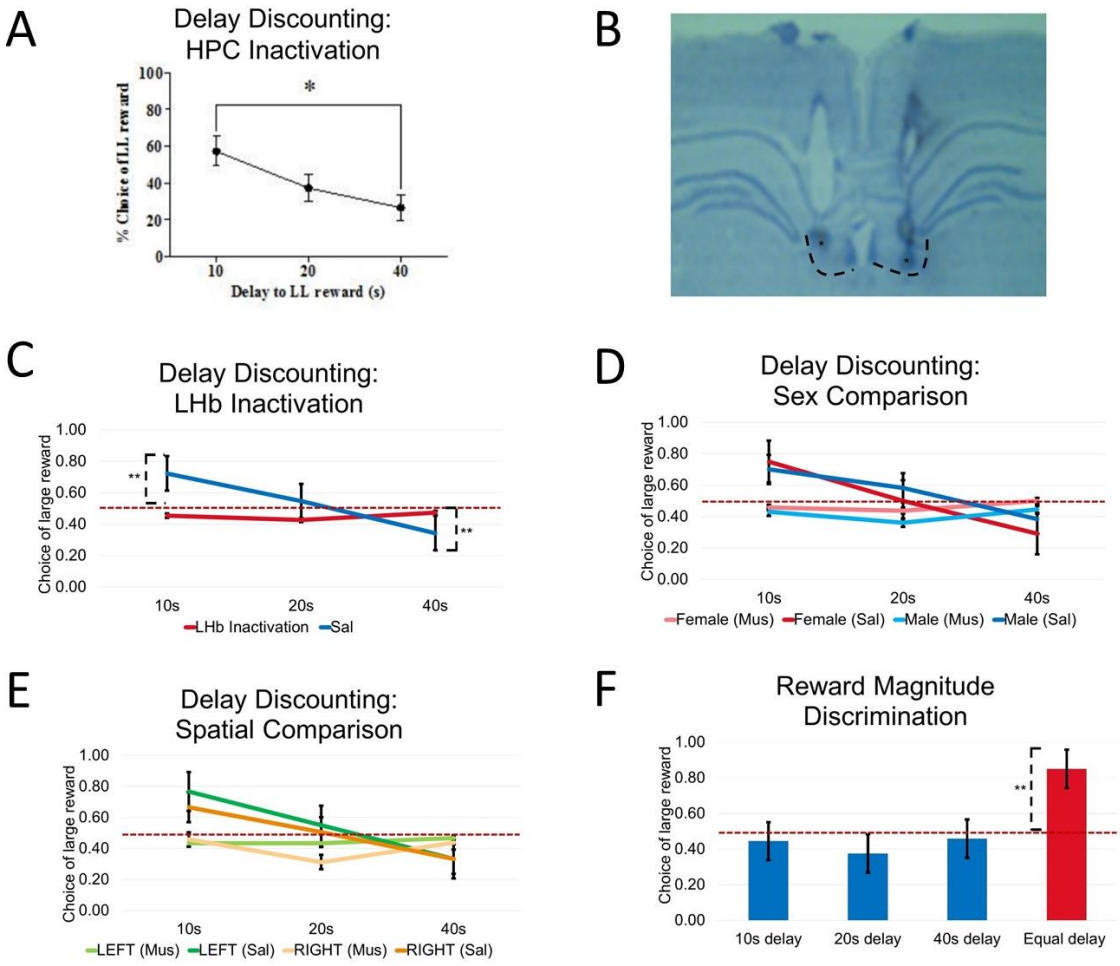

Figure 3. Bilateral LHb inactivation resulted in impaired performance in delay-discounting task.

(A) Bilateral HPC inactivation resulted in significant impaired performance in delay-discounting task. Figure modified from (Davis & Mizumori., 2017).

(B) Cannula placements in the LHb.

(C) Bilateral LHb inactivation resulted in significant impairment in delay-discounting task. Significant interaction, $F(2, 16) = 34.725$, $P < 0.01$; no single out drug effect, $F(1, 8) = 3.510$, $p = 0.098$; significant delay effect, $F(2,16) = 15.561$, $p<0.01$.

(D) Impairments were not due to sex difference. No interaction under muscimol infusion: $F (2, 14) = 0.206$, $p = 0.816$, and no significant sex effect: $F (1,7) = 0.012$, $p = 0.916$; no interaction

under saline infusion: $F(2, 14) = 0.371$, $p = 0.697$, and no significant sex effect, $F(1,7) = 0.177$, $p = 0.687$

(E) Impairments were not due to spatial difference. No interaction under muscimol infusion: $F(2,14) = 0.127$, $p = 0.882$, and no spatial effect: $F(1,7) = 0.057$, $p = 0.819$; no interaction under saline infusion: $F(2, 14) = 2.268$, $p = 0.140$, and no spatial effect: $F(1,7) = 2.356$, $p = 0.169$

(F) Intact reward magnitude discrimination after LHb inactivation. Animals preferred larger reward significantly under equal delay condition, paired sample t test, $t = 10.513$, $p<0.01$.