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Acute Stress Induces Changes in Reward Processes in Lateral Habenula in Mice

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

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ABSTRACT OF THE THESIS

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Lateral habenula has been reported to carry the reward negative feature that distinguishes it from many other subcortical brain regions. Lateral habenula neurons are inhibited by rewarding events and activated by unpleasant events. Previous reports have revealed the role of LHb in encoding reward information. However, it is not clear how aversive stimulus affect reward

processes in LHb neurons. Here we used two-photon excited fluorescence scanning microscopy to record calcium signal through GRIN lens implanted in mice with GCaMP6S expression in LHb. Based on neural responsive pattern to reward and reward omission, we categorized imaged neurons into reward-selective (RS), non-specific (NS), and non-responsive (NR) neurons. By inducing acute stress (tail shock), we observed that the reward response of RS neurons was inverted, and anticipation and consumption of reward significantly decreased. Later, we also found that the reward response in LHb neurons can be manipulated by switching between presence and absence of stress.

INTRODUCTION

The habenula, located at the dorsal thalamus near the pineal gland, is composed of a medial and lateral part on each side of the brain (Proulx et al., 2014). Habenular nuclei receive input from stria medullaris and send output through fasciculus retroflexus (Hikosaka, 2010). The lateral habenula (LHb), specifically, receives input from various upstream regions such as basal ganglia, ventral pallidum, lateral hypothalamus and other regions of the limbic system. LHb is believed to be a reward-negative region where its neurons are inhibited by rewarding events and activated by aversive stimuli and a number of studies have revealed the role of LHb in reward prediction error and its influence on goal-directed behavior (Matsumoto and Hikosaka, 2007). Previous studies have shown that the efferent signal from the LHb has a major impact on the regulation of the serotonergic and dopaminergic systems, which are two of the major regulatory monoamine systems (Lecourtier and Kelly, 2007). LHb projects to the medial and dorsal raphe nuclei that contain mostly serotonergic neurons, and it is believed that LHb inhibits serotonin release through an excitatory projection on GABAergic neurons that suppresses serotonin release (Aghajanian and Wang, 1977; Nishikawa and Scatton, 1985). Similarly, LHb has inhibitory control on downstream dopaminergic release, including substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) through GABAergic projections from the mesopontine rostromedial tegmental nucleus (RMTg) that is excited by LHb efferents (Gonçalves et al., 2012; Jhou et al., 2009). The effect that LHb exerts on both serotonergic and dopaminergic neurons underlies its capability on regulating emotions and behavior.

LHb has a particularly high sensitivity to aversive stimuli, which elicit an immediate excitation in LHb neurons (Wang et al., 2017). In an earlier study, the stress induced by unpleasant stimuli was found to cause a loss of interest in an open field activity in rat models (Katz et al.,

1981). Similarly, depression-like symptoms, such as emotional changes, were also observed in animal models with a defective LHb (Yang et al., 2008; Li et al., 2011). The synaptic and cellular mechanisms of these behavioral changes were traced to the output projections to serotonergic and dopaminergic neurons of those with affective disorders and helplessness models (Morris et al., 1999; Shumake et al., 2003).

In addition, anhedonia, defined as hyposensitivity to reward, was found to be related to an alteration in reward prediction function of the LHb (Kumar et al., 2018). However, it is not clear how stress and other aversive stimuli affect the reward processes in the LHb. To address this question, we induced acute stress on reward-conditioned mice to observe changes in the neural response to reward in the presence of stress, as well as their reward consumption behavior.

RESULTS

To study reward response and reward prediction processes in LHb, we trained WT mice to consume a reward that is paired with a conditioned stimulus (CS). Sucrose was delivered through a lick spout that was positioned near the mouth of the head-fixed mouse. The licking intensity was measured by the same sucrose delivery apparatus. Animals' licking behavior was recorded and analyzed in three trial types: a CS followed by sucrose (CS+ Sucrose), a CS with sucrose omitted (CS+ Omission), and a different CS without paired unconditioned stimulus (CS-). The animals that learned the association between sucrose and CS+ showed anticipatory licking before the onset of sucrose delivery in both sucrose and sucrose omission trials. Animals also showed prolonged licking behavior during sucrose trials, indicating reward consumption (1A).

To measure neural activity in the LHb, we used a two-photon excited fluorescence scanning microscopy system to record transient calcium signals in neurons. Animals were injected with AAV-GCaMP and td-Tomato and a GRIN lens was surgically implanted over the LHb, this allowed us to successfully record both channels from the same group of cells across days (1B, 1C).

To test whether neurons in the LHb have the same response pattern to reward, we recorded and analyzed neural activity from a total of 42 neurons in all three types of trials, including CS+ Sucrose, CS+ Omission, and CS- (control). The GCaMP signal was normalized to the td-Tomato signal, and to the baseline from the beginning of each trial. Thirty-seven neurons imaged showed responses that are time-locked to the given stimulus (1E–1L). Eighteen of these cells showed different activity during sucrose consumption and omission (1F, 1G). There was typically little response on control trials (CS-). These RS neurons had a significant change in activity during reward consumption and also a significant change in activity between reward consumption and reward omission, which indicates that these neurons are able to compute the difference between

reward, neutral, and aversive stimuli, so we classified these neurons as reward-selective (RS) neurons (1J, 1E). The non-selective (NS) neurons had a change in activity during reward consumption compared to baseline but no significant difference between responses to reward and omission. In the population of cells we analyzed, there were 5 neurons with both GCaMP and td-Tomato expression that showed no response to stimuli therefore we classified them as non-responsive (NR) neurons (1M–1P).

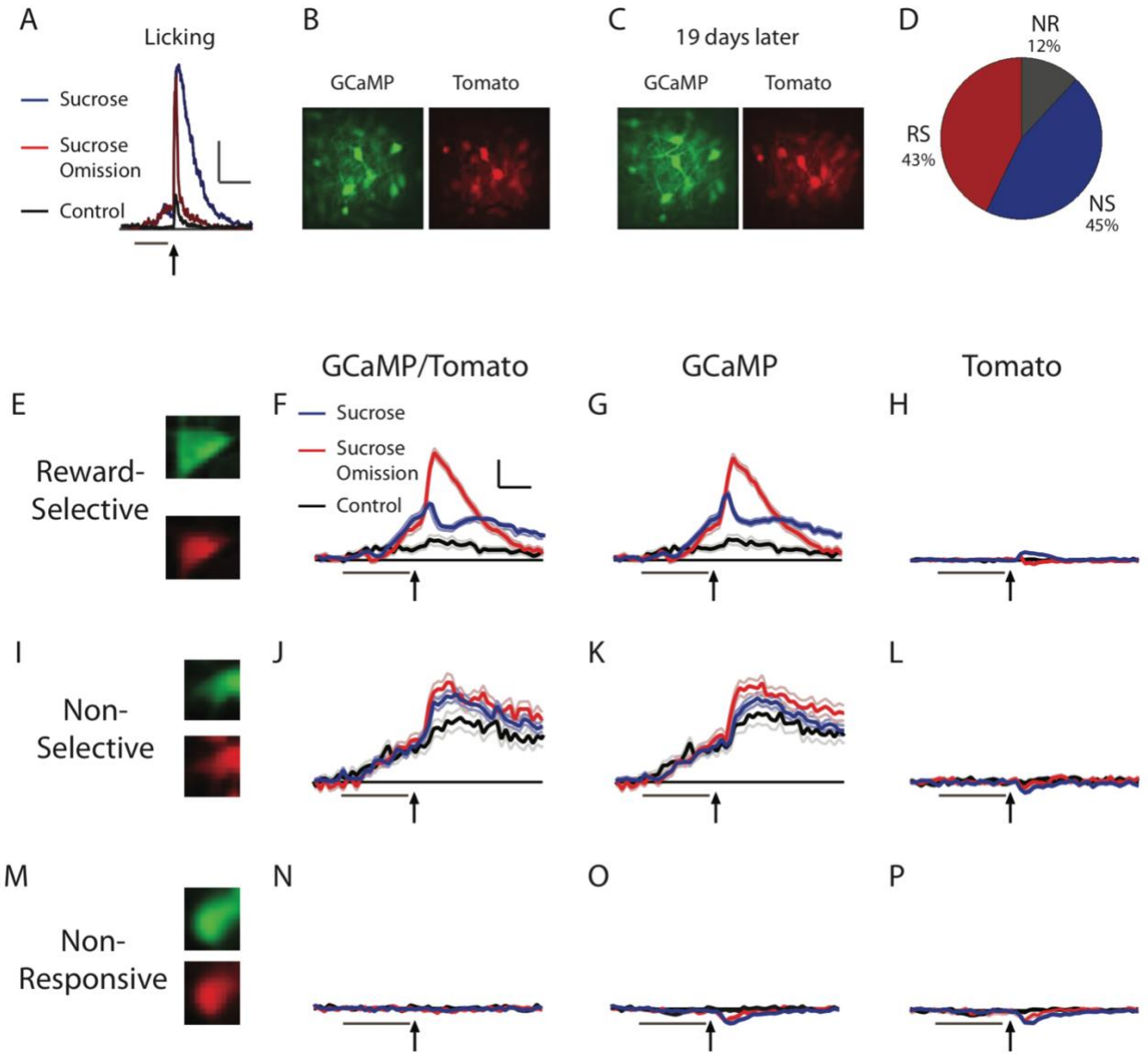

Figure 1. Dual-color, 2-photon imaging of LHb neuronal activity during reward and reward omission. (A) Mean licking during CS/Sucrose (blue), CS/Sucrose Omission (red), and Control Stimulus (black; n = 4 mice). Scale bars: 10 s, 20% licking. Arrow: Sucrose or Sucrose Omission. Gray bar: CS or Control Stimulus. (B) Image of GCaMP- and Tomato-expressing LHb neurons through GRIN lens. (C) Image of same region as (B) 19 days later. (D) Classification of LHb neurons: Reward-selective (RS, 18/42); Non-selective (NS, 19/42); Non-responsive (NR, 5/42). Example images (E, I, M; green, GCaMP; red, Tomato) and mean responses (F, G, H, J, K, L, N, O, P; 84-312 trials per trial type) for indicated cell type, trial type and channel. Arrow and gray bar, as in A. Scale bars: 5 seconds, 20 %. Response traces: ΔF/F, dark colors; ±SEM, light colors, for all figures.

To test the effects of stress on reward consumption behavior, we incorporated an electric shock paired with the control stimulus and interleaved with sucrose and sucrose omission trials. The shock was delivered through metal cuffs to tails of mice at a current of 0.6 mA for 0.3 seconds. We then measured animals' licking intensities during sucrose and sucrose omission trials. Prior to introducing tail shock, we observed that animals showed anticipatory licking during CS+ preceding the onset of sucrose delivery, as well as robust sucrose consumption per trial (2A, 2B, 2D, 2E). We then proceeded to analyze the licking behavior after the tail shock was introduced. The average of all 4 mice showed a significant decrease in anticipatory licking, as well as sucrose consumption, which indicates an inhibitory effect from tail shock on animals' expectations and reward consumption (2D, 2E). In addition, each individual mouse also displayed a decrease in sucrose consumption per trial after tail shock was introduced (2G). We also observed a significant change in licking within sucrose omission trials but not during control trials (2H, 2I).

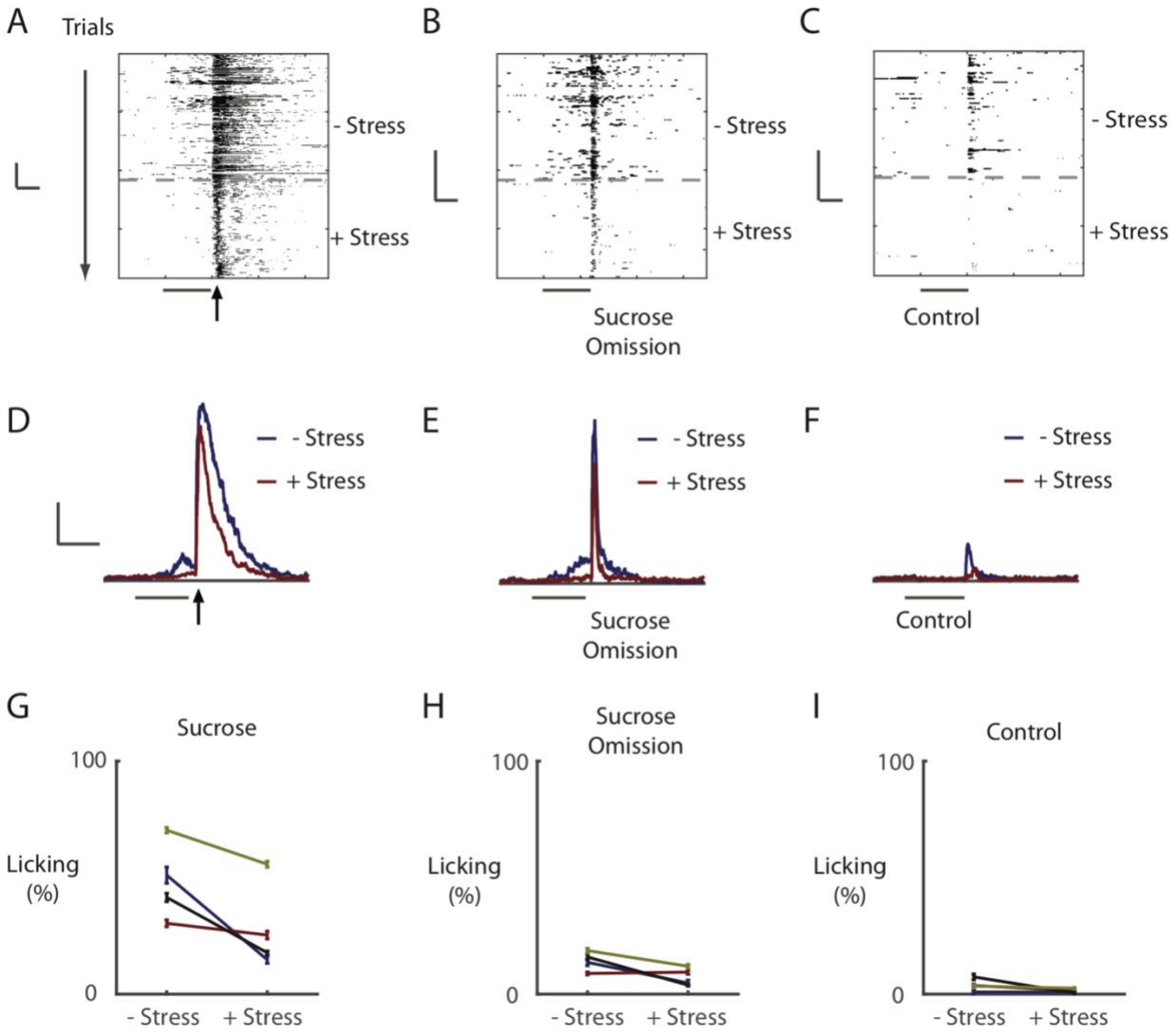

Figure 2. Stress decreases licking for reward. (A-C) Example licking data from one mouse before and during stress. Dashed line, beginning of tail shock stress. Scale bars: 5 seconds, 50 trials. Gray bar: CS (A,B) or Control Stimulus (C). Ticks denote licks. (D-F) Mean licking before and during stress for indicated trial type (n = 4 mice). Scale bars: 10 s , 20% . Gray bars as in A-C. (G-I) Mean (peak, ± SEM) licking for each mouse (different color), for indicated trial type, before and during stress.

The previous behavioral results indicate that the introduction of shock trials diminished both reward anticipation and reward consumption. To examine the potential neural basis, we studied the impact of introducing shock trials to neural responses during sucrose and sucrose omission trials. We first analyzed the RS neurons and their change in response to reward and reward omission. Before tail shock was introduced, RS neurons exhibited an inhibitory response to sucrose presentation and an excitatory response to omission of sucrose (3A). These neurons, however, showed a change in polarity of response to sucrose, so that after the introduction of tail shock, both trial types elicited an upward activity (2B). Furthermore, an average of responses to both sucrose and sucrose omission showed a net increase after shock was introduced (3E, 3F). This shows that introduction of shock alters the reward processing by these neurons; that is, shock trials make the response to reward and its absence similar. Next, we attempted to measure how quickly the changes in response due to tail shock occur. Among the same population of RS neurons, the increased activity in both sucrose and omission trials from the shock was observed immediately during the first session that shock was incorporated and remained stable for the following sessions, which suggests that these RS neurons are sensitive to the presence of strong aversive stimuli (3J).

We also studied NS neurons that did not initially exhibit a difference in responses to sucrose and omission of sucrose (1I-1L. 3C). We did not see shock-induced changes in the average response to either sucrose or omission trials (3D). Moreover, when examining individual cells, some neurons increased and others decreased in response to reward and reward omission. (3G, 3H).

Overall, incorporation of tail shock produced small changes in identities of three main types of neurons. Within the same population, the number of NS neurons decreased from 45% to 35%, NR neurons increased from 12% to 18% (3L, 3M) and RS neurons increased from 43% to 47%. The most dramatic change was in the number of reward prediction error (RPE) neurons; this category decreased sharply from 36% to only 3% which shifted this group of cells from RPE dominant to non-RPE dominant. This suggests that an aversive stimulus such as shock is able to affect how LHb neurons predict and process reward.

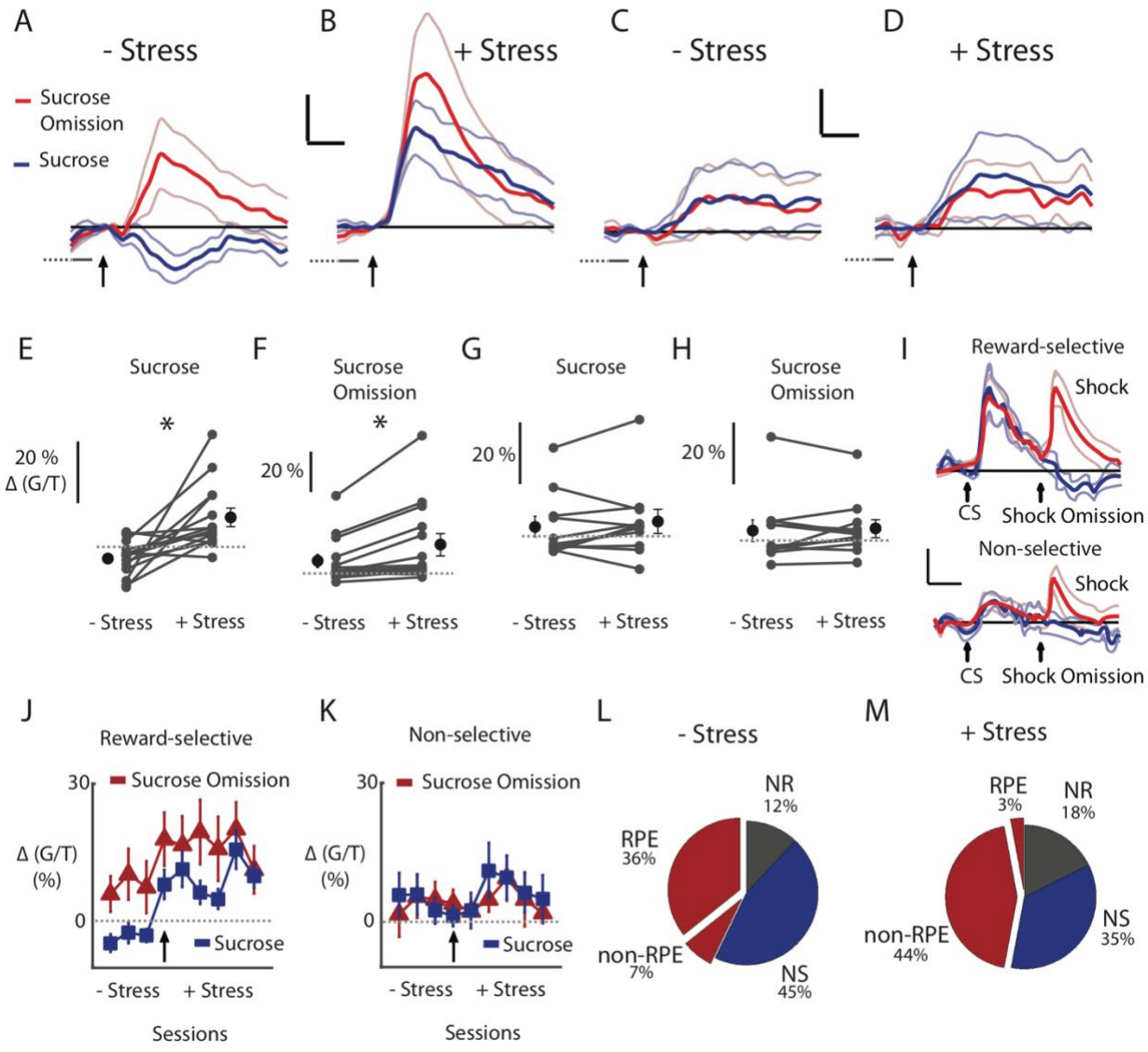

Figure 3. Stress inverts LHb neuronal reward responses. (A-D) Mean ΔF/F (9-24 trials over 3 or 6 days each) in GCaMP/Tomato for Reward-selective (A,B) and Non-selective neurons (C,D) during indicated trial type , before and during stress (RS, n = 14; NS, n=11). Scale bars: 2s, 5%. Dotted-solid lines indicate end of CS. (E-H) Mean peak response of indicated neuron type and trial type, for individual neurons (connected with line) before and during stress. (I) Mean responses of indicated neuron type during CS/shock and CS/Shock Omission trials (RS, n=8; NS, n=10). Scale bars 5s, 10%. (J,K) Mean peak response of indicated neuron type during indicated trial type for consecutive sessions before and during stress. Arrows: first session with stress. (L, M) % of neurons behaving as indicated class before (n=42) and during stress (n=34); RS neurons (red) displaying (RPE) or not (non-RPE) reward prediction error, is indicated. *, P < .05; NS, non-significant; t-test.

Next, we removed tail shock from our experiment to test if the effect of stress on neural response is reversible. We analyzed sucrose responses in the presence of either signaled stress or unsignaled stress. Among all the RS cells, we observed that the mean response to predicted sucrose (CS-paired) was reversed to the response in no stress state (4B). Additionally, introducing tail shock that occurred without a CS (unsignaled stress) also had similar effects as introduction of predictable shock (4B). The same phenomenon was observed in the individual RS neurons (4A, 4C). We then examined the neural response to unpredicted sucrose. Similarly, it showed an increase with the presence of stress, compared to no stress (4E). Furthermore, removing the stress was able to reverse the response back towards the no stress state, which was observed in the mean response and in responses from individual RS cells (4D-4F). This indicates that not only can the effects of stress on reward processes in LHb be reversed, it can also be freely manipulated through presence and absence of stress factors.

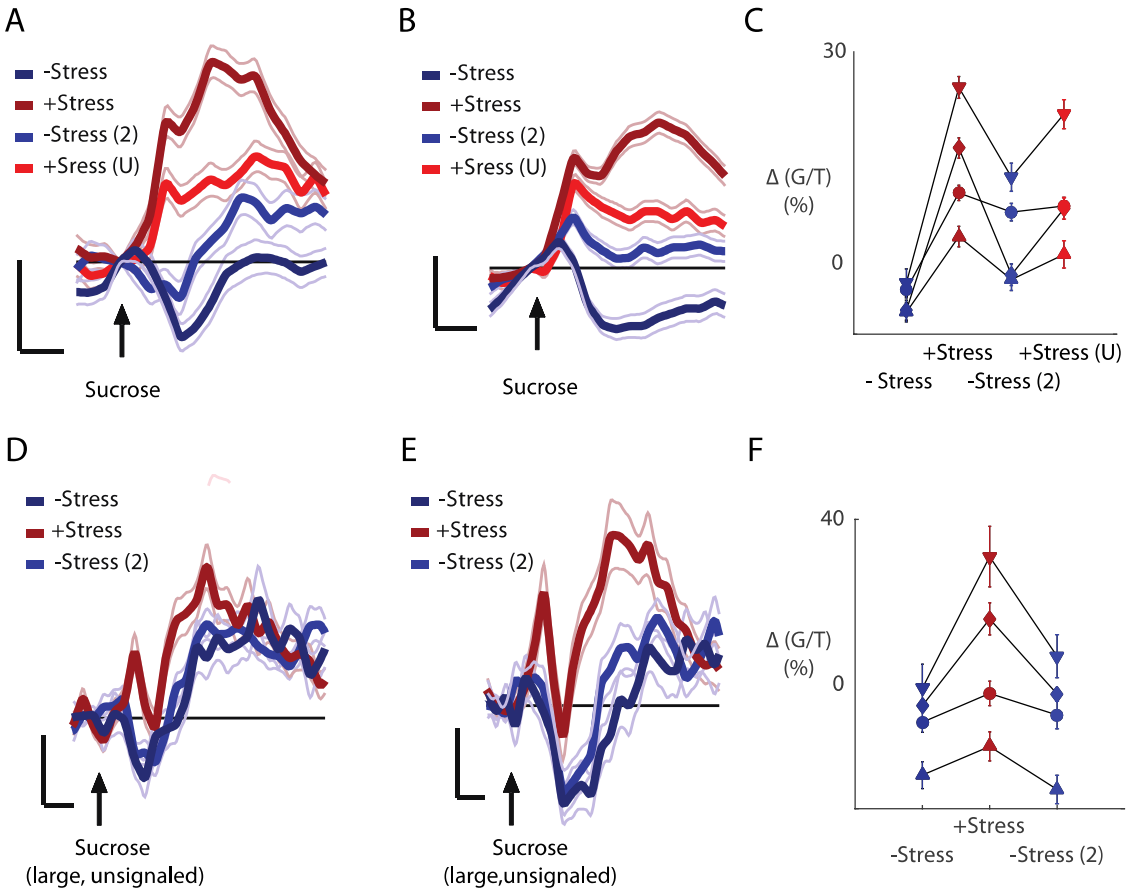

Figure 4. Effect of stress on LHb neuronal responses is reversible by stress removal. (A-C) Neuronal responses to sucrose with and without stress. –Stress(2), stress removal. +Stress(U), unsignaled stress. (A) Mean response of an example Reward-selective cell (ttests,P<.05). Scale 2s, 10%. Dotted-solid line indicates end of CS. (B) Mean response of all Reward-selective cells (n=4; ttests,P<.05). Scale 2s, 10%. Dotted-solid line as in A. (C) Mean neuronal responses. Symbols denote individual cells. (D-F) Neuronal responses to large, unsignaled sucrose with and without stress. –Stress(2), stress removal. (D) Mean response of an example Reward-selective cell (ttests, P<.05). Scale 2s, 10%. (E) Mean response of all Reward-selective cells (n=4; ttests,P<.05). Scale 2s, 10%. (F) Mean neuronal responses. Symbols as in (C).

DISCUSSION

Using a two-photon imaging system and GRIN lens technology, we have obtained GCaMP signal recording from the same group of neurons in LHb across days. Our observation was consistent with previous reports on RPE property in LHb neurons where we recorded a decrease in activity when reward was successfully predicted, and an increase in activity when reward was omitted (Schultz, 1998; Proulx, 2014). However, we have also found heterogeneity exists in LHb neurons, that is, not the entire population of LHb neurons showed RPE processing. Although some of the neurons in our recording showed responses to reward and punishment, they were not able to differentiate between reward and omission of reward. Thus, these neurons might possess other functions in local or downstream regulations other than RPE computation. In spite of reward processing, all responsive neurons showed excitation to the presence of shock, which reinforces the reward-negative nature of LHb.

Shock increased the activity of RS cells, which is consistent with previous findings (Wang et al., 2017). More importantly, we also observed inversion of response to reward from decrease to increase in this population of neurons. This change in direction in LHb activity suggests a decrease in the value of reward after the stress was introduced. This is also supported by an immediate decrease in reward prediction and consumption with induced stress, which could be explained by the shift in the neural response pattern. Combined with a universal increase in response to reward omission, the data show anhedonia in the recorded animals, as measured by a reduced behavioral response to a reward. The reversal of reward responses in the LHb may be fundamental to the diminished vigor to reward. Previously, it was reported that the outputs from LHb strongly regulate the downstream monoamine systems such as VTA and Raphe nuclei (Matsumoto and Hikosaka, 2007; Hong et al., 2011; Herkenham and Nauta, 1979; Goncalves et

al., 2012). Hyperactivity in LHb output onto RMTg nuclei eventually leads to inhibition on dopamine and serotonin release (Matsumoto and Hikosaka, 2007; Goncalves et al., 2012). Although it is not clear the underlying mechanism that caused the switch in reward response direction, we have seen that removing and re-introducing stress can manipulate LHb activity back and forth, so we hypothesize that this plasticity must reside in the inputs to LHb. In previous reports, GABA and glutamate were found to be co-released onto LHb from basal ganglia (Shabel et al., 2014). Therefore, it is possible that a reduction of GABA input contributes to the hyper-excitation of LHb. Further experiments could be done with electrophysiology and *in vivo* recording to test whether it is the presynaptic neurotransmitter switch that underlies the change in LHb neural activity.

These results implicate the LHb as a key player in the regulation of mental health. From what we observed, induced acute stress caused hyposensitivity to reward along with negative bias, which are two key indications of mental disorders such as depression, and patients with depression often carry life-long symptoms (Kumar et al., 2018). Therefore, if previous reported synaptic plasticity induced by stress can account for the changes in reward responses in LHb neurons, then we can have a greater understanding of the roles of LHb neurons in mental health mediation and in developing psychiatric disorder treatments.

CONCLUSION

Using a calcium imaging technique, we were able to record neuronal activity from the same group of LHb cells and the plasticity induced by external stimuli. By analyzing these neurons' response patterns to reward and reward omission, we classified LHb neurons into RS, NS, and NR neurons. Within the RS neurons, it was shown that induced acute stress inverted reward responses, bringing it closer to reward omission response, which altered their functions in RPE encoding. It was further demonstrated that removal of stress could reverse the effect of the stress. These observations suggest there is heterogeneity among the population of LHb neurons, and, more importantly, their manipulability through external stimuli.

METHODS

Surgery and Preparation

Age of about 65 days of mice were anesthetized through inhaling isoflurane and oxygen mixture, followed by 10% ketamine administered intraperitoneally. Heating pad was placed under animal during surgery to maintain body temperature. Craniotomy was performed to through the dorsal side of brain. GCaMP and td-Tomato viruses were injected through a glass pipette into one side of the LHb, 2.8mm ventral of bregma. 15 minutes after the injection, GRIN lens was implanted into LHb with injection. Dental cement was applied to hold the GRIN lens in place. Headbar was placed parallel and posteriorly to the eye level and anteriorly to GRIN lens, fixed by clear cement. All animals were given Carprofen post-surgery for pain control and were monitored daily.

All post-op mice were water deprived for five days before entering experiment. Water was given immediately after each imaging session for 15 minutes. Animals' body weights were closely monitored.

Two-photon Imaging

Image stack was acquired from two-photon imaging system. We used Coherent's Chameleon two-photon laser source and Bruker's scanner and Prairie View for image acquisition. The mouse was head-fixed and placed under the microscope, to which the GRIN lens is aligned with 20x objective (Olympus). The laser excitation wavelength was set at 1000 nanometers to acquire both GCaMP and td-Tomato signals. The excitation power through the objective was around 20mW, and images were acquired at frequency of 28.8Hz.

Behavioral Conditioning Experiments

The duration of the conditioned stimulus, tone or white noise, was 10 seconds, and was followed by one drop of a 10% sucrose solution, 0.5 seconds after the end of the CS+. The sucrose was delivered through a lick spout that was positioned near the mouth of the head-fixed mouse. The intensity of licking was measured by the same sucrose delivery apparatus.

Electric shock was applied from a control box and was delivered through two metal cuffs that tightly fit on mouse's tail. Conducting paste was applied in the contact area with electrodes. The current was consistent and was set at 0.6mA.

Histology

The mice were anesthetized with isoflurane followed by an injection of ketamine. Mice were perfused with saline followed by 4% paraformaldehyde solution. The perfusion was administered through the ventricle. The coronal brain slices were prepared with 20um in thickness. GCaMP and td-Tomato expression and lens path was then determined to verify the location of neurons imaged.

Data Analysis

Image stacks were motion corrected and averaged on a 1:100 ratio within each session using MATLAB program. Within each animal, ROIs were drawn for individual neurons that have both GCaMP and tdTomato expression and traced across days. GCaMP signal was normalized to tdTomato signal by division. For each trial, $\Delta F/F$ was calculated by $(F_{stimulus} - F_{baseline})/F_{baseline}$.

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