THE HABENULA AND ITS ROLE IN NEUROPSYCHIATRIC SYMPTOMS

EDITED BY: Jurgen Germann, Phillip Michael Baker,
Flavia Venetucci Gouveia and Manuel Mameli

PUBLISHED IN: Frontiers in Behavioral Neuroscience, Frontiers in Psychiatry
and Frontiers in Systems Neuroscience

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88976-380-1
DOI 10.3389/978-2-88976-380-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

THE HABENULA AND ITS ROLE IN NEUROPSYCHIATRIC SYMPTOMS

Topic Editors:
Jurgen Germann, University Health Network (UHN), Canada
Phillip Michael Baker, Seattle Pacific University, United States
Flavia Venetucci Gouveia, University of Toronto, Canada
Manuel Mameli, The University of Lausanne, Switzerland

Citation: Germann, J., Baker, P. M., Gouveia, F. V., Mameli, M., eds. (2022). The Habenula and Its Role in Neuropsychiatric Symptoms. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88976-380-1

Table of Contents

frontiers | Frontiers in Behavioral Neuroscience

EDITORIAL
published: 24 May 2022
doi: 10.3389/fnbeh.2022.929507

Editorial: The Habenula and Its Role in Neuropsychiatric Symptoms

Flavia Venetucci Gouveia[1]*, *Phillip Michael Baker*[2], *Manuel Mameli*[3,4] and *Jurgen Germann*[5]*

[1] Neuroscience and Mental Health, The Hospital for Sick Children Research Institute, Toronto, ON, Canada, [2] Department of Psychology, Seattle Pacific University, Seattle, WA, United States, [3] The Department of Fundamental Neuroscience, The University of Lausanne, Lausanne, Switzerland, [4] INSERM, UMR-S 839, Paris, France, [5] Division of Neurosurgery, Department of Surgery, University Health Network and University of Toronto, Toronto, ON, Canada

Keywords: habenula, psychiatric disorder, reward & motivation, monoaminergic system, neuroimaging, neuromodulation

Editorial on the Research Topic

The Habenula and Its Role in Neuropsychiatric Symptoms

The habenula (Hb) is a small epithalamic structure that, through its downstream connectivity controls major neurotransmitters, such as the cholinergic and monoaminergic systems (Hikosaka, 2010; Hu et al., 2020). The Hb contributes to a wide range of behaviors e.g. social behavior (van Kerkhof et al., 2013; Ogawa and Parhar, 2021), circadian rhythms (Liu et al., 2021; Salaberry and Mendoza, 2022), reward processing (Lalive et al., 2022; Mondoloni et al., 2022), decision-making (Stopper et al., 2014; Baker et al., 2015, 2017; Nuno-Perez et al., 2021), cognitive flexibility (Vadovičová, 2014; Baker et al., 2015, 2017), and is implicated in the neurobiology of a number of psychiatric disorders and neuropsychiatric symptoms (Hu et al., 2020). Recent findings confirmed the importance of the Hb in schizophrenia (Schafer et al., 2018; Li et al., 2019; Germann et al., 2020; Wang et al., 2020), bipolar disorder (Schafer et al., 2018; Zhang et al., 2019; Germann et al., 2020; Sonkusare et al., 2022), autism (Germann et al., 2021; Murru et al., 2021), depression (Yang et al., 2018; Barreiros et al., 2022; Zhang et al., 2022; Young et al.), and eating disorders (Maldonado et al., 2018; Wills et al., 2020; Carlson et al., 2022), and implicated the Hb in neuropsychiatric symptoms such as sleep disturbances (Aizawa et al., 2013; Ge et al., 2021), and agitation/aggressive behavior (Flanigan et al., 2017, 2020; Gan et al., 2019).

Thus, this timely special issue provided the space and opportunity for both clinical and pre-clinical researchers to have an up to date discussion of the important and broad role of the Hb in the various neuropsychiatric disorders and symptoms. In total, 60 authors from 8 different countries participated.

Emphasizing the broad role of the Hb, Baker et al. highlight some of the less explored aspects of lateral habenula (LHb) function in contextual memory, sleep, and behavioral flexibility, by providing evidence that the LHb is well-situated to integrate different internal states and multimodal sensory information. The authors focus on the impact of early life stress on LHb function to illustrate how dysregulations on LHb systems promote anhedonia and motivational deficits, and stress the importance of ethologically-relevant behaviors to further understand LHb involvement in a wide range of psychiatric illnesses. Illustrating the important role of the LHb in motivation Sevigny et al. using a unique behavioral paradigm that requires rats to climb a physical barrier in order to receive a large reinforcement or to opt for a smaller reward without the need to climb a barrier, show that pharmacological inactivation of the LHb results in fewer choices for the high-effort-high-reward option, demonstrating that the LHb is part of the circuit responsible for integrating external information on a trial-by-trial basis. This work points to the involvement of the LHb in the ability to discriminate rewards specifically when contingencies change in an unpredictable manner. This supports a growing body of experimental evidence arguing for a

OPEN ACCESS

Edited and reviewed by:
Rainer Spanagel,
University of Heidelberg, Germany

***Correspondence:**
Flavia Venetucci Gouveia
fvenetucci@gmail.com
Jurgen Germann
germannj@gmail.com

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *26 April 2022*
Accepted: *02 May 2022*
Published: *24 May 2022*

Citation:
Gouveia FV, Baker PM, Mameli M and Germann J (2022) Editorial: The Habenula and Its Role in Neuropsychiatric Symptoms. Front. Behav. Neurosci. 16:929507. doi: 10.3389/fnbeh.2022.929507

relevant contribution of the Hb in diverse facets of reward encoding (Stopper et al., 2014; Lalive et al., 2022).

Considering the important role of eye contact as the starting point of interactions in many social animals, Lee and Hikosaka recorded eye movement and LHb activity while monkeys viewed faces in the context of Pavlovian and instrumental conditioning tasks. The results show that faces associated with larger rewards elicited longer periods of eye contact and are associated with suppression of LHb neurons. Faces signaling low values are associated with excitation of LHb neurons. The authors conclude that the reward encoding of LHb contributes to social behavior and disorders, as a sequential goal-directed behavior. Webster et al., in their review, provide an up-to-date summary of the current state of knowledge on LHb neuronal activity and its association with Major Depressive Disorder (MDD). They discuss the growing body of literature on LHb excitatory and inhibitory neurons, downstream connections with the rostromedial tegmental nucleus, and involvement of the reward system, arguing that normalizing inhibitory signaling within the LHb may be a potential therapeutic strategy for MDD. Further studies are necessary to better understand the exact pharmacological and neural circuit mechanisms underlying inhibitory signaling within the LHb.

Another line of research associating Hb activity and MDD, involves circadian rhythms and light signals that affect the LHb. Young et al. review the literature regarding neuronal activity in the LHb during altered circadian rhythms and link it to mental disorders, including depression. The authors, however, highlight the need for further research before firm conclusions can be drawn regarding the importance of changes in the circadian function of the LHb in the etiology of depression and antidepressant treatments. New research by Elias et al. on the role of the Hb in the therapeutic effect of Deep Brain Stimulation (DBS) for MDD, showed clinical response to treatment was significantly associated with Hb volume changes, with responders showing increased Hb volume over time, and non-responders showing the opposite. Furthermore, functional MRI analysis showed DBS treatment to be significantly associated with increased Hb connectivity to several prefrontal and corticolimbic regions, areas previously implicated in the neurocircuitry of depression.

DBS targeting the Hb has been trialed for a number of psychiatric disorders as outlined in the review article by Germann et al. Merging the knowledge from pre-clinical and clinical observations, and using both the published literature as well as registered clinical trials the work highlights the important role of the Hb in mental health. The outcomes of the ongoing clinical trials for treating schizophrenia, depression, obsessive-compulsive disorder, and bipolar disorder will provide further knowledge that will be necessary to confirm the clinical benefit of this promising intervention. To investigate possible mechanisms of action of Hb-DBS, Zhang et al. explored the transient effects of Hb stimulation in patients with bipolar disorder and schizophrenia. Commonly elicited effects of stimulation were numbness, heart rate changes, pain, and involuntary movements and these showed a pattern suggesting a potential somatosensory organization of the Hb.

Expanding on the involvement of the Hb in psychiatric disorders, Lee and Goto in their perspective review hypothesize that an initially hypoactive Hb during childhood in individuals with Attention-Deficit-Hyperactivity-Disorder (ADHD) may undergo compensatory changes during development, priming the Hb to be hyperactive in response to stress exposure and thereby increasing vulnerability to MDD in adulthood. They suggest that the Hb is involved in the neural network of both MDD and ADHD, via direct and indirect connections with dopaminergic and serotonergic neurons in midbrain nuclei. Suggesting a role of the Hb in anxiety disorders, Liu et al. find that ovariectomized (OVX)-induced anxiety-like behavior is associated with increased LHb activity. Moreover, their results showed that estrogen-treated OVX rats present less anxiety-like behavior, higher levels of monoamines in dopaminergic and serotonergic nuclei, and reduced neuronal activity in the LHb, as compared to non-treated OVS rats. This effect is also observed following intra-LHb injections of estrogen receptor agonist in OVX rats. Gouveia and Ibrahim explore the anatomical organization of the Hb and discuss several distinct mechanisms by which the Hb is involved in the modulation of aggressive behaviors. They propose new investigations for the development of innovative neuromodulatory techniques targeting the Hb to reduce aggressive behaviors. Along those lines, Marks et al. propose that the LHb plays a critical role in the transition from suicidal ideations to self- harm. The authors argue that a multidisciplinary group of researchers is necessary to better understand the role of the LHb, and its long-term modulation, in response to the negative affect in suicidal behavior, to discern the underlying neural mechanisms of this contribution.

The studies presented in this special topic, highlight broad and important roles of the Hb in the neural-networks of several psychiatric disorders and neuropsychiatric symptoms, in both animal models and humans. This body of research points to the new experimental actions needed to further shed light on Hb cellular and molecular mechanisms, and its repercussions for physiological and pathological behaviors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

Aizawa, H., Cui, W., Tanaka, K., and Okamoto, H. (2013). Hyperactivation of the habenula as a link between depression and sleep disturbance. *Front. Hum. Neurosci.* 7, 826. doi: 10.3389/fnhum.2013.00826

Baker, P. M., Oh, S. E., Kidder, K. S., and Mizumori, S. J. Y. (2015). Ongoing behavioral state information signaled in the lateral habenula guides choice flexibility in freely moving rats. *Front. Behav. Neurosci.* 9, 295. doi: 10.3389/fnbeh.2015.00295

Baker, P. M., Raynor, S. A., Francis, N. T., and Mizumori, S. J. Y. (2017). Lateral habenula integration of proactive and retroactive

information mediates behavioral flexibility. *Neuroscience* 345, 89–98. doi: 10.1016/j.neuroscience.2016.02.010

Barreiros, A. R., Breukelaar, I., Mayur, P., Andepalli, J., Tomimatsu, Y., Funayama, K., et al. (2022). Abnormal habenula functional connectivity characterizes treatment-resistant depression. *Neuroimage. Clin.* 34, 102990. doi: 10.1016/j.nicl.2022.102990

Carlson, H. N., Christensen, B. A., and Pratt, W. E. (2022). Stimulation of mu opioid, but not GABAergic, receptors of the lateral habenula alters free feeding in rats. *Neurosci. Lett.* 771, 136417. doi: 10.1016/j.neulet.2021.136417

Flanigan, M., Aleyasin, H., Takahashi, A., Golden, S. A., and Russo, S. J. (2017). An emerging role for the lateral habenula in aggressive behavior. *Pharmacol. Biochem. Behav.* 162, 79–86. doi: 10.1016/j.pbb.2017.05.003

Flanigan, M. E., Aleyasin, H., Li, L., Burnett, C. J., Chan, K. L., LeClair, K. B., et al. (2020). Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice. *Nat. Neurosci.* 23, 638–650. doi: 10.1038/s41593-020-0617-7

Gan, G., Zilverstand, A., Parvaz, M. A., Preston-Campbell, R. N., d'Oleire Uquillas, F., Moeller, S. J., et al. (2019). Habenula-prefrontal resting-state connectivity in reactive aggressive men - a pilot study. *Neuropharmacology* 156, 107396. doi: 10.1016/j.neuropharm.2018.10.025

Ge, F., Mu, P., Guo, R., Cai, L., Liu, Z., Dong, Y., et al. (2021). Chronic sleep fragmentation enhances habenula cholinergic neural activity. *Mol. Psychiat.* 26, 941–954. doi: 10.1038/s41380-019-0419-z

Germann, J., Gouveia, F. V., Brentani, H., Bedford, S. A., Tullo, S., Chakravarty, M. M., et al. (2021). Involvement of the habenula in the pathophysiology of autism spectrum disorder. *Sci. Rep.* 11, 21168. doi: 10.1038/s41598-021-00603-0

Germann, J., Gouveia, F. V., Martinez, R. C. R., Zanetti, M. V., de Souza Duran, F. L., Chaim-Avancini, T. M., et al. (2020). Fully automated habenula segmentation provides robust and reliable volume estimation across large magnetic resonance imaging datasets, suggesting intriguing developmental trajectories in psychiatric disease. *Biol. Psychiat. Cogn. Neurosci. Neuroimag.* 5, 923–929. doi: 10.1016/j.bpsc.2020.01.004

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.* 11, 503–513. doi: 10.1038/nrn2866

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Lalive, A. L., Congiu, M., Lewis, C., Groos, D., Clerke, J. A., Tchenio, A., et al. (2022). Synaptic inhibition in the lateral habenula shapes reward anticipation. *Curr. Biol.* 32, 1829–36.e4. doi: 10.1016/j.cub.2022.02.035

Li, J., Yang, S., Liu, X., Han, Y., Li, Y., Feng, J., et al. (2019). Hypoactivity of the lateral habenula contributes to negative symptoms and cognitive dysfunction of schizophrenia in rats. *Exp. Neurol.* 318, 165–173. doi: 10.1016/j.expneurol.2019.05.005

Liu, H., Rastogi, A., Narain, P., Xu, Q., Sabanovic, M., Alhammadi, A. D., et al. (2021). Blunted diurnal firing in lateral habenula projections to dorsal raphe nucleus and delayed photoentrainment in stress-susceptible mice. *PLoS Biol.* 19, e3000709. doi: 10.1371/journal.pbio.3000709

Maldonado, M., Molfese, D. L., Viswanath, H., Curtis, K., Jones, A., Hayes, T. G., et al. (2018). The habenula as a novel link between the homeostatic and hedonic pathways in cancer-associated weight loss: a pilot study. *J. Cachexia Sarcopenia Muscle* 9, 497–504. doi: 10.1002/jcsm.12286

Mondoloni, S., Mameli, M., and Congiu, M. (2022). Reward and aversion encoding in the lateral habenula for innate and learned behaviours. *Transl. Psychiat.* 12, 3. doi: 10.1038/s41398-021-01774-0

Murru, L., Ponzoni, L., Longatti, A., Mazzoleni, S., Giansante, G., Bassani, S., et al. (2021). Lateral habenula dysfunctions in Tm4sf2 mice model for neurodevelopmental disorder. *Neurobiol. Dis.* 148, 105189. doi: 10.1016/j.nbd.2020.105189

Nuno-Perez, A., Trusel, M., Lalive, A. L., Congiu, M., Gastaldo, D., Tchenio, A., et al. (2021). Stress undermines reward-guided cognitive performance through synaptic depression in the lateral habenula. *Neuron* 109, 947–956.e5. doi: 10.1016/j.neuron.2021.01.008

Ogawa, S., and Parhar, I. S. (2021). Role of habenula in social and reproductive behaviors in fish: comparison with mammals. *Front. Behav. Neurosci.* 15, 818782. doi: 10.3389/fnbeh.2021.818782

Salaberry, N. L., and Mendoza, J. (2022). The circadian clock in the mouse habenula is set by catecholamines. *Cell Tissue Res.* 387, 261–274. doi: 10.1007/s00441-021-03557-x

Schafer, M., Kim, J.-W., Joseph, J., Xu, J., Frangou, S., and Doucet, G. E. (2018). Imaging habenula volume in schizophrenia and bipolar disorder. *Front. Psychiat.* 9, 456. doi: 10.3389/fpsyt.2018.00456

Sonkusare, S., Ding, Q., Zhang, Y., Wang, L., Gong, H., Mandali, A., et al. (2022). Power signatures of habenular neuronal signals in patients with bipolar or unipolar depressive disorders correlate with their disease severity. *Transl. Psychiatry* 12, 72. doi: 10.1038/s41398-022-01830-3

Stopper, C. M., Tse, M. T. L., Montes, D. R., Wiedman, C. R., and Floresco, S. B. (2014). Overriding phasic dopamine signals redirects action selection during risk/reward decision making. *Neuron* 84, 177–189. doi: 10.1016/j.neuron.2014.08.033

Vadovičová, K. (2014). Affective and cognitive prefrontal cortex projections to the lateral habenula in humans. *Front. Hum. Neurosci.* 8, 819. doi: 10.3389/fnhum.2014.00819

van Kerkhof, L. W. M., Damsteegt, R., Trezza, V., Voorn, P., and Vanderschuren, L. J. M. J. (2013). Functional integrity of the habenula is necessary for social play behaviour in rats. *Eur. J. Neurosci.* 38, 3465–3475. doi: 10.1111/ejn.12353

Wang, Y., Zhang, C., Zhang, Y., Gong, H., Li, J., Jin, H., et al. (2020). Habenula deep brain stimulation for intractable schizophrenia: a pilot study. *Neurosurg. Focus* 49, E9. doi: 10.3171/2020.4.FOCUS20174

Wills, K. E., Gosnell, S. N., Curtis, K. N., Velasquez, K., Fowler, J. C., and Salas, R. (2020). Altered habenula to locus coeruleus functional connectivity in past anorexia nervosa suggests correlation with suicidality: a pilot study. *Eat. Weight Disord.* 25, 1475–1480. doi: 10.1007/s40519-019-00746-0

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., et al. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nat. Publish. Group.* 554, 317–322. doi: 10.1038/nature25509

Zhang, C., Kim, S.-G., Li, D., Zhang, Y., Li, Y., Husch, A., et al. (2019). Habenula deep brain stimulation for refractory bipolar disorder. *Brain Stimul.* 12, 1298–1300. doi: 10.1016/j.brs.2019.05.010

Zhang, C., Zhang, Y., Luo, H., Xu, X., Yuan, T.-F., Li, D., et al. (2022). Bilateral Habenula deep brain stimulation for treatment-resistant depression: clinical findings and electrophysiological features. *Transl. Psychiatry* 12, 52. doi: 10.1038/s41398-022-01818-z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Psychiatry

BRIEF RESEARCH REPORT
published: 23 June 2021
doi: 10.3389/fpsyt.2021.674962

Deep Brain Stimulation-Induced Transient Effects in the Habenula

Chencheng Zhang[1,2], *Yijie Lai*[2], *Yingying Zhang*[2], *Xinmeng Xu*[2], *Bomin Sun*[1,2] *and Dianyou Li*[1,2]*

[1] Clinical Neuroscience Center, Ruijin Hospital Luwan Branch, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2] Department of Neurosurgery, Center for Functional Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

The habenula, located in the epithalamus, has been implicated in various psychiatric disorders including mood disorders and schizophrenia. This study explored the transient effects of deep brain stimulation in the habenula. Each of the four patients (two with bipolar disorder and two with schizophrenia) was tested with eight deep brain stimulation contacts. Patients were examined via transient electrical stimulation 1 month after deep brain stimulation surgery. The pulse width was 60 μs and the voltage ranged from 0 V to a maximum of 10 V, increasing in increments of 1 V. Each patient received stimulation at two frequencies, 60 and 135 Hz. A total of 221 out of 385 active trials elicited stimulation-induced effects. The three most common transient effects were numbness, heart rate changes, and pain. The incidence of numbness, heart rate changes, pain, and involuntary movements increased with the increase in stimulation voltage. Through contralateral stimulation, numbness was triggered in all parts of the body except the scalp. The obtained stimulus-response maps suggested a possible somatosensory organization of the habenula.

Keywords: deep brain stimulation, habenula, acute electrical stimulation, bipolar disorder, schizophrenia

OPEN ACCESS

Edited by:
Jurgen Germann, University Health Network (UHN), Canada

Reviewed by:
Gavin Elias, University of Toronto, Canada
Mario Cristancho, University of Pennsylvania, United States

***Correspondence:**
Dianyou Li
ldy11483@rjh.com.cn

Specialty section:
This article was submitted to Neuroimaging and Stimulation, a section of the journal Frontiers in Psychiatry

Received: *02 March 2021*
Accepted: *17 May 2021*
Published: *23 June 2021*

Citation:
Zhang C, Lai Y, Zhang Y, Xu X, Sun B and Li D (2021) Deep Brain Stimulation-Induced Transient Effects in the Habenula. Front. Psychiatry 12:674962. doi: 10.3389/fpsyt.2021.674962

INTRODUCTION

The habenula is a paired, evolutionarily conserved structure located in the epithalamus. It connects more recently evolved structures in the neocortex and limbic forebrain that are responsible for executive functions with ancient areas in the midbrain and hindbrain that process sleep, pain, and reward (1). Many preclinical studies have acknowledged this structure in psychiatric disorders such as mood disorders and schizophrenia (2).

Deep brain stimulation (DBS) involves the implantation of electrodes within specific areas of the brain and the generation of electrical impulses that control abnormal brain activity. The amount of stimulation in DBS is controlled by a pacemaker-like device which is usually placed under the skin in the upper chest. A wire traveling beneath the skin connects the device to the electrodes in the brain. DBS has been approved for the treatment of various conditions including dystonia, epilepsy, essential tremor, obsessive-compulsive disorder, and Parkinson's disease (3). DBS is also investigated as a potential treatment for addiction (4), major depression (5), and Tourette syndrome (6). Physicians may use DBS to treat movement disorders or neuropsychiatric

disorders if medications become less effective or if poorly tolerated. Unlike other surgical options, an advantage of DBS is that it is reversible and does not cause permanent damage to any part of the brain (7). Therefore, deep brain stimulation is seen as a promising treatment for psychiatric disorders. In two clinical trials targeting the habenula (5, 8), we systematically assessed transient responses to focal electrical stimulation.

METHODS

Four patients with refractory psychiatric disorders [**Table 1**, two with schizophrenia (8) and two with bipolar disorder (5)] underwent DBS placement in the bilateral habenula (**Figures 1**, **2A,B**). All the patients were male with a mean age of 31 years. The test was conducted at 1 month after surgery. The stimulation pulse width was fixed at 60 μs and the voltage was systematically increased from 0 V to a maximum of 10 V in increments of 1 V. Our test stimulation was delivered unilaterally on each contact in each patient. The contact was tested from ventral to dorsal areas and the frequency was set at low frequency (60 Hz) and titrated to high frequency (135 Hz) later in the test. If the patient reported an unacceptable side-effect, trials with higher voltage amplitudes at that contact were waived. Meanwhile, expecting that 0 V stimulation (no stimulation) would cause little effects on the patients, we decided to reduce the number of sham trials to facilitate the progression of the experiment. Patients underwent single blind tests during which they sat in a chair facing a video camera and their heart rates were recorded. A programmer sat behind the patient and a recorder sat behind the camera. The programmer used gestures to inform the recorder of the voltage used. At the beginning of each trial, parameters of DBS were changed, and the patient was asked to describe what they felt in a time window of 2 min. The association between stimulation parameters and different stimulation-induced effects was examined using logistic regression and a two tailed $p < 0.05$ was considered significant. Positions of the electrodes in the nucleus were reconstructed using the Lead-DBS toolbox in MATLAB according to the methods described by Horn et al. (9). For each patient, we also explored the affected body parts in numbness trials. All participants provided written consent and the experimental protocol was approved by the ethics committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

TABLE 1 | Conditions of the different trials.

Variables	Specific conditions	Trials	Proportion (%)
Frequency (Hz)	60	195	50.6%
	135	190	49.4%
Diagnosis	BP	211	54.8%
	SZ	174	45.2%
Voltage (V)	1	25	6.5%
	2	58	15.1%
	3	38	9.9%
	4	60	15.6%
	5	41	10.6%
	6	56	14.5%
	7	26	6.8%
	8	39	10.1%
	9	18	4.7%
	10	24	6.2%
Patient	NO. 1	127	33.0%
	NO. 2	84	21.8%
	NO. 3	91	23.6%
	NO. 4	83	21.6%
Cathode	0	42	10.9%
	1	51	13.2%
	2	49	12.7%
	3	47	12.2%
	8	42	10.9%
	9	51	13.2%
	10	51	13.2%
	11	52	13.5%

BP, bipolar disorder; SZ, schizophrenia.

RESULTS

A total of 385 active and 9 sham trials were tested (**Table 2**); 219 active and no sham trials induced transient stimulation-induced effects. The most common responses were numbness (66.7% of 219 trials), heart rate changes (36.1%), pain (16.9%) and dizziness (16.0%). Other transient effects included eye closure (11.0%), involuntary movements (8.2%), giddiness (8.2%), chest pain (6.4%), nausea (5.5%), discomfort (3.2%), a feeling of relaxation (2.3%), and a feeling of heaviness in some body parts (0.9%). One patient with bipolar disorder also reported in some trials a feeling of electrical shock (5.9%) and palpitation (1.8%) (**Table 3**). No severe adverse events were observed.

The incidence of numbness ($p = 0.015$), heart rate change ($p = 0.071$), pain ($p = 0.425$), and involuntary movements ($p = 0.534$) increased as the DBS voltage increased. It appeared more often for the 60-Hz voltage pulse to evoke an increase in heart rate ($p < 0.0001$) and less often to induce dizziness than for the 135-Hz voltage pulse ($p = 0.003$). DBS elicited heart rate change ($p < 0.0001$) and pain ($p < 0.0001$) more often in patients with schizophrenia than with bipolar disorder. On the other hand, patients with bipolar disorder exhibited giddiness ($p = 0.001$) and involuntary movements ($p = 0.001$) more often. Specifically, under active stimulation, one patient with schizophrenia reported feelings of pain in almost all trials (36 out of 37 = 97.3%); the other patient with schizophrenia experienced eye closure in more than half of the trials (15 out of 24 = 62.5%); one patient with bipolar disorder reported involuntary

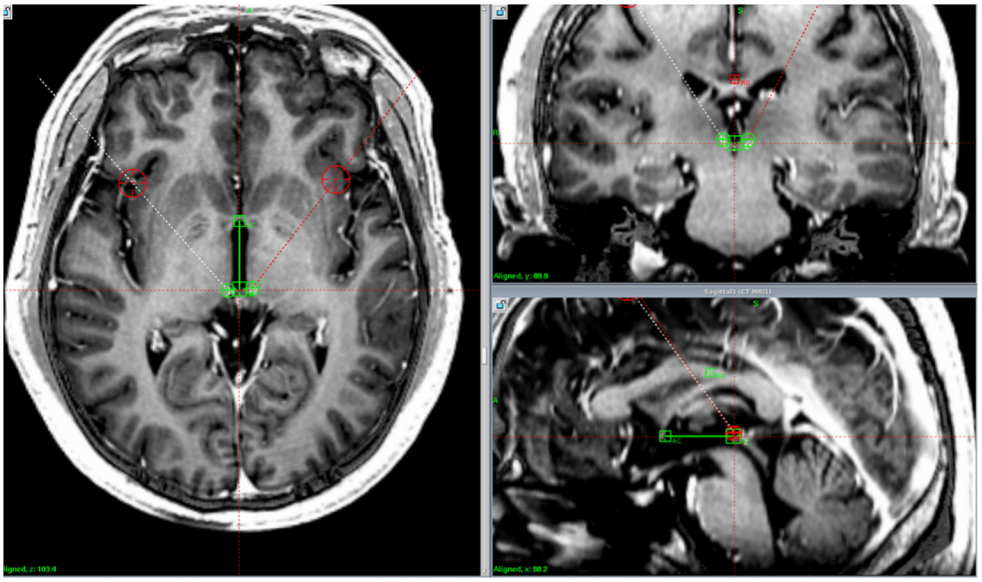

FIGURE 1 | Lead location. Postoperative computed tomography images fused with preoperative magnetic resonance imaging demonstrating the position of the implanted electrodes in the habenula in one patient.

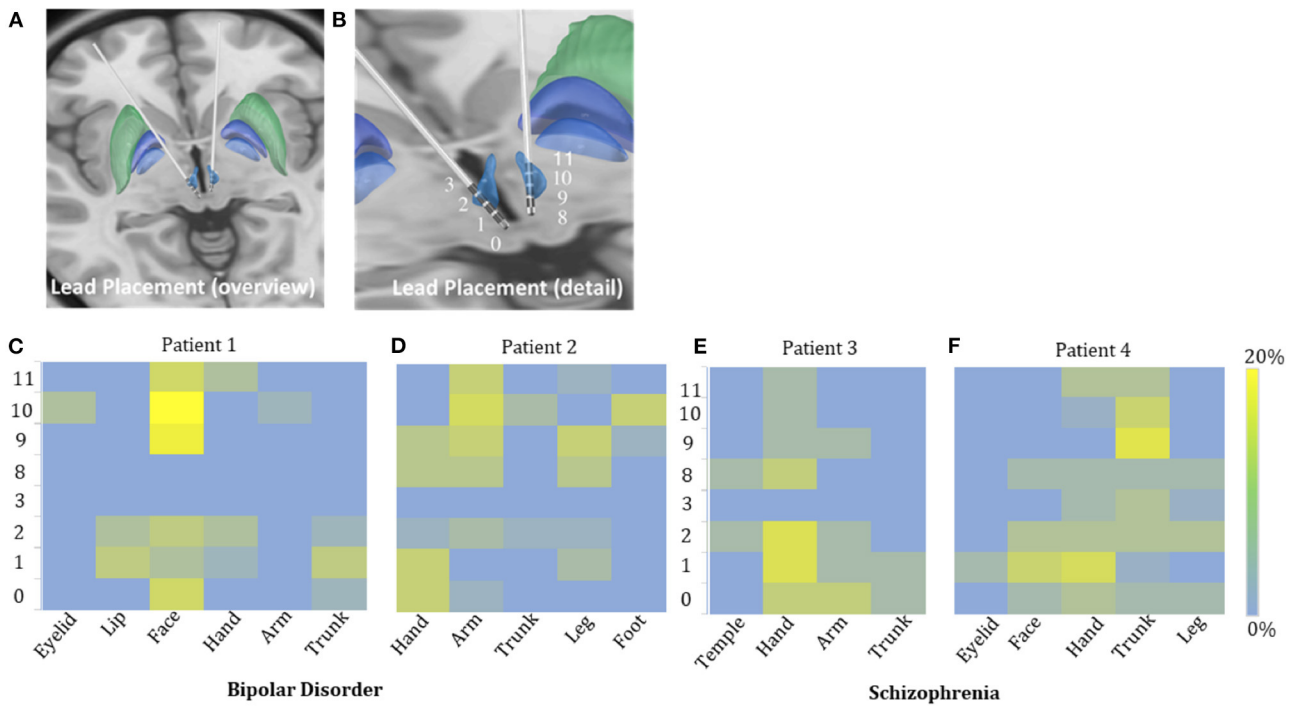

FIGURE 2 | Possible sensory projections in the habenula. Overview **(A)** and detailed view **(B)** of the deep brain stimulation lead placement. The letters marked beside the cathodes **(C–F)** indicate the most frequently reported body part affected by numbness. The colors box indicated the percentage of numbness occurrence at different contact and body parts in numbness trials for each patient. The contacts 0, 1, 2, and 3 were located in the right habenula from ventral to dorsal, and the contacts 8, 9, 10, and 11 were located in the left habenula from ventral to dorsal.

movements in 12 out of 18 trials (66.7%). The experience of numbness except on scalp was predominantly triggered by contralateral stimulation. The most frequently affected body parts in these four patients were face (26 out of 35 = 74.3%), arm (19 out of 41 = 46.3%), hand (13 out of 22 = 59.1%) and trunk (26 out of 48 = 54.2%), respectively (**Figures 2C–F**).

DISCUSSION

Somatosensory Organization of the Habenula

The safety and feasibility of DBS in the habenula for various refractory psychiatric disorders have been demonstrated in previous studies. In this study, we observed a lateralized pattern of stimulation-induced responses: the left side of the habenula appeared to correlate only with sensations in the right arm, while the right side of the habenula correlated with sensations in the left face, leg, and hand. Subject variability in the location of the active contact of each electrode could be a confounding factor. Unfortunately, the post-surgical imaging was not helpful in clarifying this issue.

TABLE 2 | Relationships of trial conditions with responses.

Response	Number of responses	Prop. of responses (%)	Lead contact	Voltage	Frequency	Diagnosis	Patient
Numbness	146	66.7	0.197	0.015*	0.702	0.848	<0.0001***
Changes in heart rate	79	36.1	0.635	0.071	<0.0001***	<0.0001***	<0.0001***
Pain	37[a]	16.9	0.573	0.425	0.454	<0.0001***	<0.0001***
Dizziness	35	16	0.002**	0.001**	0.003**	0.447	0.001**
Eye closure	24[b]	11	0.595	0.335	0.157	0.058	<0.0001***
Giddiness	18	8.2	0.036*	<0.0001***	<0.0001***	0.001**	<0.0001***
Involuntary movements	18[c]	8.2	0.008**	0.534	0.553	0.001**	<0.0001***

*Responses that occurred in <5% of the trials or in only one patient were omitted. Prop, proportion. *p < 0.05; **p < 0.01; ***p < 0.001. The total number of trials was 219. [a]36 reported in a patient with schizophrenia; [b]15 reported in another patient with schizophrenia; [c]12 reported in a patient with bipolar disorder.*

TABLE 3 | Demographic and clinical information.

Patient	NO.1	NO.2	NO.3	NO.4
Diagnosis	Bipolar disorder I	Bipolar disorder I	Schizophrenia	Schizophrenia
Comorbidity	No	Substance dependence (clonazepam, alcohol gambling disorder)	No	No
Age at surgery	41	46	26	21
Gender	Male	Male	Male	Male
Education (years)	12	9	10	10
Marriage	Married	Divorced	Single	Single
Duration of disease (years)	21	10	9	4
Current episode duration (years)	3.5	10	9	4
Past ETC	Yes	Yes	Yes	Yes
Medications per day	Clomipramine hydrochloride,150 m; Ozanpine, 5 mg	Lamotrigine, 100 mg; seroquel, 100 mg; magnesium valproate, 0.75 g; amfebutamone, 0.3 g; clonazepam, 16–20 mg	Ozanpine,10 mg; lithium carbonate, 0.25 g	Quetiapine, 0.2 g; benzhexol, 4 mg; ziprasidone hydrochloride, 60 mg
HAMD	24	23	NA	NA
YMRS	0	11	NA	NA
PANSS	NA	NA	74	66
Positive subscale	NA	NA	13	14
Negative subscale	NA	NA	23	19
General subscale	NA	NA	38	33

HAMD, Hamilton depression rating scale; YMRS, Yong mania rating scale; PANSS, Positive and negative syndrome scale; ECT, Electroconvulsive therapy; NA, Not available.

Various Effects Induced by Different Combination of Stimulation Parameters

Parameter settings have been discussed extensively in terms of movement disorders. For instance, 130 Hz is used as a standard frequency for DBS in Parkinson's disease because it balances between power consumption and clinical efficiency. A different choice of frequency may be applied to treat mood disorders, especially when the electrodes are placed at newly discovered brain targets. Low frequency or high frequency DBS in the habenula has been suggested to induce physiological effects relevant to habenula functions such as mood regulation in bipolar disorders. The patients' reactions to the two tested frequencies (60 and 135 Hz), namely changes in heart rate and feelings of dizziness, were significantly different, suggesting that the lower stimulation frequency was more likely to affect the heart rate while the higher frequency was more likely to affect sensations of dizziness.

Presumed Mechanisms Behind the Various Transient Effects

DBS is used to treat neuropsychiatric conditions by providing certain electrical stimulation to certain brain regions. However, DBS affects not just the targeted area but also other components in the neural network. The lateral habenula is involved in pain transmission by receiving pain signals from the spinal cord and interacting with canonical pain modulatory regions. The lateral habenula is also connected with hypothalamus which is known as an autonomic regulatory region (2). Therefore, the heart rate changes may be induced by the regulation of the central autonomic regulatory regions from the habenula.

Limitations

First, only four patients with psychiatric disorders were tested: the small sample size might limit the validity and generality of the study. Second, our results were based mainly on the patients' subjective descriptions which potentially introduced self-report bias. Also, specific psychiatric responses (i.e., mood, psychosis, and anxiety) were not monitored during testing.

To our knowledge, this is the first study to investigate transient effects induced by systematic electrical stimulation of the habenula. We have demonstrated the effect profile and have proposed a possible somatosensory organization in the habenula. The most common transient effect was numbness, followed by heart rate changes and pain. Different frequencies appeared to elicit similar responses in most cases. The relationship between transient effects and long-term clinical outcomes requires further investigation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ruijin Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DL and BS designed the study. DL conducted the testing. YZ collected the data. YL analyzed the data. CZ wrote the manuscript. YZ and YL drafted the figures. XX reviewed and revised the manuscript and wrote the abstract. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the STAR project (2019105).

ACKNOWLEDGMENTS

The authors would like to thank the participants that took part in this study.

REFERENCES

1. Ables JL, Antolin-Fontes B, Ibañez-Tallon I. Chapter 10 - Habenular synapses and nicotine. In: Preedy VR, editor. *Neuroscience of Nicotine*. Waltham, MA: Academic Press (2020). doi: 10.1016/B978-0-12-813035-3.00010-1
2. Hu H, Cui Y, Yang Y. Circuits and functions of the lateral habenula in health and in disease. *Nat Rev Neurosci.* (2020) 21:277–95. doi: 10.1038/s41583-020-0292-4
3. Lozano AM, Lipsman N, Bergman H, Brown P, Chabardes S, Chang JW, et al. Deep brain stimulation: current challenges and future directions. *Nat Rev Neurol.* (2019) 15:148–60. doi: 10.1038/s41582-018-0128-2
4. Ma S, Zhang C, Yuan T, Steele D, Voon V, Sun B. Neurosurgical treatment for addiction: Lessons from an untold story in China and a path forward. *Natl Sci Rev.* (2019) 7:702–12. doi: 10.1093/nsr/nwz207
5. Zhang C, Kim S-G, Li D, Zhang Y, Li Y, Husch A, et al. Habenula deep brain stimulation for refractory bipolar disorder. *Brain Stimulation.* (2019) 12:1298–300. doi: 10.1016/j.brs.2019.05.010
6. Xu W, Zhang C, Deeb W, Patel B, Wu Y, Voon V, et al. Deep brain stimulation for Tourette's syndrome. *Transl Neurodegener.* (2020) 9:4. doi: 10.1186/s40035-020-0183-7
7. Cagnan H, Denison T, McIntyre C, Brown P. Emerging technologies for improved deep brain stimulation. *Nat Biotechnol.* (2019) 37:1024–33. doi: 10.1038/s41587-019-0244-6
8. Wang Y, Zhang C, Zhang Y, Gong H, Li J, Jin H, et al. Habenula deep brain stimulation for intractable schizophrenia: a pilot study. *Neurosurgical Focus.* (2020) 49:E9. doi: 10.3171/2020.4.FOCUS20174
9. Horn A, Li N, Dembek TA, Kappel A, Boulay C, Ewert S, et al. Lead-DBS v2: towards a comprehensive pipeline for deep brain stimulation imaging. *NeuroImage.* (2019) 184:293–316. doi: 10.1016/j.neuroimage.2018.08.068

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

PERSPECTIVE
published: 24 June 2021
doi: 10.3389/fnbeh.2021.699691

The Habenula in the Link Between ADHD and Mood Disorder

*Young-A Lee[1] and Yukiori Goto[2]**

[1] Department of Food Science and Nutrition, Daegu Catholic University, Gyeongsan, South Korea, [2] Primate Research Institute, Kyoto University, Inuyama, Japan

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset, neurodevelopmental disorder, whereas major depressive disorder (MDD) is a mood disorder that typically emerges in adulthood. Accumulating evidence suggests that these seemingly unrelated psychiatric disorders, whose symptoms even appear antithetical [e.g., psychomotor retardation in depression vs. hyperactivity (psychomotor acceleration) in ADHD], are in fact associated with each other. Thus, individuals with ADHD exhibit high comorbidity with MDD later in life. Moreover, genetic studies have shown substantial overlaps of susceptibility genes between ADHD and MDD. Here, we propose a novel and testable hypothesis that the habenula, the epithalamic brain region important for the regulation of monoamine transmission, may be involved in both ADHD and MDD. The hypothesis suggests that an initially hypoactive habenula during childhood in individuals with ADHD may undergo compensatory changes during development, priming the habenula to be hyperactive in response to stress exposure and thereby increasing vulnerability to MDD in adulthood. Moreover, we propose a new perspective on habenular deficits in psychiatric disorders that consider the habenula a neural substrate that could explain multiple psychiatric disorders.

Keywords: neurodevelopmental disorder, depression, animal model, dopamine, serotonin, p-factor

OPEN ACCESS

Edited by:
Manuel Mameli,
The University of Lausanne, Switzerland

Reviewed by:
Luca Murru,
National Research Council, Consiglio Nazionale delle Ricerche, Italy
Christian Wozny,
Medical School Hamburg, Germany

***Correspondence:**
Yukiori Goto
goto.yukiori.5c@kyoto-u.ac.jp

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *24 April 2021*
Accepted: *25 May 2021*
Published: *24 June 2021*

Citation:
Lee YA and Goto Y (2021) The Habenula in the Link Between ADHD and Mood Disorder. Front. Behav. Neurosci. 15:699691. doi: 10.3389/fnbeh.2021.699691

INTRODUCTION

The current diagnostic manuals of psychiatric disorders, such as the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (Americal Psychiatric Association [APA], 2013) and International Classification of Diseases, 11th Edition (ICD-11) (World Health Organization [WHO], 2018), classify psychiatric disorders into categories as distinct entities. However, patients who have one category of a psychiatric disorder are often diagnosed with other comorbid disorders in other categories. Such observations raise the possibility that psychiatric disorders may be dimensional rather than categorical. Thus, a few or perhaps a single factor, such as a general psychopathological factor, i.e., the p-factor, may explain all psychiatric conditions (Wright et al., 2013; Caspi et al., 2014; Kotov et al., 2017; Caspi and Moffitt, 2018).

Attention-deficit/hyperactivity disorder (ADHD) is a childhood-onset neurodevelopmental disorder (Biederman and Faraone, 2005). In contrast, major depressive disorder (MDD) is a mood disorder that typically emerges in adulthood (Kupfer et al., 2012). Accumulating evidence suggests

that both disorders may involve deficits in the habenula. The habenula is a set of epithalamic nuclei consisting of the medial and lateral parts that receive inputs and integrate information from limbic structures and the basal ganglia. The habenula in turn sends outputs to midbrain nuclei where dopamine (DA) and serotonin (5-HT) neurons are located (Hikosaka et al., 2008; Boulos et al., 2017; Fakhoury, 2017; Hu et al., 2020). Relationships between ADHD and MDD, which are distinct categories of disorders and seemingly unrelated to each other, would be worth considering in relation to the roles of the habenula in the regulation of mesocorticolimbic DA and 5-HT transmission in the context of a dimensional model.

In this article, we first briefly summarize the literature demonstrating correlations between ADHD and MDD, followed by a discussion of the effects of habenular deficits in these disorders based primarily on animal models. Then, we further propose that investigations examining habenular dysfunction not in the context of a unitary, categorized disorder but as a common factor underlying multiple psychiatric conditions would be a thriving future direction.

ADHD AND MDD COMORBIDITY

ADHD comprises the core symptoms of hyperactivity, impulsivity, and attention deficit that is classified into three types, depending on which symptoms are prominent: inattentive, hyperactive/impulsive, and combined types (Biederman and Faraone, 2005). MDD is a mood disorder involving depressed mood and loss of pleasure and interest (Kupfer et al., 2012). MDD typically occurs in adulthood, although nowadays a significant number of children and adolescents are also diagnosed with MDD (Luby, 2009; Maughan et al., 2013).

Some MDD symptoms could be antithetical to those of ADHD. For instance, psychomotor retardation (Bennabi et al., 2013) in MDD could be considered the opposite of hyperactivity as psychomotor acceleration in ADHD. Rumination (Figueroa et al., 2019) is the focused and persistent thoughts of negative content causing emotional distress, whereas ADHD subjects exhibit excessive spontaneous mind wandering (Bozhilova et al., 2018). Moreover, abnormally augmented behavioral inhibition has been reported as a risk factor for MDD (Kasch et al., 2002; Gladstone and Parker, 2006). In contrast, impulsivity is a core symptom of ADHD.

There are other interesting coincidences between ADHD and MDD. Both disorders involve sleep disturbances, such as insomnia (Konofal et al., 2010; Hvolby, 2015; Pandi-Perumal et al., 2020), although hypersomnia is also often observed in MDD (Lopez et al., 2017). Circadian rhythms are also compromised in ADHD (Kooij and Bijlenga, 2013; Wynchank et al., 2016; Lunsford-Avery and Kollins, 2018). In MDD, symptom severity fluctuates within a day and even across seasons, with more severe symptoms in the winter (Germain and Kupfer, 2008; Boyce and Barriball, 2010). Numerous studies have demonstrated that the habenula plays critical roles in the regulation of both sleep and circadian rhythms (Valjakka et al., 1998; Salaberry and Mendoza, 2015; Bano-Otalora and Piggins, 2017; Mendoza, 2017; Aizawa and Zhu, 2019).

Subjects with ADHD are frequently diagnosed with other comorbid disorders, such as autism spectrum disorder, mood and anxiety disorder, drug addiction, and personality disorder (Katzman et al., 2017; Gnanavel et al., 2019). Epidemiological surveys in the United States have reported that, although the prevalence of MDD in typically developing children is only approximately 1%, it approaches approximately 15% among children with ADHD (Larson et al., 2011). The prevalence of MDD in adult ADHD subjects is twice as high (19%) as that in subjects without ADHD (8%) (Kessler et al., 2006). Longitudinal and meta-analysis studies have also demonstrated that childhood ADHD increases the risk of MDD during adolescence and young adulthood with an odds ratio of approximately 1.2–1.3 (Meinzer et al., 2014; Bron et al., 2016; Riglin et al., 2020).

Various mediators have been suggested regarding the comorbidity of ADHD and MDD. These include psychosocial factors, such as parent management (Ostrander and Herman, 2006), peer problems (Powell et al., 2020), academic attainment (Powell et al., 2020), emotion regulation (Seymour et al., 2012), anxiety (Roy et al., 2014), and disruptive behaviors (Roy et al., 2014). Neuroimaging studies have also reported neuronal mediators, such as decreased left hippocampal volume, and impairments in intrinsic functional connectivity between the hippocampus and orbitofrontal cortex (Posner et al., 2014) and between the anterior cingulate cortex and dorsolateral prefrontal cortex (PFC) (Whitfield-Gabrieli et al., 2020).

GENETIC CORRELATIONS BETWEEN ADHD AND MDD

Recent genetic studies have substantiated associations between ADHD and MDD. In a genome-wide association study (GWAS) with ADHD subjects, Ebejer et al. (2013) identified the strongest association with the gene for GPR139. GPR139 is an orphan G-protein coupled receptor whose role has been suggested to be a sensor of L-tryptophan and L-phenylalanine (Liu et al., 2015; Vedel et al., 2020). GPR139 has also been suggested to be relevant in MDD (Vedel et al., 2020). GPR139 signaling in the habenula was recently found to play an important role in fear learning in zebrafish (Roy et al., 2021).

Direct evidence of the associations between ADHD and MDD comes from GWAS meta-analyses examining genetic correlations with several different psychiatric disorders. A GWAS meta-analysis with ADHD subjects by Demontis et al. identified 12 genome-wide significant loci that were modestly, but significantly, correlated with depressive symptoms and MDD (r_g = 0.42) (Demontis et al., 2019). A similar meta-analysis of GWASs with an even larger sample size of MDD patients by Wray et al. identified 44 loci that were correlated with ADHD at a similar strength (r_g = 0.42) (Wray et al., 2018). Thus, similar strengths in the associations between ADHD and MDD have been observed in analyses using different cohorts, suggesting that the association between these disorders is highly consistent. Two GWAS meta-analyses by the Cross-Disorder Group of the

Psychiatric Genomics Consortium that examined associations in five and eight psychiatric disorders have demonstrated genetic correlations of similar strengths between ADHD and MDD to those reported in other meta-analysis studies, along with identification of associations of single nucleotide polymorphisms (SNPs) on chromosomes 3p21 and 10q24 and CACNB2, the gene encoding a voltage-gated L-type calcium channel, suggesting that L-type calcium channels could be a candidate molecule linking MDD and ADHD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013, 2019). A GWAS meta-analysis by Powell et al. also compared ADHD and MDD, identifying 14 SNPs with concordant directions of effect for both disorders, with the estimated genetic correlation being $r_g = 0.52$ (Powell et al., 2021).

Collectively, these GWASs have demonstrated modest, but highly consistent, SNP-based genetic correlations between ADHD and MDD, consolidating the associations between these disorders.

HABENULA IN ADHD AND MDD

Extensive research has been conducted to reveal the molecular and cellular mechanisms in the habenula that influence cognitive and affective functions as well as dysfunction implicated in psychiatric disorders. There are many comprehensive reviews that have summarized these studies (Hikosaka et al., 2008; Boulos et al., 2017; Fakhoury, 2017; Hu et al., 2020), such that here we refer only briefly to some findings about habenular deficits relevant to the pathophysiology of MDD and ADHD.

Using animal models of MDD, hyperactivity in the lateral nucleus of the habenula has consistently been demonstrated (Browne et al., 2018; Yang et al., 2018; Aizawa and Zhu, 2019; Gold and Kadriu, 2019). Specific patterns of acute and chronic electrical stimulation (Li et al., 2011; Meng et al., 2011; Tchenio et al., 2017) or pharmacological inhibition (Winter et al., 2011) of the habenula attenuate MDD-like behaviors in animal models. This is further supported by a recent case report study showing improvement of symptoms with deep brain stimulation of the habenula in treatment-resistant MDD patients (Wang et al., 2020). In contrast, human neuroimaging and postmortem studies have been inconsistent. Some studies have reported larger, and others found smaller, volumes of the habenula in MDD patients than in healthy subjects (Ranft et al., 2010; Savitz et al., 2011; Carceller-Sindreu et al., 2015; Schmidt et al., 2017). Functional imaging studies have demonstrated higher or lower than normal habenular activation in MDD patients (Roiser et al., 2009; Furman and Gotlib, 2016; Lawson et al., 2017). Such inconsistent findings are not surprising, given the heterogeneous nature of symptoms across MDD patients (Kupfer et al., 2012). Thus, there are huge gaps between animal model studies and the realm of human psychiatric conditions, and findings with animal models are unlikely to be directly translatable into human situations (Lee and Goto, 2013; Planchez et al., 2019; Baker et al., 2020; Stanford, 2020).

Compared with MDD and other psychiatric disorders, such as schizophrenia and drug addiction (Lecourtier and Kelly, 2005; Heldt and Ressler, 2006; Lecourtier et al., 2006; Velasquez et al., 2014; Boulos et al., 2017; Fakhoury, 2017; Mathuru, 2018; Li et al., 2019; Mathis and Kenny, 2019; Hu et al., 2020), research examining habenular deficits in ADHD pathophysiology are scarce. When we investigated the effects of neonatal habenular lesions (NHLs) in rats, there were unexpected findings with NHLs causing an assortment of behavioral alterations resembling ADHD symptoms. Thus, rats with NHLs exhibit spontaneous hyperlocomotion, more impulsive choices in decision-making tasks, and shorter attention spans in object exploration, all of which were ameliorated by amphetamine (Lee and Goto, 2011). Moreover, these behavioral alterations dynamically changed through development, with hyperlocomotion and impulsivity apparent only in childhood, whereas attention deficits persisted up until adulthood. Such developmental patterns are consistent with the waxing and waning of ADHD symptoms over development (Biederman and Faraone, 2005; Spencer et al., 2007). This novel aspect of the NHL model distinguished it from other conventional animal models of ADHD, such as spontaneously hypertensive rats (Russell, 2011).

NHLs also cause an assortment of neural alterations, such as a smaller PFC volume (Lee and Goto, 2011) and abnormally augmented amygdala–PFC connectivity (Kim et al., 2021), which are also consistent with those found in ADHD individuals (Plessen et al., 2006; Shaw et al., 2007; Batty et al., 2010; Posner et al., 2011; Batty et al., 2015; Van Dessel et al., 2018). We further found that tissue concentrations of DA and 5-HT were balanced in mesocorticolimbic regions of normal rats, but these levels were disrupted in NHL rats, suggesting that imbalances between DA and 5-HT may be more important than alterations in DA or 5-HT levels alone in NHL-induced behavioral alterations (Lee et al., 2021). There has been only one human neuroimaging study that investigated habenular deficits in ADHD subjects to date (Arfuso et al., 2019). This study demonstrated that intrinsic functional connectivity between the habenula and putamen was impaired in ADHD subjects. Additional human studies are needed to identify habenular deficits in ADHD.

Although a neonatal "lesion" gives an impression of damage in the habenula, NHLs result in smaller nuclear sizes of both medial and lateral habenula than those of normal rats, which could be due to the manipulation during early brain development. This raises a couple of issues to be further examined. First, since both medial and lateral nuclei of the habenula are affected by NHL, it has remained elusive whether and in what way neonatal manipulations of either the medial or lateral nucleus alone would yield distinct alterations. For instance, ADHD symptoms are grouped into hyperactive-impulsive dominant, inattention dominant, and mixed types (Biederman and Faraone, 2005). NHLs affecting both the medial and lateral nuclei induce behavioral alterations consistent with the mixed types. Thus, a selective neonatal lesion to either the medial or lateral nucleus may induce hyperactive-impulsive or inattention dominant types of alterations. Another issue is whether animals with smaller habenular nuclei as naturally occurring individual variations may also exhibit more hyperactive, impulsive, and inattentive traits than those of

larger habenular nuclei. On the other hand, smaller habenular nuclei caused by NHL may produce a condition equivalent to the hypoactive state of the habenula, such that volume size itself may not be the important factor. This is supported by inconsistent findings regarding anatomical and functional habenular changes in MDD patients (Roiser et al., 2009; Ranft et al., 2010; Savitz et al., 2011; Carceller-Sindreu et al., 2015; Furman and Gotlib, 2016; Lawson et al., 2017; Schmidt et al., 2017).

Taken together, a hypothesis has emerged that may explain the pathophysiology of comorbid ADHD and MDD (**Figure 1**). In particular, hypoactivity of the habenula early in development may initially produce ADHD-like behaviors with molecular alterations, such as differences in GPR139 and L-type calcium channels as suggested in GWASs. As the brain develops and matures into adulthood, such hypoactive habenula undergoes compensatory changes that subsequently increases vulnerability to MDD by priming the habenula for hyperactivation on exposure to stress.

HABENULA AS A NEURONAL P-FACTOR?

In addition to ADHD and MDD, habenular deficits have been implicated in other psychiatric disorders. As is the case with MDD, although a relatively large number of animal model studies have provided support for habenular deficits in schizophrenia (Lecourtier and Kelly, 2005; Heldt and Ressler, 2006; Lecourtier et al., 2006; Boulos et al., 2017; Fakhoury, 2017; Li et al., 2019; Hu et al., 2020) and drug addiction (Velasquez et al., 2014; Boulos et al., 2017; Fakhoury, 2017; Mathuru, 2018; Mathis and Kenny, 2019; Hu et al., 2020), there has been little or inconsistent evidence regarding habenular deficits in human patients with these disorders (Shepard et al., 2006; Ranft et al., 2010; Boulos et al., 2017; Fakhoury, 2017; Zhang et al., 2017; Schafer et al., 2018; Germann et al., 2020; Hu et al., 2020).

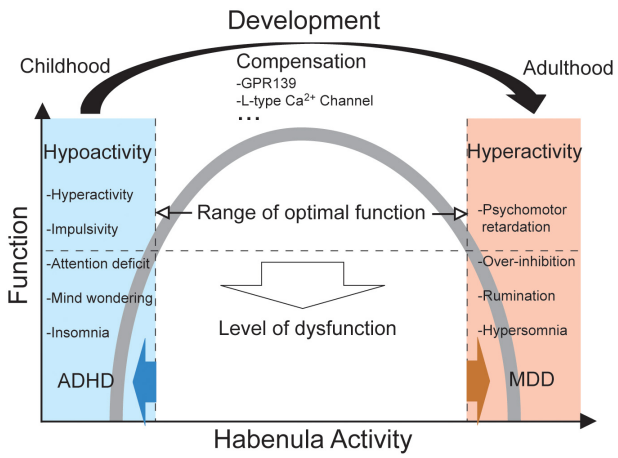

FIGURE 1 | A diagram illustrating the hypothesis. In terms of habenular function in relation to its activity (i.e., an inverted U-shaped relationship), ADHD and MDD may be antithetical. A dysfunctional level of hypoactivity in the habenula may induce an assortment of symptoms relevant to ADHD, whereas hyperactivity of the habenula may cause those relevant to MDD. A transition from hypoactivity to hyperactivity, but not vice versa, may take place over the course of brain development from childhood to adulthood, as compensation. Such a process may involve several molecules, such as GPR139 and L-type calcium channels.

Implications of habenular deficits in such an assortment of psychiatric disorders are not surprising, given the function of the habenula (Hikosaka et al., 2008). Thus, the lateral habenular nucleus regulates DA and 5-HT neuron activities in the midbrain nuclei directly and indirectly, respectively, through the rostrotegmental nucleus. Moreover, the medial habenular nucleus regulates the DA and 5-HT systems indirectly through the interpeduncular nucleus. DA and 5-HT are in turn neurochemical substances whose alterations are implicated in most, if not all, psychiatric disorders (Esposito et al., 2008). However, such a notion raises concern about how habenular deficits should be considered in the categorical model of psychiatric disorders.

A current diagnosis of psychiatric disorders is based on the categorical model (Americal Psychiatric Association [APA], 2013; World Health Organization [WHO], 2018). In this model, each disorder has unique symptoms and causes that are independent from other disorders. Accordingly, most studies investigating the neural mechanisms of psychiatric disorders, including those related to habenular deficits, follow this model and attempt to elucidate a pattern of deficits unique to a single psychiatric disorder. Such a categorical model does not comply with the idea that deficits of a single brain area, such as the habenula, could be involved in multiple disorders.

As an alternative to the categorical model, the dimensional model has been considered, especially in childhood psychiatry (Achenbach and Edelbrock, 1981; Sourander and Helstela, 2005; Wright et al., 2013; Willner et al., 2016; Kotov et al., 2017). In this model, two factors, internalizing and externalizing dimensions, are considered to underlie different psychiatric disorders. The internalizing dimension explains anxious and depressive symptoms, whereas the externalizing dimension explains aggressive, antisocial, and hyperactive-impulsive symptoms. It is interesting to note that the symptoms in the internalizing and externalizing dimensions are often discussed in relation to the functions of 5-HT and DA transmission, respectively. Thus, although this is highly speculative, DA/5-HT imbalance may explain internalizing and externalizing dimensions (e.g., imbalance toward DA- and 5-HT-predominant conditions lead to externalizing and internalizing symptoms, respectively).

Although it has been suggested that patients are more likely to have comorbidities of psychiatric disorders within the same dimension, correlations have also been observed between externalizing and internalizing symptoms (Wright et al., 2013; Willner et al., 2016), which corresponds to the relationship between ADHD and MDD, as ADHD is related to the externalizing dimension, whereas MDD is related to the internalizing dimension. Thus, a one-step higher and more generalized factor that is inclusive of both internalizing and externalizing dimensions may be required to explain

the ADHD and MDD relationships. Such a latent factor has recently been proposed that is mutually involved in the diagnoses of all the different psychiatric disorders, which is denoted as a general psychopathological factor or p-factor (Caspi et al., 2014; Caspi and Moffitt, 2018). The presence of the p-factor is supported by the number of studies, including investigations that have demonstrated substantial genetic overlaps between different psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013, 2019; Golovina et al., 2020). The habenula, which links externalizing and internalizing dimensions, along with its involvement in other psychiatric disorders, could therefore be a promising candidate for a neuronal substrate of the p-factor.

Future investigations that clarify the impacts of habenula deficits in psychiatric disorders may be fertile in the context of general psychopathological factors, such as how habenular deficits can explain the comorbidity of multiple disorders, rather than being associated with deficits in a particular psychiatric disorder.

CONCLUSION

We have proposed a hypothesis that habenular hypoactivity early in development may produce ADHD-like behaviors. The habenula may subsequently go through compensatory changes across development that leads to hyperactivity with an increased vulnerability to stress and MDD. Thus, the habenula may be a crucial brain region linking ADHD and MDD. Moreover, the roles of the habenula could be generalized across multiple psychiatric disorders beyond ADHD and MDD as a neural substrate of the p-factor.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YAL and YG conceived the idea and wrote the article. Both authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the research grants from National Research Foundation of Korea (NRF)-2020R1F1A1048370 funded by the Ministry of Science and ICT, NRF-2017R1D1A1B03028486 funded by Ministry of Education, JSPS Grant-in-Aid for Challenging Exploratory Research (19K22511), the Ichiro Kanehara Foundation for the Promotion of Medical Sciences and Medical Care, and the Institute of Seizon and Life Sciences.

ACKNOWLEDGMENTS

We thank Yui Asaoka for valuable comments on the article.

REFERENCES

Achenbach, T. M., and Edelbrock, C. S. (1981). Behavioral problems and competencies reported by parents of normal and disturbed children aged four through sixteen. *Monogr. Soc. Res. Child Dev.* 46, 1–82. doi: 10.2307/1165983

Aizawa, H., and Zhu, M. (2019). Toward an understanding of the habenula's various roles in human depression. *Psychiatry Clin. Neurosci.* 73, 607–612. doi: 10.1111/pcn.12892

Americal Psychiatric Association [APA]. (2013). *Diagnostic And Statistical Manual Of Mental Disorders*, 5th Edn. Arlington, VA: Americal Psychiatric Association.

Arfuso, M., Salas, R., Castellanos, F. X., and Krain Roy, A. (2019). Evidence of Altered Habenular Intrinsic Functional Connectivity in Pediatric ADHD. *J. Atten. Disord.* 25, 749–757.

Baker, M., Hong, S. I., Kang, S., and Choi, D. S. (2020). Rodent models for psychiatric disorders: problems and promises. *Lab. Anim. Res.* 36:9.

Bano-Otalora, B., and Piggins, H. D. (2017). Contributions of the lateral habenula to circadian timekeeping. *Pharmacol. Biochem. Behav.* 162, 46–54. doi: 10.1016/j.pbb.2017.06.007

Batty, M. J., Liddle, E. B., Pitiot, A., Toro, R., Groom, M. J., Scerif, G., et al. (2010). Cortical gray matter in attention-deficit/hyperactivity disorder: a structural magnetic resonance imaging study. *J. Am. Acad. Child Adolesc. Psychiatry* 49, 229–238. doi: 10.1097/00004583-201003000-00006

Batty, M. J., Palaniyappan, L., Scerif, G., Groom, M. J., Liddle, E. B., Liddle, P. F., et al. (2015). Morphological abnormalities in prefrontal surface area and thalamic volume in attention deficit/hyperactivity disorder. *Psychiatry Res.* 233, 225–232. doi: 10.1016/j.pscychresns.2015.07.004

Bennabi, D., Vandel, P., Papaxanthis, C., Pozzo, T., and Haffen, E. (2013). Psychomotor retardation in depression: a systematic review of diagnostic, pathophysiologic, and therapeutic implications. *Biomed. Res. Int.* 2013:158746.

Biederman, J., and Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet* 366, 237–248.

Boulos, L. J., Darcq, E., and Kieffer, B. L. (2017). Translating the Habenula-From Rodents to Humans. *Biol. Psychiatry* 81, 296–305. doi: 10.1016/j.biopsych.2016.06.003

Boyce, P., and Barriball, E. (2010). Circadian rhythms and depression. *Aust. Fam. Physician* 39, 307–310.

Bozhilova, N. S., Michelini, G., Kuntsi, J., and Asherson, P. (2018). Mind wandering perspective on attention-deficit/hyperactivity disorder. *Neurosci. Biobehav. Rev.* 92, 464–476. doi: 10.1016/j.neubiorev.2018.07.010

Bron, T. I., Bijlenga, D., Verduijn, J., Penninx, B. W., Beekman, A. T., and Kooij, J. J. (2016). Prevalence of ADHD symptoms across clinical stages of major depressive disorder. *J. Affect. Disord.* 197, 29–35. doi: 10.1016/j.jad.2016.02.053

Browne, C. A., Hammack, R., and Lucki, I. (2018). Dysregulation of the Lateral Habenula in Major Depressive Disorder. *Front. Synaptic Neurosci.* 10:46. doi: 10.3389/fnsyn.2018.00046

Carceller-Sindreu, M., De Diego-Adelino, J., Serra-Blasco, M., Vives-Gilabert, Y., Martin-Blanco, A., Puigdemont, D., et al. (2015). Volumetric MRI study of the habenula in first episode, recurrent and chronic major depression. *Eur. Neuropsychopharmacol.* 25, 2015–2021. doi: 10.1016/j.euroneuro.2015.08.009

Caspi, A., Houts, R. M., Belsky, D. W., Goldman-Mellor, S. J., Harrington, H., Israel, S., et al. (2014). The p Factor: one General Psychopathology Factor in

the Structure of Psychiatric Disorders? *Clin. Psychol. Sci.* 2, 119–137. doi: 10.1177/2167702613497473

Caspi, A., and Moffitt, T. E. (2018). All for One and One for All: mental Disorders in One Dimension. *Am. J. Psychiatry* 175, 831–844. doi: 10.1176/appi.ajp.2018.17121383

Cross-Disorder Group of the Psychiatric Genomics Consortium (2013). Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 381, 1371–1379. doi: 10.1016/s0140-6736(12)62129-1

Cross-Disorder Group of the Psychiatric Genomics Consortium (2019). Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders. *Cell* 179, 1469–1482e1411.

Demontis, D., Walters, R. K., Martin, J., Mattheisen, M., Als, T. D., Agerbo, E., et al. (2019). Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. *Nat. Genet.* 51, 63–75.

Ebejer, J. L., Duffy, D. L., Van Der Werf, J., Wright, M. J., Montgomery, G., Gillespie, N. A., et al. (2013). Genome-wide association study of inattention and hyperactivity-impulsivity measured as quantitative traits. *Twin Res. Hum. Genet.* 16, 560–574. doi: 10.1017/thg.2013.12

Esposito, E., Di Matteo, V., and Di Giovanni, G. (2008). Serotonin-dopamine interaction: an overview. *Prog. Brain Res.* 172, 3–6. doi: 10.1016/s0079-6123(08)00901-1

Fakhoury, M. (2017). The habenula in psychiatric disorders: more than three decades of translational investigation. *Neurosci. Biobehav. Rev.* 83, 721–735. doi: 10.1016/j.neubiorev.2017.02.010

Figueroa, C. A., Dejong, H., Mocking, R. J. T., Fox, E., Rive, M. M., Schene, A. H., et al. (2019). Attentional control, rumination and recurrence of depression. *J. Affect. Disord.* 256, 364–372. doi: 10.1016/j.jad.2019.05.072

Furman, D. J., and Gotlib, I. H. (2016). Habenula responses to potential and actual loss in major depression: preliminary evidence for lateralized dysfunction. *Soc. Cogn. Affect. Neurosci.* 11, 843–851. doi: 10.1093/scan/nsw019

Germain, A., and Kupfer, D. J. (2008). Circadian rhythm disturbances in depression. *Hum. Psychopharmacol.* 23, 571–585. doi: 10.1002/hup.964

Germann, J., Gouveia, F. V., Martinez, R. C. R., Zanetti, M. V., De Souza Duran, F. L., Chaim-Avancini, T. M., et al. (2020). Fully Automated Habenula Segmentation Provides Robust and Reliable Volume Estimation Across Large Magnetic Resonance Imaging Datasets, Suggesting Intriguing Developmental Trajectories in Psychiatric Disease. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 5, 923–929. doi: 10.1016/j.bpsc.2020.01.004

Gladstone, G. L., and Parker, G. B. (2006). Is behavioral inhibition a risk factor for depression? *J. Affect. Disord.* 95, 85–94. doi: 10.1016/j.jad.2006.04.015

Gnanavel, S., Sharma, P., Kaushal, P., and Hussain, S. (2019). Attention deficit hyperactivity disorder and comorbidity: a review of literature. *World J. Clin. Cases* 7, 2420–2426.

Gold, P. W., and Kadriu, B. (2019). A Major Role for the Lateral Habenula in Depressive Illness: physiologic and Molecular Mechanisms. *Front. Psychiatry* 10:320. doi: 10.3389/fpsyt.2019.00320

Golovina, E., Vickers, M. H., Erb, C. D., and O'sullivan, J. M. (2020). GWAS SNPs Impact Shared Regulatory Pathways Amongst Multimorbid Psychiatric Disorders and Cognitive Functioning. *Front. Psychiatry* 11:560751. doi: 10.3389/fpsyt.2020.560751

Heldt, S. A., and Ressler, K. J. (2006). Lesions of the habenula produce stress- and dopamine-dependent alterations in prepulse inhibition and locomotion. *Brain Res.* 107, 229–239. doi: 10.1016/j.brainres.2005.12.053

Hikosaka, O., Sesack, S. R., Lecourtier, L., and Shepard, P. D. (2008). Habenula: crossroad between the basal ganglia and the limbic system. *J. Neurosci.* 28, 11825–11829. doi: 10.1523/jneurosci.3463-08.2008

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Hvolby, A. (2015). Associations of sleep disturbance with ADHD: implications for treatment. *Atten. Defic. Hyperact. Disord.* 7, 1–18. doi: 10.1007/s12402-014-0151-0

Kasch, K. L., Rottenberg, J., Arnow, B. A., and Gotlib, I. H. (2002). Behavioral activation and inhibition systems and the severity and course of depression. *J. Abnorm. Psychol.* 111, 589–597. doi: 10.1037/0021-843x.111.4.589

Katzman, M. A., Bilkey, T. S., Chokka, P. R., Fallu, A., and Klassen, L. J. (2017). Adult ADHD and comorbid disorders: clinical implications of a dimensional approach. *BMC Psychiatry* 17:302. doi: 10.1186/s12888-017-1463-3

Kessler, R. C., Adler, L., Barkley, R., Biederman, J., Conners, C. K., Demler, O., et al. (2006). The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. *Am. J. Psychiatry* 163, 716–723. doi: 10.1176/ajp.2006.163.4.716

Kim, Y. J., Jeon, S. Y., Choi, J. S., Kim, N. H., Goto, Y., and Lee, Y. A. (2021). Alterations of amygdala-prefrontal cortical coupling and attention deficit/hyperactivity disorder-like behaviors induced by neonatal habenula lesion: normalization by Ecklonia stolonifera extract and its active compound fucosterol. *Behav. Pharmacol.* 32, 308–320. doi: 10.1097/fbp.0000000000000620

Konofal, E., Lecendreux, M., and Cortese, S. (2010). Sleep and ADHD. *Sleep Med.* 11, 652–658.

Kooij, J. J., and Bijlenga, D. (2013). The circadian rhythm in adult attention-deficit/hyperactivity disorder: current state of affairs. *Expert Rev. Neurother.* 13, 1107–1116. doi: 10.1586/14737175.2013.836301

Kotov, R., Krueger, R. F., Watson, D., Achenbach, T. M., Althoff, R. R., and Bagby, R. M. (2017). The Hierarchical Taxonomy of Psychopathology (HiTOP): a dimensional alternative to traditional nosologies. *J. Abnorm. Psychol.* 126, 454–477.

Kupfer, D. J., Frank, E., and Phillips, M. L. (2012). Major depressive disorder: new clinical, neurobiological, and treatment perspectives. *Lancet* 379, 1045–1055. doi: 10.1016/s0140-6736(11)60602-8

Larson, K., Russ, S. A., Kahn, R. S., and Halfon, N. (2011). Patterns of comorbidity, functioning, and service use for US children with ADHD, 2007. *Pediatrics* 127, 462–470. doi: 10.1542/peds.2010-0165

Lawson, R. P., Nord, C. L., Seymour, B., Thomas, D. L., Dayan, P., Pilling, S., et al. (2017). Disrupted habenula function in major depression. *Mol. Psychiatry* 22, 202–208. doi: 10.1038/mp.2016.81

Lecourtier, L., Deschaux, O., Arnaud, C., Chessel, A., Kelly, P. H., and Garcia, R. (2006). Habenula lesions alter synaptic plasticity within the fimbria-accumbens pathway in the rat. *Neuroscience* 141, 1025–1032. doi: 10.1016/j.neuroscience.2006.04.018

Lecourtier, L., and Kelly, P. H. (2005). Bilateral lesions of the habenula induce attentional disturbances in rats. *Neuropsychopharmacology* 30, 484–496. doi: 10.1038/sj.npp.1300595

Lee, Y. A., and Goto, Y. (2011). Neurodevelopmental disruption of cortico-striatal function caused by degeneration of habenula neurons. *PLoS One* 6:e19450. doi: 10.1371/journal.pone.0019450

Lee, Y. A., and Goto, Y. (2013). Reconsideration of animal models of schizophrenia and other psychiatric disorders with evolutionary perspective. *Med. Hypotheses* 81, 1120–1126. doi: 10.1016/j.mehy.2013.10.017

Lee, Y. A., Kim, Y. J., Lee, J. S., Lee, S. H., and Goto, Y. (2021). Imbalance between dopamine and serotonin caused by neonatal habenula lesion. *Behav. Brain Res.* 409:113316. . doi: 10.1016/j.bbr.2021.113316

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C. D., Schulz, D., et al. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* 470, 535–539. doi: 10.1038/nature09742

Li, J., Yang, S., Liu, X., Han, Y., Li, Y., Feng, J., et al. (2019). Hypoactivity of the lateral habenula contributes to negative symptoms and cognitive dysfunction of schizophrenia in rats. *Exp. Neurol.* 318, 165–173. doi: 10.1016/j.expneurol.2019.05.005

Liu, C., Bonaventure, P., Lee, G., Nepomuceno, D., Kuei, C., Wu, J., et al. (2015). GPR139, an Orphan Receptor Highly Enriched in the Habenula and Septum, Is Activated by the Essential Amino Acids L-Tryptophan and L-Phenylalanine. *Mol. Pharmacol.* 88, 911–925. doi: 10.1124/mol.115.100412

Lopez, R., Barateau, L., Evangelista, E., and Dauvilliers, Y. (2017). Depression and Hypersomnia: a Complex Association. *Sleep Med. Clin.* 12, 395–405. doi: 10.1016/j.jsmc.2017.03.016

Luby, J. L. (2009). Early childhood depression. *Am. J. Psychiatry* 166, 974–979.

Lunsford-Avery, J. R., and Kollins, S. H. (2018). Editorial Perspective: delayed circadian rhythm phase: a cause of late-onset attention-deficit/hyperactivity disorder among adolescents? *J. Child Psychol. Psychiatry* 59, 1248–1251. doi: 10.1111/jcpp.12956

Mathis, V., and Kenny, P. J. (2019). From controlled to compulsive drug-taking: the role of the habenula in addiction. *Neurosci. Biobehav. Rev.* 106, 102–111. doi: 10.1016/j.neubiorev.2018.06.018

Mathuru, A. S. (2018). A little rein on addiction. *Semin. Cell Dev. Biol.* 78, 120–129. doi: 10.1016/j.semcdb.2017.09.030

Maughan, B., Collishaw, S., and Stringaris, A. (2013). Depression in childhood and adolescence. *J. Can. Acad. Child Adolesc. Psychiatry* 22, 35–40.

Meinzer, M. C., Pettit, J. W., and Viswesvaran, C. (2014). The co-occurrence of attention-deficit/hyperactivity disorder and unipolar depression in children and adolescents: a meta-analytic review. *Clin. Psychol. Rev.* 34, 595–607. doi: 10.1016/j.cpr.2014.10.002

Mendoza, J. (2017). Circadian neurons in the lateral habenula: clocking motivated behaviors. *Pharmacol. Biochem. Behav.* 162, 55–61. doi: 10.1016/j.pbb.2017.06.013

Meng, H., Wang, Y., Huang, M., Lin, W., Wang, S., and Zhang, B. (2011). Chronic deep brain stimulation of the lateral habenula nucleus in a rat model of depression. *Brain Res.* 1422, 32–38. doi: 10.1016/j.brainres.2011.08.041

Ostrander, R., and Herman, K. C. (2006). Potential cognitive, parenting, and developmental mediators of the relationship between ADHD and depression. *J. Consult. Clin. Psychol.* 74, 89–98. doi: 10.1037/0022-006x.74.1.89

Pandi-Perumal, S. R., Monti, J. M., Burman, D., Karthikeyan, R., Bahammam, A. S., Spence, D. W., et al. (2020). Clarifying the role of sleep in depression: a narrative review. *Psychiatry Res.* 291:113239. doi: 10.1016/j.psychres.2020.113239

Planchez, B., Surget, A., and Belzung, C. (2019). Animal models of major depression: drawbacks and challenges. *J. Neural Transm.* 126, 1383–1408. doi: 10.1007/s00702-019-02084-y

Plessen, K. J., Bansal, R., Zhu, H., Whiteman, R., Amat, J., Quackenbush, G. A., et al. (2006). Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. *Arch. Gen. Psychiatry* 63, 795–807.

Posner, J., Nagel, B. J., Maia, T. V., Mechling, A., Oh, M., Wang, Z., et al. (2011). Abnormal amygdalar activation and connectivity in adolescents with attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 50, 828–837.e3.

Posner, J., Siciliano, F., Wang, Z., Liu, J., Sonuga-Barke, E., and Greenhill, L. (2014). A multimodal MRI study of the hippocampus in medication-naive children with ADHD: what connects ADHD and depression? *Psychiatry Res.* 224, 112–118. doi: 10.1016/j.pscychresns.2014.08.006

Powell, V., Martin, J., Thapar, A., Rice, F., and Anney, R. J. L. (2021). Investigating regions of shared genetic variation in attention deficit/hyperactivity disorder and major depressive disorder: a GWAS meta-analysis. *Sci. Rep.* 11:7353.

Powell, V., Riglin, L., Hammerton, G., Eyre, O., Martin, J., Anney, R., et al. (2020). What explains the link between childhood ADHD and adolescent depression? Investigating the role of peer relationships and academic attainment. *Eur. Child Adolesc. Psychiatry* 29, 1581–1591. doi: 10.1007/s00787-019-01463-w

Ranft, K., Dobrowolny, H., Krell, D., Bielau, H., Bogerts, B., and Bernstein, H. G. (2010). Evidence for structural abnormalities of the human habenular complex in affective disorders but not in schizophrenia. *Psychol. Med.* 40, 557–567. doi: 10.1017/s0033291709990821

Riglin, L., Leppert, B., Dardani, C., Thapar, A. K., Rice, F., O'donovan, M. C., et al. (2020). ADHD and depression: investigating a causal explanation. *Psychol. Med.* 1–8.[Epub Online ahead of print]. doi: 10.1017/S0033291720000665

Roiser, J. P., Levy, J., Fromm, S. J., Nugent, A. C., Talagala, S. L., Hasler, G., et al. (2009). The effects of tryptophan depletion on neural responses to emotional words in remitted depression. *Biol. Psychiatry* 66, 441–450. doi: 10.1016/j.biopsych.2009.05.002

Roy, A., Oldehinkel, A. J., Verhulst, F. C., Ormel, J., and Hartman, C. A. (2014). Anxiety and disruptive behavior mediate pathways from attention-deficit/hyperactivity disorder to depression. *J. Clin. Psychiatry* 75, e108–e113.

Roy, N., Ogawa, S., Maniam, R., and Parhar, I. (2021). Habenula GPR139 is associated with fear learning in the zebrafish. *Sci. Rep.* 11:5549.

Russell, V. A. (2011). Overview of animal models of attention deficit hyperactivity disorder (ADHD). *Curr. Protoc. Neurosci.* 9:Unit9.35.

Salaberry, N. L., and Mendoza, J. (2015). Insights into the Role of the Habenular Circadian Clock in Addiction. *Front. Psychiatry* 6:179. doi: 10.3389/fpsyt.2015.00179

Savitz, J. B., Nugent, A. C., Bogers, W., Roiser, J. P., Bain, E. E., Neumeister, A., et al. (2011). Habenula volume in bipolar disorder and major depressive disorder: a high-resolution magnetic resonance imaging study. *Biol. Psychiatry* 69, 336–343. doi: 10.1016/j.biopsych.2010.09.027

Schafer, M., Kim, J. W., Joseph, J., Xu, J., Frangou, S., and Doucet, G. E. (2018). Imaging Habenula Volume in Schizophrenia and Bipolar Disorder. *Front. Psychiatry* 9:456. doi: 10.3389/fpsyt.2018.00456

Schmidt, F. M., Schindler, S., Adamidis, M., Strauss, M., Trankner, A., Trampel, R., et al. (2017). Habenula volume increases with disease severity in unmedicated major depressive disorder as revealed by 7T MRI. *Eur. Arch. Psychiatry Clin. Neurosci.* 267, 107–115. doi: 10.1007/s00406-016-0675-8

Seymour, K. E., Chronis-Tuscano, A., Halldorsdottir, T., Stupica, B., Owens, K., and Sacks, T. (2012). Emotion regulation mediates the relationship between ADHD and depressive symptoms in youth. *J. Abnorm. Child Psychol.* 40, 595–606. doi: 10.1007/s10802-011-9593-4

Shaw, P., Eckstrand, K., Sharp, W., Blumenthal, J., Lerch, J. P., Greenstein, D., et al. (2007). Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19649–19654.

Shepard, P. D., Holcomb, H. H., and Gold, J. M. (2006). Schizophrenia in translation: the presence of absence: habenular regulation of dopamine neurons and the encoding of negative outcomes. *Schizophr. Bull.* 32, 417–421. doi: 10.1093/schbul/sbj083

Sourander, A., and Helstela, L. (2005). Childhood predictors of externalizing and internalizing problems in adolescence. A prospective follow-up study from age 8 to 16. *Eur. Child. Adolesc. Psychiatry* 14, 415–423. doi: 10.1007/s00787-005-0475-6

Spencer, T. J., Biederman, J., and Mick, E. (2007). Attention-deficit/hyperactivity disorder: diagnosis, lifespan, comorbidities, and neurobiology. *Ambul. Pediatr.* 7, 73–81. doi: 10.1016/j.ambp.2006.07.006

Stanford, S. C. (2020). Some Reasons Why Preclinical Studies of Psychiatric Disorders Fail to Translate: what Can Be Rescued from the Misunderstanding and Misuse of Animal 'Models'? *Altern. Lab. Anim.* 48, 106–115. doi: 10.1177/0261192920939876

Tchenio, A., Lecca, S., Valentinova, K., and Mameli, M. (2017). Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. *Nat. Commun.* 8:1135.

Valjakka, A., Vartiainen, J., Tuomisto, L., Tuomisto, J. T., Olkkonen, H., and Airaksinen, M. M. (1998). The fasciculus retroflexus controls the integrity of REM sleep by supporting the generation of hippocampal theta rhythm and rapid eye movements in rats. *Brain Res. Bull.* 47, 171–184. doi: 10.1016/s0361-9230(98)00006-9

Van Dessel, J., Sonuga-Barke, E., Mies, G., Lemiere, J., Van Der Oord, S., Morsink, S., et al. (2018). Delay aversion in attention deficit/hyperactivity disorder is mediated by amygdala and prefrontal cortex hyper-activation. *J. Child Psychol. Psychiatry* 59, 888–899. doi: 10.1111/jcpp.12868

Vedel, L., Nohr, A. C., Gloriam, D. E., and Brauner-Osborne, H. (2020). Pharmacology and function of the orphan GPR139 G protein-coupled receptor. *Basic Clin. Pharmacol. Toxicol.* 126, 35–46. doi: 10.1111/bcpt.13263

Velasquez, K. M., Molfese, D. L., and Salas, R. (2014). The role of the habenula in drug addiction. *Front. Hum. Neurosci.* 8:174. doi: 10.3389/fnhum.2014.00174

Wang, Z., Cai, X., Qiu, R., Yao, C., Tian, Y., Gong, C., et al. (2020). Case Report: lateral Habenula Deep Brain Stimulation for Treatment-Resistant Depression. *Front. Psychiatry* 11:616501. doi: 10.3389/fpsyt.2020.616501

Whitfield-Gabrieli, S., Wendelken, C., Nieto-Castanon, A., Bailey, S. K., Anteraper, S. A., Lee, Y. J., et al. (2020). Association of Intrinsic Brain Architecture With Changes in Attentional and Mood Symptoms During Development. *JAMA Psychiatry* 77, 378–386. doi: 10.1001/jamapsychiatry.2019.4208

Willner, C. J., Gatzke-Kopp, L. M., and Bray, B. C. (2016). The dynamics of internalizing and externalizing comorbidity across the early school years. *Dev. Psychopathol.* 28, 1033–1052. doi: 10.1017/s0954579416000687

Winter, C., Vollmayr, B., Djodari-Irani, A., Klein, J., and Sartorius, A. (2011). Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. *Behav. Brain Res.* 216, 463–465.

World Health Organization [WHO]. (2018). *International classification of diseases for mortality and morbidity statistics (11th Revision)*. Available Online at: https://icd.who.int/browse11/l-m/en (accessed April 20, 2021).

Wray, N. R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E. M., Abdellaoui, A., et al. (2018). Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nat. Genet.* 50, 668–681.

Wright, A. G., Krueger, R. F., Hobbs, M. J., Markon, K. E., Eaton, N. R., and Slade, T. (2013). The structure of psychopathology: toward an expanded quantitative empirical model. *J. Abnorm. Psychol.* 122, 281–294.

Wynchank, D. S., Bijlenga, D., Lamers, F., Bron, T. I., Winthorst, W. H., Vogel, S. W., et al. (2016). ADHD, circadian rhythms and seasonality. *J. Psychiatr. Res.* 81, 87–94.

Yang, Y., Wang, H., Hu, J., and Hu, H. (2018). Lateral habenula in the pathophysiology of depression. *Curr. Opin. Neurobiol.* 48, 90–96.

Zhang, L., Wang, H., Luan, S., Yang, S., Wang, Z., Wang, J., et al. (2017). Altered Volume and Functional Connectivity of the Habenula in Schizophrenia. *Front. Hum. Neurosci.* 11:636. doi: 10.3389/fnhum.2017.00636

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 10 August 2021
doi: 10.3389/fnbeh.2021.652793

Lateral Habenula Inactivation Alters Willingness to Exert Physical Effort Using a Maze Task in Rats

*Joshua P. Sevigny, Emily N. Bryant, Érica Encarnacion, Dylan F. Smith, Rudith Acosta and Phillip M. Baker**

Department of Psychology, Seattle Pacific University, Seattle, WA, United States

An impairment in willingness to exert physical effort in daily activities is a noted aspect of several psychiatric conditions. Previous studies have supported an important role for the lateral habenula (LHb) in dynamic decision-making, including decisions associated with discounting costly high value rewards. It is unknown whether a willingness to exert physical effort to obtain higher rewards is also mediated by the LHb. It also remains unclear whether the LHb is critical to monitoring the task contingencies generally as they change, or whether it also mediates choices in otherwise static reward environments. The present study indicates that the LHb might have an integrative role in effort-based decision-making even when no alterations in choice contingencies occur. Specifically, pharmacological inactivation of the LHb showed differences in motivational behavior by reducing choices for the high effort (30cm barrier) high reward (2 pellets) choice versus the low effort (0 cm) low reward (1 pellet) choice. In sessions where the barrier was removed, rats demonstrated a similar preference for the high reward arm under both control and LHb inactivation. Further, no differences were observed when accounting for sex as a biological variable. These results support that effort to receive a high-value reward is considered on a trial-by-trial basis and the LHb is part of the circuit responsible for integrating this information during decision-making. Therefore, it is likely that previously observed changes in the LHb may be a key contributor to changes in a willingness to exert effort in psychiatric conditions.

Keywords: discounting, depression, effort, decision-making, reward

OPEN ACCESS

Edited by:
Martine Ammassari-Teule, National Research Council (CNR), Italy

Reviewed by:
Aki Takahashi, University of Tsukuba, Japan
Ja Wook Koo, Korea Brain Research Institute, South Korea

***Correspondence:**
Phillip M. Baker
bakerp5@spu.edu

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *13 January 2021*
Accepted: *14 July 2021*
Published: *10 August 2021*

Citation:
Sevigny JP, Bryant EN, Encarnacion É, Smith DF, Acosta R and Baker PM (2021) Lateral Habenula Inactivation Alters Willingness to Exert Physical Effort Using a Maze Task in Rats. Front. Behav. Neurosci. 15:652793. doi: 10.3389/fnbeh.2021.652793

INTRODUCTION

Changes in motivation and willingness to exert physical effort are noted across psychiatric conditions including Major Depressive Disorder, addiction, and chronic fatigue (Cohen et al., 2001; Sharpe and Wilks, 2002; Demyttenaere et al., 2005; Bachleda and Darhiri, 2018). For example, patients with Major Depressive Disorder report trying harder even though they objectively exert less effort (Cléry-Melin et al., 2011). Understanding the neurobiological causes of changes in willingness to exert effort to obtain rewards can offer insight into addressing this debilitating aspect of psychiatric conditions. Prior research has revealed a role for the anterior cingulate cortex, the amygdala, the nucleus accumbens, and the dopamine and adrenergic systems in tasks that assess physical effort in rodents (Salamone et al., 1994; Floresco and Ghods-Sharifi, 2007; Bardgett et al., 2009; Yohn et al., 2015).

Common to many of these brain areas is a functional connection with the lateral habenula (LHb) (Lecourtier and Kelly, 2007; Quina et al., 2015). The lateral habenula is proposed to be a key integrator of ongoing context into a wide range of decision behaviors due to its unique position between frontal and midbrain regions involved in motivation and motor behavior (Sutherland, 1982; Lecourtier and Kelly, 2007; Baker et al., 2015). The LHb has also been connected to a growing list of psychiatric conditions (Lecca et al., 2014; Zhang et al., 2017; Schafer et al., 2018). Further, manipulation of the LHb has relieved some of these conditions in human pilot studies (Sartorius et al., 2010; Zhang et al., 2019; Wang et al., 2020), although further research is required.

To date, it remains unclear whether the LHb is involved in the willingness to exert effort during dynamic decision-making tasks. The LHb is known to play a central role in aversive and reward-oriented behavior, particularly when outcomes change dynamically (Baker and Mizumori, 2017; Flanigan et al., 2017; Lecca et al., 2020). The majority of previous work has demonstrated that manipulation of the LHb results in disrupted behavior when outcomes are changed dynamically but not when task outcomes remain stable (Stopper and Floresco, 2014; Baker and Mizumori, 2017). For example, in an operant chamber version of delay or probability discounting, rather than rats altering behavior-based changes in probability for a high reward or a delay to high reward, LHb inactivation resulted in chance performance (Stopper and Floresco, 2014). However, when the discrimination between the high and low reward was tested without changing contingencies, the same rats performed similarly to controls.

The present study sought to examine whether the LHb is involved in a trial by trial consideration of a willingness to exert effort, or more generally, in the ability to discriminate rewards specifically when contingencies change in an unpredictable manner. To test this, we used a unique behavioral paradigm that requires rats to climb a physical barrier in order to receive a large reinforcement or to opt for a smaller reward without the need to climb a barrier. Importantly, the location of the high reward, high effort arm remains constant throughout the task. This allowed us to examine the willingness to exert effort straightforwardly in an ethologically relevant manner. If the habenula is required to integrate a willingness to expend effort to obtain a high reward on a trial-by-trial basis, we should observe changes in behavior when the LHb is inactivated. Alternatively, if the habenula is only required to recognize an alteration in contingencies, then no changes in behavior should be observed. Either result would serve to clarify the larger role the LHb plays in behavioral selection under conditions of physical effort and reinforcement more generally.

MATERIALS AND METHODS

Animals

The rats acquired for this study were 12 female and 12 male *Sprague Dawley* rats from Envigo Labs. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Seattle Pacific University (protocol # 201819-05-R). After a minimum of five days from entry into the lab, rats were handled daily and food-restricted to approximately 85% of their free-feeding weight. Once rats' weights were stabilized, they began discrimination training on a plexiglass maze.

Apparatus and Training

The maze consisted of four arms 5.5 cm in width, 60 cm in length and with walls 15 cm high. The arms were arranged in a plus shape with blocks preventing access to a given arm resulting in a T-shaped maze (**Figure 1**). Initially, all arms of the maze were baited with 1 sucrose pellet (45 mg pellet, Bio Serve F0042), and rats were placed in a random arm and allowed to explore until consuming all pellets. Once the rats explored and consumed the pellets at least seven times in less than 15 min for two consecutive days, they were advanced to discrimination training. In the discrimination training, the rats were initially trained to discriminate between two choice arms (designated N and S) containing either 1 or 2 sucrose pellets. The location of the high reward was counterbalanced between rats. The remaining arms (E and W) were used as start arms and were pseudo-randomly alternated on each trial with the other blocked off with a plexiglass barrier. Rats were trained daily on 30 trials in this stage until they chose the high reward on at least 80% of trials for two consecutive days. Toward the end of the first week, the rats learned the initial task, reaching two consecutive days with 80% or greater high reward choices. The rats would then progress to the next stage wherein a 15 cm ramp was placed in the arm containing the high reward. The same acquisition criterion was used in this and all following stages as the rat progressed through a further 20 cm and a final 30 cm barrier. The rats had to reach the criteria of $>80\%$ for all barriers to be ready for surgery (**Figure 1A**).

Surgery

Following completion of training on the 30cm ramp, rats were returned to free feed and then given surgery to place a bilateral cannula in the LHb to inactivate it during subsequent tests using the GABAa agonist muscimol (Sigma). Briefly, rats were placed in an induction chamber and anesthetized with vaporized isoflurane (5%) prior to surgery. Rats were then placed in a stereotaxic apparatus and maintained on isoflurane with a nose cone (1–3%). Once the skull was exposed, four partial pilot holes were drilled and screws were inserted into each. These acted as anchors to keep the headcap and cannula firmly in place. Two holes were drilled bilaterally and the guide cannula was inserted (A–P: -3.5, M–L: $\pm$ 0.9, and D–V: 4.35 mm) dorsal to the LHb. Dental acrylic was used to secure the guide cannula to the anchor screws, completing the headcap. Analgesic (Meloxicam) was administered subcutaneously prior to rats waking up and 24 h after surgery.

Testing and Inactivation Procedure

Once recovered from surgery, subjects went through a short re-acquisition phase where they again had to demonstrate a preference of $>80\%$ for the high reward with the 30cm ramp in place (one day). Once completed, rats were moved to the treatment phase of the experiment. Treatments were

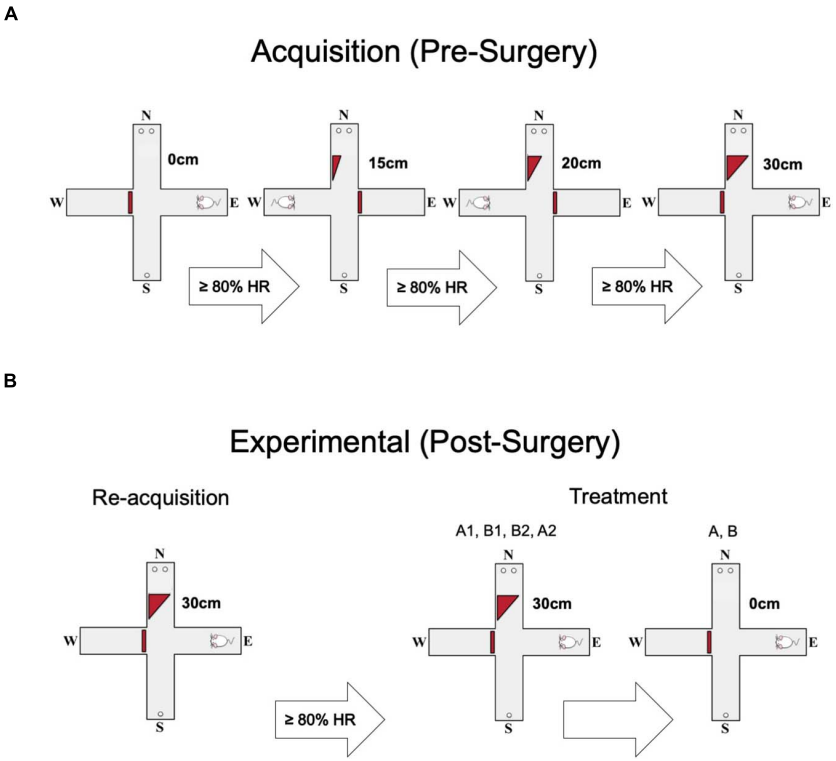

FIGURE 1 | Behavioral protocol. **(A)** Each animal went through four stages of acquisition, progressing to the next ramp height (0 cm, 15 cm, 20 cm, 30 cm) after making ≥80% high reward (HR) choices. The animals' starting arm varied pseudorandomly. **(B)** Each animal was required to pass a short re-acquisition phase post-surgery, but before experimental trials. Again they were required to make ≥80% HR choices. The experimental phase consisted of two parts. Each animal did two control sessions (A1, A2) and two inactivation sessions (B1, B2). Then, the ramp was removed and the animals did two more sessions, one inactivation and one saline (A, B).

administered in *ABBA* order. *A* was an injection of vehicle (saline) and *B* was the GABAa receptor agonist muscimol (50 ng per side). Infusions were administered 5 min prior to a test session. A total volume of 0.25 uL was injected at a rate of 0.15 uL/min with a microinfusion pump (74,900 Series Cole Palmer) loaded with two identical 10-uL syringes. The infusion traveled through polyethylene tubing to a 32-gauge injection cannula which extended 1 mm below the guide cannula. The cannula was left in place for an additional minute to allow for full diffusion, after which the cannula was removed. Seven minutes later the subject completed a full session of 30 trials with the 30 cm ramp. Rats were run each day through the sequence until completed. Directly after the ABBA sequence, a second inactivation trial and a second saline trial were conducted with the ramp completely removed. This was done in order to confirm that any observed alteration in high reward preference was due to the presence of the ramp and not due to an inability to discriminate between the reward contingencies. The time to complete each session (session duration) was recorded as an indirect measure of gross motor function.

Histology

Post-experiment, rats were euthanized by carbon dioxide-induced hypoxia. Following respiratory cessation, rats were perfused first with a 0.9% saline solution and then with a 4% formaldehyde solution. Brains were removed, sliced with a cryostat into 40um sections, and mounted on slides. Slices were stained with cresyl violet (Sigma-Aldrich) in order to visually confirm cannula placement. Any rats that did not have bilaterally accurate placements of internal cannula tips within the borders of the habenula were not included in the analysis.

Analysis

Statistical analysis was conducted using JASP v 0.14.1 (JASP Team, 2020). Data visualizations were crafted in R v 4.0.2, in R-studio v 1.3.1073, using packages ggplot2 v 3.3.2, and readxl v 1.3.1. (Wickham, 2016; R Core Team, 2017; Wickham et al., 2019). Figures were arranged using Microsoft PowerPoint for Mac v 16.43.

A mixed model, two-way, repeated measures ANOVA was conducted to compare high-reward data across order, treatment and sex. A *post hoc* Student's *t*-test was used to compare high-reward in combined drug and saline groups. A single sample Student's *t*-test was used to specifically compare the muscimol, with ramp treatment to a chance outcome (50% high reward). High-reward data in the no ramp condition was compared across muscimol and saline treatments using Student's *t*-test.

Session duration in the no ramp condition was compared between treatments using Wilcoxen's signed-rank test. Session duration was similarly compared using a mixed model, two-way, repeated measures ANOVA.

RESULTS

Acquisition

A summary of the initial training performance as the rats progressed through the stages is shown in **Figure 2A**. Two animals were excluded from the study due to failing to meet the acquisition criterion. After surgery, the acquisition of the 30 cm ramp was repeated as a reorientation, before the treatment sessions (**Figure 1B**).

Testing

Results of the histological examination indicated there were a total of 12 rats with accurate cannula placements (6 male and 6 female) to be included in the analysis (**Figure 2B**). Because both male and female rats were tested in an ABBA order, a mixed model, two-way, repeated measures ANOVA test of differences was conducted, with treatment and order as repeated measures factors and sex as a between-subjects factor. There were no effects of trial order [$F(1, 10) = 0.016$, $p = 0.902$]. Muscimol inactivation of the LHb significantly decreased choice for the high reward in comparison to saline treatment [$F(1, 10) = 11.602$, $p < 0.01$] (**Figure 3A**). Due to the significant result in the ANOVA, a *post hoc t*-test, comparing combined drug and saline replicates was performed using the Bonferroni correction [$t(11) = -3.280$, $p < 0.01$, $d = -0.947$] confirming that muscimol treatment significantly reduced high reward, high arm preference. The proportion of high-reward responses did not differ between sex when considering treatment condition, $F(1, 10) = 2.841$, $p = 0.123$ (**Figures 4A,B**). No interaction effects were observed for order, treatment or sex.

One possibility was that the reduction in choices for the high reward was due to an impaired ability to discriminate reward conditions generally. To control for this possibility, a single sample *t*-test was used to test whether rats differed from chance ($\mu_o = 0.5$) in their high-reward choices. Results revealed that high-reward choices did not differ significantly from chance with the muscimol treatment [$t(11) = 0.624$, $p = 0.545$]. High-reward choices did significantly deviate from chance, however, ($\mu_o = 0.5$) in the saline condition [$t(11) = 14.327$, $p < 0.01$, $d = 11.783$]. As a follow-up, rats were also tested on the following days without the ramp present to determine whether preferences for the high-reward were altered in the absence of effort as a factor. Student's *t*-test found no difference in proportion high-reward choice between inactivation and control treatments when no ramp was present in the high-reward arm [$t(11) = -0.063$, $p = 0.951$], indicating that reward discrimination ability remained intact despite the muscimol manipulation (**Figure 3B**). Wilcoxen's signed-rank test found no difference in session duration between inactivation and control treatments when no ramp was present in the high reward arm ($W = 29.000$, $p = 0.456$).

To further examine whether LHb inactivation altered other aspects of rats' performance, especially gross motor function, the time taken to complete sessions (session duration) was examined. A mixed model, two-way, repeated measures ANOVA was used with treatment and order as repeated measures factors, and sex as a between-subjects factor. No difference in session duration was observed in regards to order [$F(1, 10) = 4.435$, $p = 0.061$]. No difference in session duration was observed between inactivation and control treatments [$F(1, 10) = 0.029$, $p = 0.869$] (**Figure 3C**). There was also no difference in time to session completion observed between sexes, $F(1, 10) = 0.003$, $p = 0.958$ (**Figure 4C**). No interaction effects with time to complete a session were observed for order, treatment, or sex.

DISCUSSION

The present study sought to determine whether the LHb is important when animals are required to consider physical effort as a factor in obtaining a higher value reward in an ethologically relevant maze based task. Inactivation of the LHb led to an overall reduction in preference for the high effort, high reward arm. These findings suggest that when animals are faced with an effort-based decision, the LHb is required for optimizing rewards. This was further evidenced as there was no difference between treatments when the ramp was removed, demonstrating that the rats had not forgotten which arm contained the high reward, despite reducing preference to chance levels when the ramp was present. Instead, these results demonstrate a decreased willingness to exert the effort required to obtain the reward even when reward conditions are held constant. Additional analyses revealed there were no differences between the time taken to complete a given session regardless of treatment, indicating that the deficit was likely not due to any motor impairments. In addition, there were no differences in choice preference or magnitude of decrease in high reward choices when accounting for sex as a biological variable.

The use of the maze based effort task reveals an important contribution of the LHb to common behavioral conditions associated with psychiatric conditions. Fatigue, increased perception of effort, and apathy are important behavioral hallmarks in several disorders (Sharpe and Wilks, 2002; Cléry-Melin et al., 2011; Konstantakopoulos et al., 2011). These same disorders also result in alterations of both structure and function of the LHb (Lecca et al., 2014; Schafer et al., 2018; Germann et al., 2020). The present results suggest that novel treatments including deep brain stimulation may lead to a specific alleviation of effort related behavioral symptoms in addition to other noted behavioral aspects of habenular function.

Prior electrophysiological and calcium imaging experiments reveal that the LHb plays an integrative role in the incorporation of context that influences decision-making. For example, the same LHb neurons can encode both action-locking and escape behavior in response to a looming stimulus in mice (Lecca et al., 2020). These signals are likely related to the velocity correlated neurons that have previously been observed while rats demonstrate motivated reward searching (Sharp et al., 2006; Baker et al., 2015). In zebrafish, brain-wide calcium imaging has also revealed that stress recruits neural ensemble

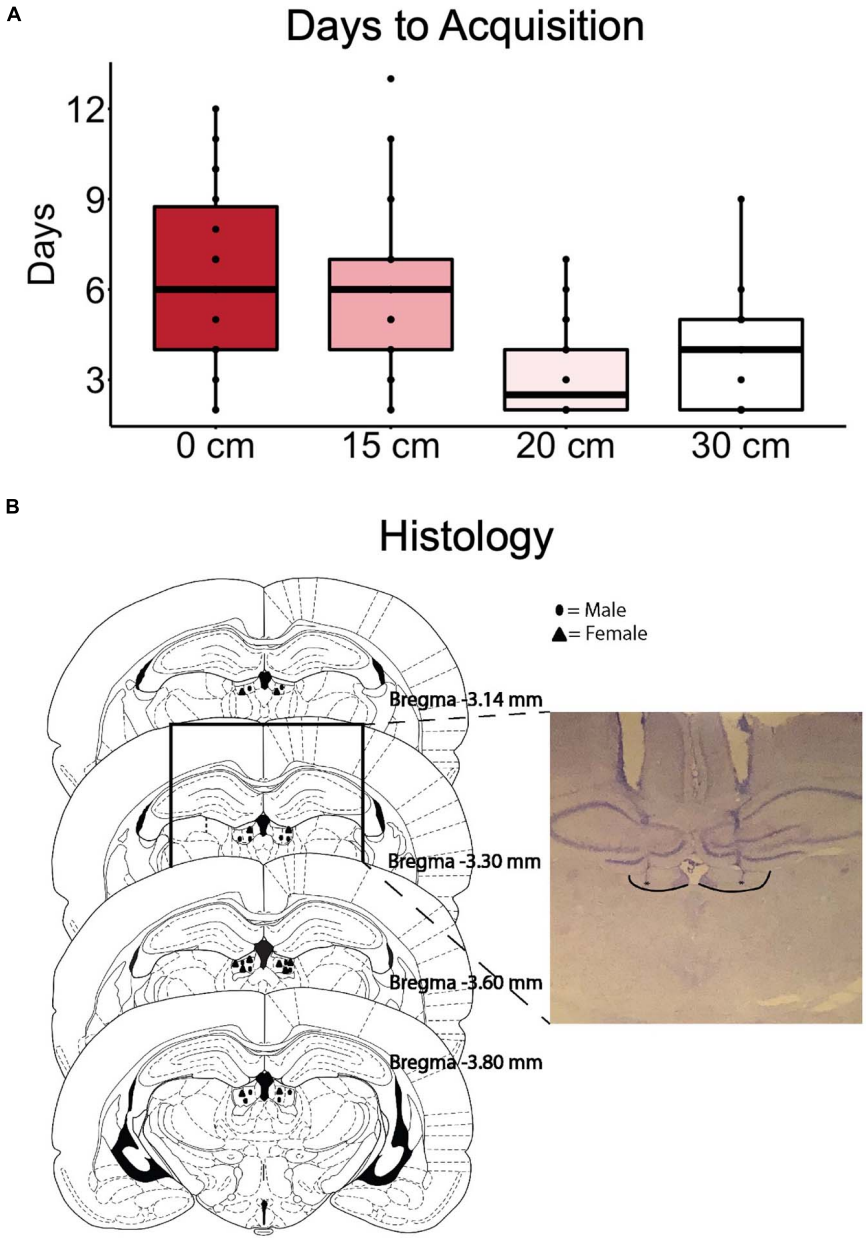

FIGURE 2 | The number of days required to reach criteria (high reward choices ≥80) at each training stage is indicated in **(A)**. **(B)** Summary of correct cannula placements in the LHb with an inset example of a successful placement of cannula aimed at the LHb. Borders of LHb added and (*)'s indicate internal cannula location.

activity to drive a transition from active to passive coping (Andalman et al., 2019). This is similar to the observation that increased activity in ventral tegmental area projecting LHb neurons was associated with increased passive coping in response to chronic mild stress (Cerniauskas et al., 2019). Together, these results support the integrative function of the LHb when deciding how to act in response to many contexts including physical effort in the present study. Likely, changes in habenular activity in freely moving animals facilitate changes in choice behavior.

In contrast to many prior studies of behavioral flexibility involving the LHb (Stopper and Floresco, 2014; Baker and Mizumori, 2017), the present study sought to specifically hold task contingencies constant in the task isolating the effort aspect of the task from any need to recognize changes in behavioral requirements. Stopper and Floresco (2014) found that when the requirements of probability or delay were held constant, no changes in behavior related to LHb manipulation were observed. This contrasts with the findings of the present study

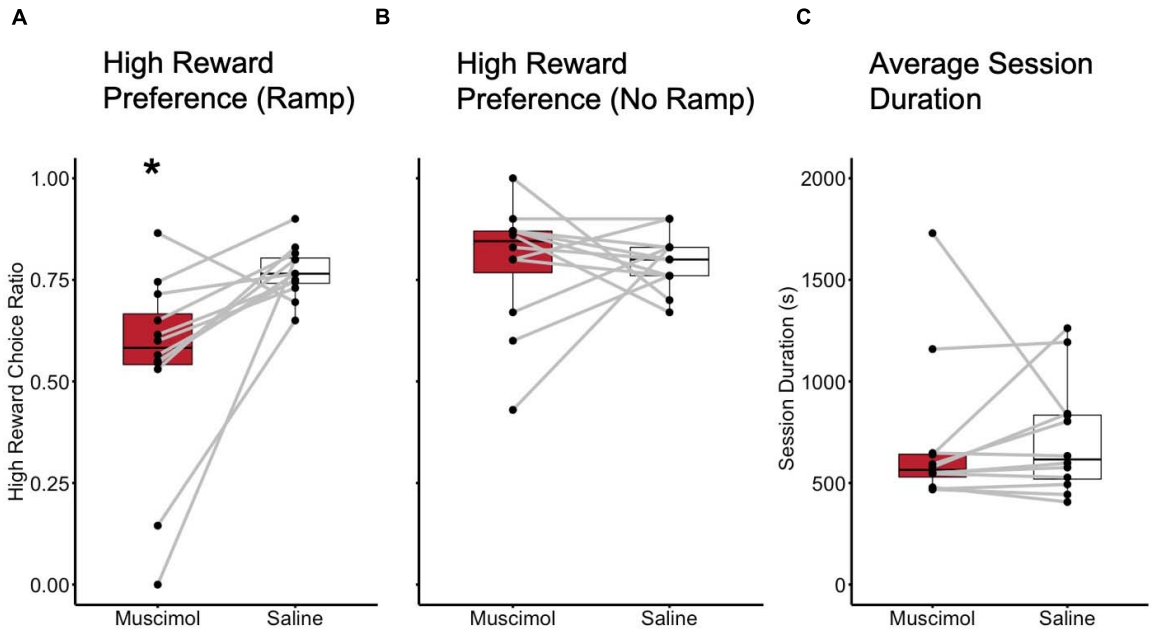

FIGURE 3 | Inactivation of the LHb decreased preference for the high effort, high reward arm. **(A)** High reward choice comparison between inactivation (Muscimol) and control (Saline) sessions with the high 30 cm ramp in place. **(B)** High reward choice comparison between inactivation and control sessions with no ramp in place. **(C)** Session duration compared between inactivation and control sessions. * = $p < 0.01$.

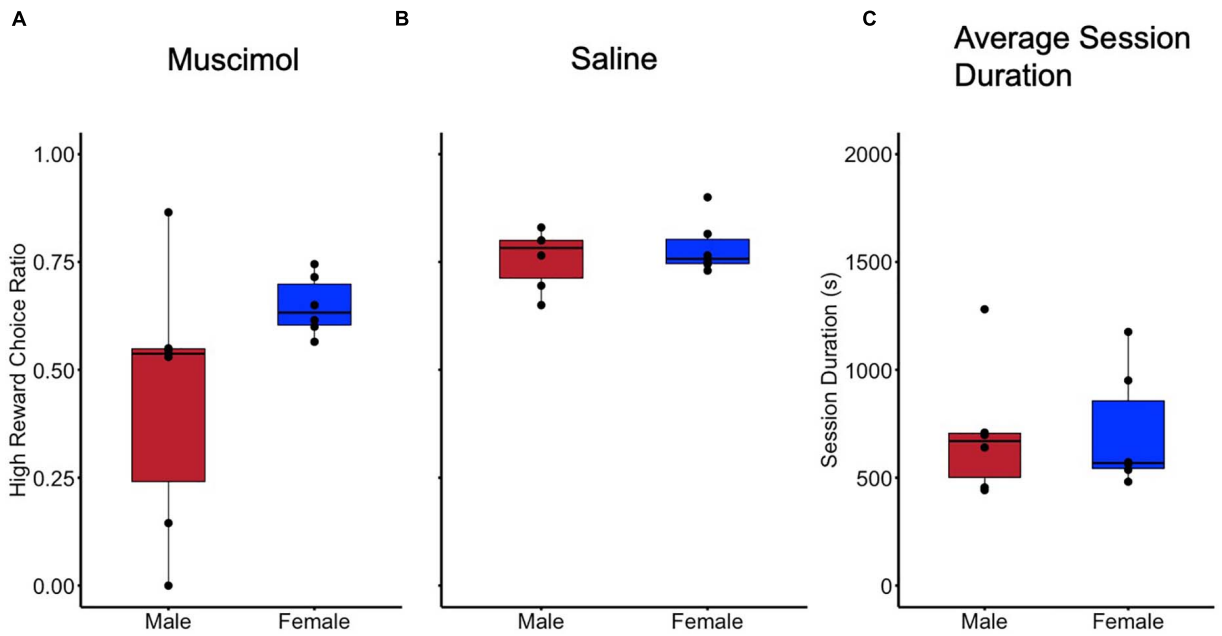

FIGURE 4 | No difference in behavior was observed in reward preferences when considering sex as a biological variable. **(A)** Preference for high reward compared during inactivation (Muscimol) sessions compared by sex. **(B)** Preference for high reward compared for sex during control (Saline) sessions. **(C)** Session duration in seconds for all sessions compared between sex.

suggesting that physical effort is specifically considered on a trial-by-trial basis in the LHb as rats perform ethologically relevant tasks such as reward-seeking in a maze based environment.

Decreases in a willingness to exert effort in the maze based effort task are associated with both dopaminergic and adrenergic systems (Bardgett et al., 2009; Mott et al., 2009). Prior work has shown that the LHb has a prominent influence

on dopaminergic neural function (Ji and Shepard, 2007). For example, during aversive experiences excitatory drive in the habenula influences the rostromedial tegmental area, which in turn inhibits dopamine neurons. Driving these neurons using optogenetics leads to avoidance behaviors seemingly simulating the aversive experiences (Stamatakis and Stuber, 2012). In addition, norepinephrine modulation in the LHb alters both motor and arousal associated behaviors (Purvis et al., 2018). Considering norepinephrine modulation alters willingness to exert physical effort to obtain reward (Mott et al., 2009), it suggests effort related changes in the LHb could be associated with signaling from norepinephrine.

The present findings suggest a possible common component, namely the LHb, across a wide variety of brain areas important for integrating effort into optimal decision-making. Further, the use of the maze based effort task also clarified that even when task aspects such as level of effort or reward location are held constant, the LHb still contributes to choices on a trial-by-trial basis. In the future it would be interesting to evaluate at what height, beyond the 30cm's in this study, rats refuse to climb given our high/low reward ratio of 2:1. Inactivation could also be done at different heights to test if size of effect changes. Regardless, present results indicate that effort is indeed an important factor that is integrated with decision-making processes within the LHb. Presently, rats did not adopt a constant strategy of one or the other arm but rather decreased the frequency of choosing the high effort, high reward arm. This is important for comprehending the LHb's role in using brain states and contextual factors to dynamically make decisions in both health and psychiatric conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Seattle Pacific University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

JS, EB, ÉE, DS, RA, and PB designed, carried out the experiments, and contributed to writing the manuscript. JS analyzed the data and prepared the figures. All authors contributed to the article and approved the submitted version.

REFERENCES

Andalman, A. S., Burns, V. M., Lovett-Barron, M., Broxton, M., Poole, B., Yang, S. J., et al. (2019). Neuronal Dynamics Regulating Brain and Behavioral State Transitions. *Cell* 177, 970.e–985.e. doi: 10.1016/j.cell.2019.02.037

Bachleda, C., and Darhiri, L. (2018). Internet addiction and mental and physical fatigue. *Int. Technol. Manage. Rev.* 7, 25–33. doi: 10.2991/itmr.7.1.3

Baker, P. M., and Mizumori, S. J. (2017). Control of behavioral flexibility by the lateral habenula. *Pharmacol. Biochem. Behav.* 162, 62–68. doi: 10.1016/j.pbb.2017.07.012

Baker, P. M., Oh, S. E., Kidder, K. S., and Mizumori, S. J. Y. (2015). Ongoing behavioral state information signaled in the lateral habenula guides choice flexibility in freely moving rats. *Front. Behav. Neurosci.* 9:295. doi: 10.3389/fnbeh.2015.00295

Bardgett, M. E., Depenbrock, M., Downs, N., Points, M., and Green, L. (2009). Dopamine modulates effort-based decision making in rats. *Behav. Neurosci.* 123:242. doi: 10.1037/a0014625

Cerniauskas, I., Winterer, J., de Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., et al. (2019). Chronic Stress Induces Activity, Synaptic, and Transcriptional Remodeling of the Lateral Habenula Associated with Deficits in Motivated Behaviors. *Neuron* 104, 899.e–915.e. doi: 10.1016/j.neuron.2019.09.005

Cléry-Melin, M.-L., Schmidt, L., Lafargue, G., Baup, N., Fossati, P., and Pessiglione, M. (2011). Why don't you try harder? An investigation of effort production in major depression. *PLoS One* 6:e23178. doi: 10.1371/journal.pone.0023178

Cohen, R., Lohr, I., Paul, R., and Boland, R. (2001). Impairments of Attention and Effort Among Patients With Major Affective Disorders. *J. Neuropsychiat. Clin. Neurosci.* 13, 385–395. doi: 10.1176/jnp.13.3.385

Demyttenaere, K., De Fruyt, J., and Stahl, S. M. (2005). The many faces of fatigue in major depressive disorder. *Int. J. Neuropsychopharmacol.* 8, 93–105. doi: 10.1017/s1461145704004729

Flanigan, M., Aleyasin, H., Takahashi, A., Golden, S. A., and Russo, S. J. (2017). An emerging role for the lateral habenula in aggressive behavior. *Pharmacol. Biochem. Behav.* 162, 79–86. doi: 10.1016/j.pbb.2017.05.003

Floresco, S. B., and Ghods-Sharifi, S. (2007). Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb. Cortex* 17, 251–260. doi: 10.1093/cercor/bhj143

Germann, J., Gouveia, F. V., Martinez, R. C. R., Zanetti, M. V., de Souza Duran, F. L., Chaim-Avancini, T. M., et al. (2020). Fully Automated Habenula Segmentation Provides Robust and Reliable Volume Estimation Across Large Magnetic Resonance Imaging Datasets, Suggesting Intriguing Developmental Trajectories in Psychiatric Disease. *Biol. Psychiat. Cognit. Neurosci. Neuroimag.* 5, 923–929. doi: 10.1016/j.bpsc.2020.01.004

JASP Team (2020). *JASP (Version 0.14.1)[Computer software]*. Amsterdam: University of Amsterdam.

Ji, H., and Shepard, P. D. (2007). Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABAA receptor-mediated mechanism. *J. Neurosci.* 27, 6923–6930. doi: 10.1523/jneurosci.0958-07.2007

Konstantakopoulos, G., Ploumpidis, D., Oulis, P., Patrikelis, P., Soumani, A., Papadimitriou, G. N., et al. (2011). Apathy, cognitive deficits and functional impairment in schizophrenia. *Schizophrenia Res.* 133, 193–198. doi: 10.1016/j.schres.2011.07.003

Lecca, S., Meye, F. J., and Mameli, M. (2014). The lateral habenula in addiction and depression: An anatomical, synaptic and behavioral overview. *Eur. J. Neurosci.* 39, 1170–1178. doi: 10.1111/ejn.12480

Lecca, S., Namboodiri, V. M., Restivo, L., Gervasi, N., Pillolla, G., Stuber, G. D., et al. (2020). Heterogeneous habenular neuronal ensembles during selection of defensive behaviors. *Cell Rep.* 31:107752. doi: 10.1016/j.celrep.2020.107752

Lecourtier, L., and Kelly, P. H. (2007). A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. *Neurosci. Biobehav. Rev.* 31, 658–672. doi: 10.1016/j.neubiorev.2007.01.004

Mott, A. M., Nunes, E. J., Collins, L. E., Port, R. G., Sink, K. S., Hockemeyer, J., et al. (2009). The adenosine A 2A antagonist MSX-3 reverses the effects of the dopamine antagonist haloperidol on effort-related decision making in a T-maze cost/benefit procedure. *Psychopharmacology* 204:103. doi: 10.1007/s00213-008-1441-z

Purvis, E. M., Klein, A. K., and Ettenberg, A. (2018). Lateral habenular norepinephrine contributes to states of arousal and anxiety in male rats. *Behav. Brain Res.* 347, 108–115. doi: 10.1016/j.bbr.2018.03.012

Quina, L. A., Tempest, L., Ng, L., Harris, J. A., Ferguson, S., Jhou, T. C., et al. (2015). Efferent pathways of the mouse lateral habenula. *J. Comparat. Neurol.* 523, 32–60. doi: 10.1002/cne.23662

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Salamone, J. D., Cousins, M. S., and Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behav. Brain Res.* 65, 221–229. doi: 10.1016/0166-4328(94)90108-2

Sartorius, A., Kiening, K. L., Kirsch, P., von Gall, C. C., Haberkorn, U., Unterberg, A. W., et al. (2010). Remission of Major Depression Under Deep Brain Stimulation of the Lateral Habenula in a Therapy-Refractory Patient. *Biol. Psychiat.* 67, e9–e11. doi: 10.1016/j.biopsych.2009.08.027

Schafer, M., Kim, J.-W., Joseph, J., Xu, J., Frangou, S., and Doucet, G. E. (2018). Imaging habenula volume in schizophrenia and bipolar disorder. *Front. Psychiat.* 9:456. doi: 10.3389/fpsyt.2018.00456

Sharp, P. E., Turner-Williams, S., and Tuttle, S. (2006). Movement-related correlates of single cell activity in the interpeduncular nucleus and habenula of the rat during a pellet-chasing task. *Behav. Brain Res.* 166, 55–70. doi: 10.1016/j.bbr.2005.07.004

Sharpe, M., and Wilks, D. (2002). Fatigue. *BMJ* 325:480. doi: 10.1136/bmj.325.7362.480

Stamatakis, A. M., and Stuber, G. D. (2012). Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. *Nat. Neurosci.* 15, 1105–1107. doi: 10.1038/nn.3145

Stopper, C. M., and Floresco, S. B. (2014). What's better for me? Fundamental role for lateral habenula in promoting subjective decision biases. *Nat. Neurosci.* 17, 33–35. doi: 10.1038/nn.3587

Sutherland, R. J. (1982). The dorsal diencephalic conduction system: A review of the anatomy and functions of the habenular complex. *Neurosci. Biobehav. Rev.* 6, 1–13. doi: 10.1016/0149-7634(82)90003-3

Wang, Y., Zhang, C., Zhang, Y., Gong, H., Li, J., Jin, H., et al. (2020). Habenula deep brain stimulation for intractable schizophrenia: A pilot study. *Neurosurg. Focus* 49:E9.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., and François, R. (2019). Welcome to the tidyverse. *J. Open Source Softw.* 4:1686. doi: 10.21105/joss.01686

Yohn, S. E., Thompson, C., Randall, P. A., Lee, C. A., Müller, C. E., Baqi, Y., et al. (2015). The VMAT-2 inhibitor tetrabenazine alters effort-related decision making as measured by the T-maze barrier choice task: Reversal with the adenosine A 2A antagonist MSX-3 and the catecholamine uptake blocker bupropion. *Psychopharmacology* 232, 1313–1323. doi: 10.1007/s00213-014-3766-0

Zhang, C., Kim, S.-G., Li, D., Zhang, Y., Li, Y., Husch, A., et al. (2019). Habenula deep brain stimulation for refractory bipolar disorder. *Brain Stimulat. Basic Translat. Clin. Res. Neuromodulat.* 12, 1298–1300. doi: 10.1016/j.brs.2019.05.010

Zhang, L., Wang, H., Luan, S., Yang, S., Wang, Z., Wang, J., et al. (2017). Altered volume and functional connectivity of the habenula in schizophrenia. *Front. Hum. Neurosci.* 11:636. doi: 10.3389/fnhum.2017.00636

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Psychiatry

SYSTEMATIC REVIEW
published: 17 August 2021
doi: 10.3389/fpsyt.2021.730931

Deep Brain Stimulation of the Habenula: Systematic Review of the Literature and Clinical Trial Registries

Jürgen Germann[1†], Manuel Mameli[2,3†], Gavin J. B. Elias[1], Aaron Loh[1], Alaa Taha[1], Flavia Venetucci Gouveia[4], Alexandre Boutet[1,5] and Andres M. Lozano[1]*

[1] Division of Neurosurgery, Department of Surgery, University Health Network and University of Toronto, Toronto, ON, Canada, [2] The Department of Fundamental Neuroscience, The University of Lausanne, Lausanne, Switzerland, [3] INSERM, UMR-S 839, Paris, France, [4] Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada, [5] Joint Department of Medical Imaging, University of Toronto, Toronto, ON, Canada

OPEN ACCESS

Edited by:
Wenbin Guo,
Central South University, China

Reviewed by:
Dianyou Li,
Shanghai Jiao Tong University, China
Giacomo Grassi,
University of Florence, Italy
Shyam Sundar Arumugham,
National Institute of Mental Health and Neurosciences (NIMHANS), India

***Correspondence:**
Andres M. Lozano
lozano@uhnresearch.ca

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Neuroimaging and Stimulation, a section of the journal Frontiers in Psychiatry

Received: *25 June 2021*
Accepted: *21 July 2021*
Published: *17 August 2021*

Citation:
Germann J, Mameli M, Elias GJB, Loh A, Taha A, Gouveia FV, Boutet A and Lozano AM (2021) Deep Brain Stimulation of the Habenula: Systematic Review of the Literature and Clinical Trial Registries. Front. Psychiatry 12:730931. doi: 10.3389/fpsyt.2021.730931

The habenula is a small bilateral epithalamic structure that plays a key role in the regulation of the main monoaminergic systems. It is implicated in many aspects of behavior such as reward processing, motivational behavior, behavioral adaptation, and sensory integration. A role of the habenula has been indicated in the pathophysiology of a number of neuropsychiatric disorders such as depression, addiction, obsessive-compulsive disorder, and bipolar disorder. Neuromodulation of the habenula using deep brain stimulation (DBS) as potential treatment has been proposed and a first successful case of habenula DBS was reported a decade ago. To provide an overview of the current state of habenula DBS in human subjects for the treatment of neuropsychiatric disorders we conducted a systematic review of both the published literature using PUBMED and current and past registered clinical trials using ClinicalTrials.gov as well as the International Clinical Trials Registry Platform. Using PRISMA guidelines five articles and five registered clinical trials were identified. The published articles detailed the results of habenula DBS for the treatment of schizophrenia, depression, obsessive-compulsive disorder, and bipolar disorder. Four are single case studies; one reports findings in two patients and positive clinical outcome is described in five of the six patients. Of the five registered clinical trials identified, four investigate habenula DBS for the treatment of depression and one for obsessive-compulsive disorder. One trial is listed as terminated, one is recruiting, two are not yet recruiting and the status of the fifth is unknown. The planned enrollment varies between 2 to 13 subjects and four of the five are open label trials. While the published studies suggest a potential role of habenula DBS for a number of indications, future trials and studies are necessary. The outcomes of the ongoing clinical trials will provide further valuable insights. Establishing habenula DBS, however, will depend on successful randomized clinical trials to confirm application and clinical benefit of this promising intervention.

Keywords: habenula, deep brain stimulation, clinical trial, depression, obsessive-compulsive disorder, schizophrenia, bipolar disorder

INTRODUCTION

Neurological and psychiatric brain disorders emerge from the aberrant activity in brain circuits (1–4). Deep Brain Stimulation (DBS) employs precisely placed electrodes to deliver current to specific brain structures in order to modulate these dysfunctional circuits (1, 5). To date, well over 200,000 patients worldwide have been treated with DBS, most commonly for the management of movement disorders, such as Parkinson's disease (PD) (1). DBS offers advantages over other neuromodulatory treatments as it is non-lesional, reversible, and stimulation parameters can be adjusted as needed. The effectiveness of DBS depends upon appropriately and selectively stimulating and modulating the intended brain circuit(s) and is contingent on the selection of the optimal anatomical target and fine tuning the stimulation. For each DBS patient, stimulation parameters have to be individually optimized to maximize clinical benefits and minimize side-effects. This parameter optimization–or "programming"–, however, remains an empirical trial-and-error process that necessitates repeated clinic visits and is thus time- and resource-intensive (6) for both the patients and healthcare systems. A number of brain structures have been proposed as targets for DBS with multiple potential targets identified for most conditions (3, 7–12).

The habenula (Hb) is a relatively new DBS target that has been proposed to treat various psychiatric disorders, including depression (7, 13). It is a small bilateral epithalamic structure located adjacent to the posterior commissure in humans (14, 15) (**Figure 1A**). Invasive studies using animal models have shown that the Hb has extensive direct connections with the hypothalamus, brainstem nuclei, basal ganglia and limbic areas–the stria medullaris being the main afferent and the fasciculus retroflexus the main efferent fiber bundle (**Figure 1B**; **Table 1**) (17–19, 25–28). Studies in humans using imaging and electrophysiological techniques have shown multiple additional cortical and cerebellar regions to be functionally connected to the habenula (**Figure 1B**; **Table 1**) (20, 22–24, 28). It plays a key role in controlling the dopaminergic, serotonergic and noradrenergic systems (25, 27, 29–32). The Hb thus has a unique position regulating the three main monoaminergic systems.

Evolutionarily preserved across vertebrae, the Hb can be divided into a medial and a lateral Hb. The medial Hb is composed mainly of glutamate producing neurons that exert influence over the serotonergic system and are involved in emotional response selection (28, 33–35). The lateral Hb (LHb) is composed mainly of glutamate producing neurons that control midbrain dopaminergic neurons and is involved in reward related behavior (17, 31). Pioneering research analyzing the role of the Hb in processing reward related information by Hikosaka and others demonstrated that the LHb plays a key role in controlling adaptive behaviors (17). Neuronal activity in the LHb diminishes upon presentation of rewards, while activity increases after the presentation of aversive stimuli or cues predicting them (36–39). A series of studies indicate that maladaptations within the LHb underlie behavioral symptoms of major depression. Indeed, several cellular, and synaptic adaptations are causally linked to LHb hyperactivity, which consequently drives the expression of anhedonia and behavioral despair, which are typical aspects of mood disorders (40–42). Playing a key role in the control of all three major monoaminergic transmitter

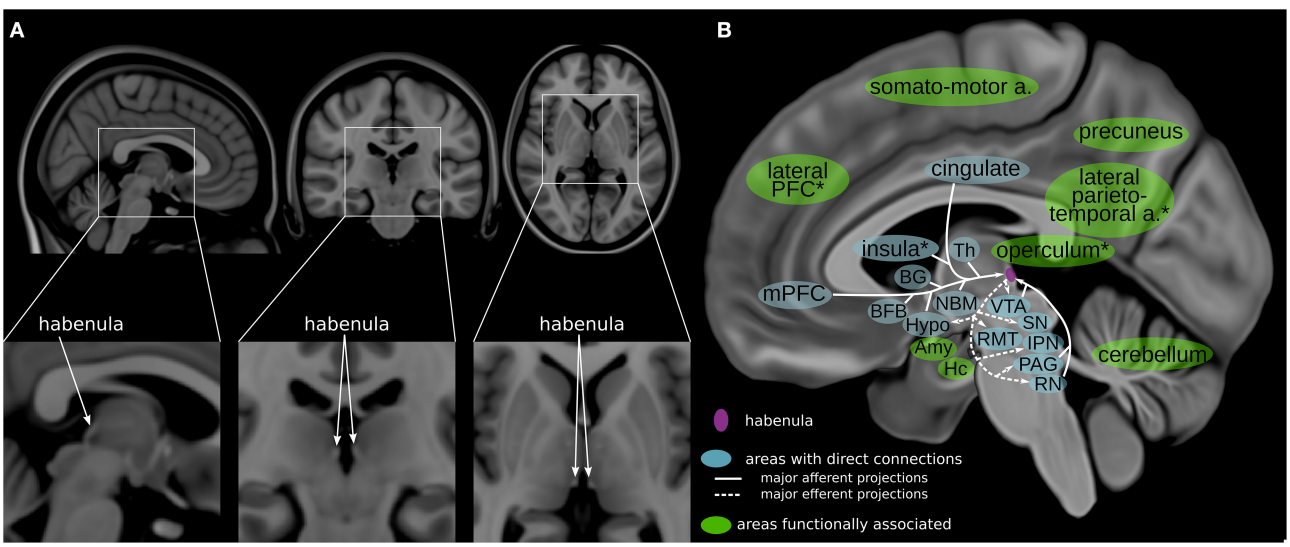

FIGURE 1 | Habenula anatomy and connections. **(A)** The human habenula illustrated in orthogonal slices using the MNI template brain (MNI152b) (16). **(B)** Highlighting regions connected with the habenula (17–20) illustrated using a medial view of a high-resolution, high contrast template (21). The habenula is highlighted in purple. Areas with direct connections to the habenula are highlighted in blue; areas that are functionally associated with the habenula are highlighted in green. a, areas; Amy, amygdala; BFB, basal forebrain; BG, basal ganglia; Hc, hippocampus; Hypo, hypothalamus; IPN, interpeduncular nucleus; mPFC, medial prefrontal cortex; NBM, nucleus basalis of Meynert; PAG, periaqueductal gray; PFC, prefrontal cortex; RMT, rostromedial tegmental nucleus; RN, raphe nuclei; SN, substantia nigra; Th, thalamus; VTA, ventral tegmental area; *approximate/example position of the region.

TABLE 1 | Connectivity of the habenula.

Structure	Type of connectivity	Model	Technique
Amygdala	Functionally connected	Clinical	Functional MRI & electrophysiology (20, 22–24)
Basal forebrain	Direct afferent	Pre-clinical	Tracer (25)
Basal ganglia	Direct afferent	Pre-clinical	Tracer (25)
Cerebellum	Functionally connected	Clinical	Functional MRI (20, 22)
Cingulate cortex	Direct efferent	Pre-clinical	Tracer (26)
Hippocampus	Functionally connected	Clinical	Functional MRI & electrophysiology (20, 22, 23)
Hypothalamus	Direct afferent + efferent	Pre-clinical	Tracer (25, 27)
Insula	Direct efferent	Pre-clinical	Tracer (26)
Intrapeduncular nucleus	Direct efferent	Pre-clinical	Tracer (27)
Lateral parieto-temporal areas	Functionally connected	Clinical	Functional MRI (20, 22)
Lateral prefrontal cortex	Functionally connected	Clinical	Functional MRI (20, 22)
Medial prefrontal cortex	Direct efferent	Pre-clinical	Tracer (26)
Nucleus basalis of Meynert	Direct afferent	Pre-clinical	Tracer (25)
Operculum	Functionally connected	Clinical	Functional MRI (20, 22)
Periaqueductal gray	Direct afferent + efferent	Pre-clinical	Tracer (25, 27)
Precuneus	Functionally connected	Clinical	Functional MRI (20, 22)
Raphe nuclei	Direct afferent + efferent	Pre-clinical	Tracer (25, 27)
Rostromedial tegmental nucleus	Direct efferent	Pre-clinical	Tracer (27)
Somato-motor areas	Functionally connected	Clinical	Functional MRI (20, 22)
Substantia nigra	Direct efferent	Pre-clinical	Tracer (27)
Thalamus	Direct afferent	Pre-clinical	Tracer (25)
Ventral tegmental area	Direct afferent + efferent	Pre-clinical	Tracer (25, 27)

MRI, magnetic resonance imaging.

systems, the Hb controls a wide range of behavior beyond reward processing and depressive symptoms. The habenula has been connected to social interaction, motivational behavior, behavioral adaptation, pain processing and sensory integration, motor activity, memory, sleep, and circadian rhythm (17–19, 22, 43–46).

In a first seminal study, DBS within the Hb successfully ameliorated depressive symptoms in a patient where classic pharmacological treatment had failed (47). The components of an implanted DBS system and an example electrode targeting the Hb are illustrated in **Figures 2A,B**. These findings prompted a series of studies to assess whether DBS was similarly effective in animal models and to evaluate the underlying therapeutic mechanisms. Using a rodent model of depression named learned helplessness, an initial study reported an increased synaptic excitation onto LHb neurons concomitantly with increased neuronal activity of LHb cells with respect to control rats (41, 42). Adapted DBS-like electrodes were then inserted in the LHb, and high frequency stimulation, similar to that employed in humans, normalized the depressive-like state typical in learned helplessness rats (41). Importantly, DBS potently produced a time-locked collapse of glutamatergic transmission onto LHb neurons (41). This initial finding represented an initial indication that changes in cellular function in the LHb causally linked to depressive states, and that LHb-targeted DBS could represent a therapeutically-relevant strategy. In support of this, a different study employed early life stress to drive the emergence of depressive-like symptoms in adulthood that included defects in coping strategies and anhedonia (50). Mechanistically, the behavioral changes were associated with a reduction in the postsynaptic function of the metabotropic GABAb receptors (**Figure 2C**). The metabotropic GABAb receptor is a key cellular module for the maintenance of neuronal activity within the LHb (40). The reduction in GABAb function led to higher neuronal firing activity in LHb neurons (40, 50).

The authors used DBS-like electrodes in acute slices to show that this also causes a presynaptic reduction in glutamate release, as well as efficient reduction in neuronal firing rate assessed using *in vivo* recordings (50). Further, the use of DBS in behaving animals submitted to early life stress produced a normalization of the depressive-like state compared to non-stimulated animals (50). These studies not only unravel mechanisms of action of DBS in the LHb but support its use for therapeutically relevant interventions.

The potential of the Hb as a target for DBS goes beyond the context of depression. For example, DBS of LHb in rats reduced sucrose seeking and cocaine seeking behavior (51, 52) consistent with the putative role of Hb in addiction (17, 34, 53, 54). Furthermore, studies have demonstrated that the Hb plays a role in the pathophysiology of a number of neuropsychiatric disorders beyond depression and addiction such as schizophrenia (15, 55, 56), bipolar disorder (BD) (15, 33, 57–59), obsessive-compulsive disorder (OCD) (60, 61) and autism (62).

Given the multitude of potential therapeutic applications of Hb DBS, we conducted a systematic review of both the published human literature and the registered clinical trials to provide an overview of the current status of Hb DBS.

METHODS

This systematic review was performed according to PRISMA guidelines (**Figure 3**). In March 2021, a literature search was conducted for original articles using PubMed/MEDLINE with the following search term: "habenula" AND ("DBS" OR "deep brain stimulation" OR "neuromodulation" OR "stimulation" OR "electrical stimulation"). No restrictions were placed on the publication date. No duplicates were found. Articles written in languages other than English, protocols, reviews, and opinion

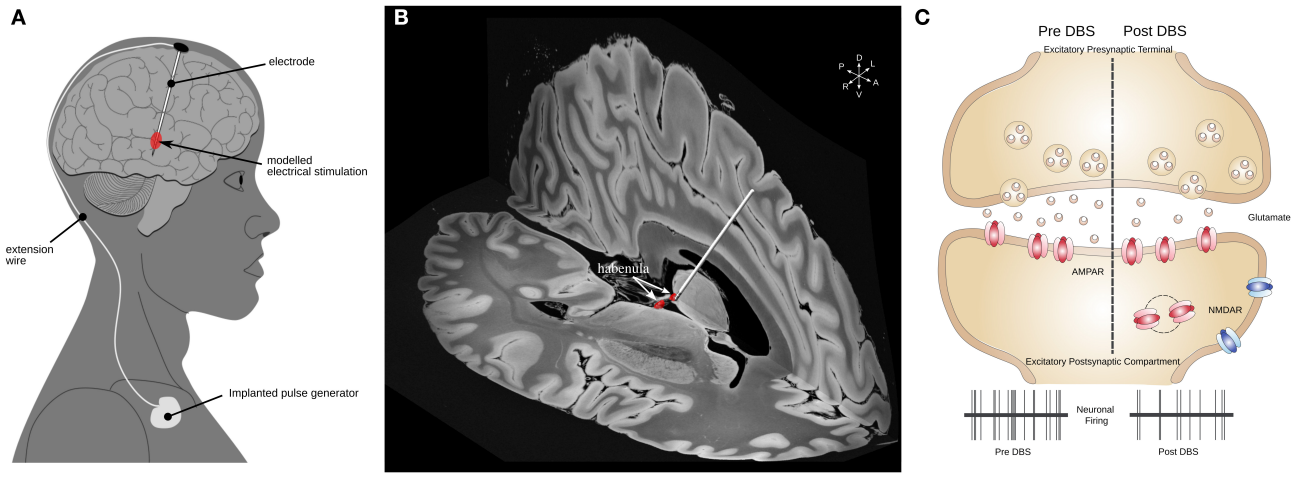

FIGURE 2 | Habenular DBS and its mechanisms of action. **(A)** Schematic illustrating the components of a DBS system. **(B)** 3-D example image of a DBS electrode targeting the left Hb (red) illustrated in MNI152 space (16) using slices from a 100 micron resolution, FLASH 7 Tesla brain (48) electrode placement performed using Lead-DBS (49). **(C)** Mechanisms of action of DBS in LHb. A schematic of excitatory synapse is presented. Human-inspired DBS protocols in rodents lead to a reduction in glutamate release, which subsequently reduces the neuronal activity of LHb neurons (41, 50). DBS, deep brain stimulation; Hb, habenula; LHb, lateral habenula; MNI, Montreal Neurological Institute.

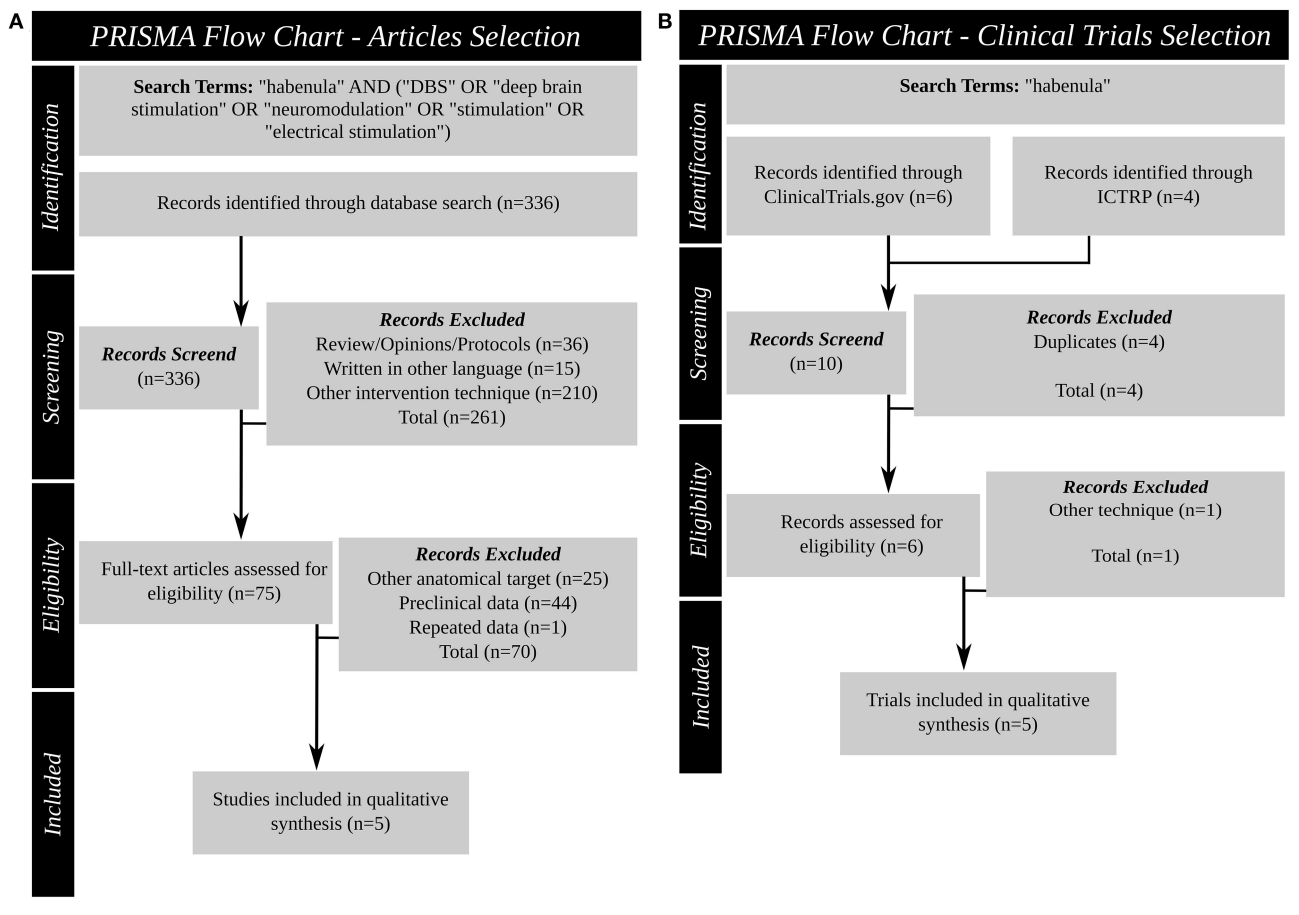

FIGURE 3 | Systematic review PRISMA figures. **(A)**: Article search **(B)**: Clinical trial database search. DBS, deep brain stimulation; ICTRP, international clinical trials registry platform; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

TABLE 2 | Articles selected.

References	Wang et al. (63)	Sartorius et al. (47)	Zhang et al. (64)	Zhang et al. (65)	Wang et al. (66)
Study type	Case series	Case study	Case study	Case study	Case study
***N* (m/f)**	2 (2/0)	1(0/1)	1(1/0)	1(1/0)	1(1/0)
Age (avg, range)	23.5 (21–26)	64	30	41	34
Disorder	Schizophrenia	TRD	OCD	BD	TRD
Disease Duration (avg/range)	6.5y (4–9)	46y	15y	21y	21y
Medication	Patient 1: olanzapine 15 mg/day from month 7 onwards (previous dose unknown); patient 2: que-tiapine 0.4 g/dayfrom month 4 onwards (previous dose unknown)	N/D	Fluvoxamine (100 mg/d); PRE-DBS: Benzhexol (2 mg/d);Olanzapine (7.5 mg/d)Magnesium Valproate (0.25 g/d)	N/D	N/D
DBS procedure	Bilateral	Bilateral	Bilateral	Bilateral	Bilateral
DBS target	Habenula	Lateral habenula	Habenula	Habenula	Lateral habenula
Follow-up	10 to 12 months	57 weeks	12 months	12 months	12 weeks
% improvement (avg/range)	11.1%(−9.5–31.7%)	100.00%	35.50%	100.00%	56.50%
Positive clinical outcome ratio	1/2	1/1	1/1	1/1	1/1
Assessment tool	PANSS	HAMD21	Y-BOCS	HAMD17	HAMD21
Secondary outcome% improvement (avg/range)	PANSS pos scale:-7.7%(-69.2–53.8%); PANSS neg scale: 13.7%(-4.3–31.7%); PANSS Gen Psychopathology: 14.35% (7.9–20.8%)	N/D	HAMD17: 36%; HARS: 31%; PSQI: −20%; EQ-5D-5L: 33%	MADRS: 100%; HARS: 87%; PSQI: 44% Plus COGSTATE, SF-36 and SDS with multiple sub-scales	MADRS: 66%; QIDS-SR: 63%; HARS: 65%; PSQI: 93%; HCL-32: 86%
Side effects	Acute: numbness, change of heart rate, pain, dizziness, eye closing, discomfort, feeling of heaviness, feeling of relaxation.	N/D	Acute: dizziness, numbness, nausea, flusteredness	Blurred vision temporary (high frequency stimulation)	Acute: dizziness, numbness, nausea, flusteredness
Stimulation settings	Patient 1: L (2.0 V, 60 us, 60 Hz); R (2.5 V, 60 μs, 60 Hz); patient 2: L (3.15 V, 80 us, 135 Hz); R (3.2 V, 60 μs, 135 Hz)	10.5V	L (1.6 V, 60 μs, 60 Hz); R (1.35 V, 60 μs, 60 Hz)	L+R (2.0 V, 60 μs, 60 Hz)	L (4.5 V, 90 μs, 160 Hz); R (2.5 V, 90 μs, 160 Hz)
Observations	At 6 months both patients showed positive treatment response, only 1 patient retained positive treatment response at 12 months	No acute antidepressant effect; interval to remission 4 months after switching to high frequency stimulation; stimulation location confirmed using FDG-PET; symptoms return when treatment accidentally discontinued	N/D	High frequency stimulation provided 46% improvement; low-frequency stimulation initiated in the fourth quarter with 100% improvement; functional connectivity compared to healthy controls at various time points	LFP results indicate decreased habenula activity with stimulation ON; magnitude of suppression associated with clinical improvements; power spectrum density decreases over time with treatment (acquired with stimulation OFF)

BD, bipolar disorder; COGSTATE, computerized cognitive assessment tool; DBS, deep brain stimulation; EQ-5D-5L, 5-level EuroQol-5D; FDG-PET, fluorodeoxyglucose positron emission tomography; HAMD, Hamilton Depression Scale; HARS, Hamilton anxiety rating Scale; HCL-32, hypomania checklist; L, left; LFP, local field potentials; MADRS, Montgomery-Asberg Depression Rating Scale; N/D, not described; OCD, obsessive-compulsive disorder; PANSS, positive and negative symptom scale; PSQI, Pittsburgh sleep quality index; QIDS-SR, Quick Inventory of Depressive Symptomatology; R, right; SDS, Sheehan disability scale; SF-36, 36-item short survey; TRD, treatment resistant major depressive disorder; Y-BOCS, yale-brown obsessive compulsive scale.

pieces as well as articles describing techniques other than DBS were excluded. All relevant articles were selected for full-text review and had to meet the following inclusion and exclusion criteria: (I) inclusion (articles reporting on the clinical outcome of Hb deep brain stimulation in humans) and (II) exclusion (studies reporting preclinical data; deep brain stimulation targeting a

TABLE 3 | Rationale for Habenula involvement in psychiatric disorders.

	Depression	Bipolar disorder	Schizophrenia	Obsessive-compulsive disorder
Evidence for involvement of habenula	Animal studies implicate the habenula in depressive-like behavior (45, 67). The LHB is found to be hyperactive and ablation of the habenula in animals alleviates depressive-like symptoms (33, 67) (proulx; Fakhoury). Altered habenula volume and function has been reported in human subjects (58, 68).	Altered habenula volume and function has been reported in human subjects (15, 58, 69). Animal models show involvement of LHB in mood disorders [see Depression].	Altered habenula volume and function has been reported in human subjects (15, 70, 71). Rodent models show LHB function important for guided decision making, and LHB hypoactivity associated with schizophrenia-like symptoms (45, 72). Antipsychotic drugs increase LHB activity in animal model (33)	Encoding and processing of aversive stimuli–processes dependent on the LHB–are impaired in OCD (36, 73, 74). Depressive symptoms are often present in OCD (75). Hb-DBS has previously been used to successfully treat depression (47)
Proposed habenula contribution to the psychiatric disorder and/or symptoms	LHB encodes negative motivational values and aversive outcomes associated with cues (37). Continuously hyperactive LHB causes depressive state (13, 47).	Hyperactive LHB causes depressive state (13, 47) [see Depression].	The habenula plays a key role in controlling the monoaminergic systems and these systems are disturbed in SZ (17, 76). Hypoactive LHB interferes with appropriate guidance of behavior and decision making (45).	Monoaminergic systems are disturbed in OCD and the habenula plays a key role in their control (17, 77).
Proposed mechanism of the beneficial effect of habenula DBS	DBS reduced LHB neuronal activity via GABAb receptors (40, 50).	DBS corrects habenula dysfunction (65) [see Depression].	DBS corrects habenula hypoactivity (63).	DBS corrects habenula dysfunction (64).

DBS, Deep brain stimulation; LHB, Lateral Habenula; OCD, Obsessive-Compulsive Disorder; SZ, Schizophrenia.

different brain region; articles reporting a patient population described previously).

Also in March 2021 two publicly available clinical trial databases were queried for past and ongoing clinical trials using "habenula" as search term: ClinicalTrials.gov (https://clinicaltrials.gov/) provided by the US National Library of Medicine, and the International Clinical Trials Registry Platform (ICTRP; https://www.who.int/ictrp/en/; https://apps.who.int/trialsearch/) of the World Health Organization (WHO). All entries since inception of the databases were queried. Duplicates were excluded. All relevant trials were selected for review and had to meet the inclusion and exclusion criteria: (I) inclusion (deep brain stimulation targeting the Hb as primary intervention) and (II) exclusion (intervention other than DBS).

Studies and trials were separately screened (JG and AT) and disagreement was resolved by consensus.

RESULTS

Of the 336 items identified in the search, 36 were reviews, technical protocols or opinion pieces, 15 were not written in English, and 210 described an intervention other than DBS (e.g., optogenetic stimulation, neurochemical stimulation). The full text search of the remaining 75 studies 25 did not target the Hb, 44 described preclinical experiments and one study described data reported in an earlier manuscript. After screening, this systematic review identified five articles (**Table 2**) (47, 63–66) that satisfied the inclusion criteria. These articles reported the results of DBS targeting the Hb for four different indications: schizophrenia (63), treatment resistant major depressive disorder (TRD) (47, 65, 66), OCD (45) and BD (46).

Table 3 outlines the evidence and rationale the studies provide for using Hb DBS as treatment in each of the various psychiatric conditions.

Four of the five were single case studies (47, 64–66), one was a small case series of two patients (63). Clinical changes ranged from −9.5 to 100% symptom improvement in the overall six patients treated, with positive clinical outcome (improvement $\geqq$31.7%) reported in 5 patients. The patients ranged in age from 21 to 64 years, disease duration at time of surgery varied from 4 to 46 years, and five of the six were male. One patient dropped out after 18 weeks for non-medical reasons (47) and all other patients were observed for at least >10 months. In all cases reported, Hb DBS stimulation was delivered via bilateral electrodes using a monopolar configuration and a pulse-width of 60 μs. Frequency of stimulation spanned from 60 to 160 Hz and voltage ranged from 1.35 to 10.5V. Studies describe numerous side effects of acute stimulation, all of which were subsequently controlled by altering the stimulation parameters (e.g., lower voltage, change contact, change frequency).

In addition to published articles, the search found 10 trial entries in the two clinical trial databases. The search results of these clinical trial databases provide insight into the current research investigating the habenula as a DBS target. Four duplicates were excluded and the records revealed that one trial did not use Hb DBS. Therefore, five clinical trials (**Table 4**) (NCT03463590, NCT03347487, NCT03254017, NCT01798407, NCT03667872) were identified using Hb DBS for two different indications: TRD (NCT03347487, NCT03254017,

TABLE 4 | Registered clinical trials of habenula DBS.

NCT number	NCT03463590	NCT03347487	NCT03254017	NCT01798407	NCT03667872
Link	https://ClinicalTrials.gov/show/NCT03463590	https://ClinicalTrials.gov/show/NCT03347487	https://ClinicalTrials.gov/show/NCT03254017	https://ClinicalTrials.gov/show/NCT01798407	https://ClinicalTrials.gov/show/NCT03667872
Title	Deep Brain Stimulation of the Bilateral Habenula for Treatment-Refractory Obsessive-Compulsive Disorder	DBS of the Habenula for Treatment- Resistant Major Depression	Remotely Programmed Deep Brain Stimulation of the Bilateral Habenula for Treatment- Resistant Major Depression: An Open Label Pilot Trial	DBS of the Lateral Habenula in Treatment-Resistant Depression	Efficacy and Safety of DBS in Patients With Treatment-Resistant Depression
Status	Unknown status	Recruiting	Terminated	Active, not recruiting	Not yet recruiting
Condition	OCD	TRD	TRD	TRD	TRD
Intervention	Device: Bilateral surgical implantation of DBS system to habenula	Procedure: Deep brain stimulation system implantation	Procedure: Bilateral surgical implantation of DBS system to Habenula Other: Follow-up Period	Device: Activa Tremor Control Sys (DBS Implant) Other: Randomized, staggered withdrawal phase	Procedure: Bilateral implantation of DBS system to Habenula
Outcome measures	Questionnaires: Y-BOCS II; OCI-R; HAMD; HAMA; WHO-BREF; SF-36 Neuropsychologic: Cogstate battery Imaging: fMRI	Questionnaires: HAMD; MADRS; YMRS; HAMA; GAF; C-SSRS; WHO-BREF; SF-36; BDI; PSQI; Q-LES-Q-SF; SDS Neuropsychologic: CANTAB tasks Others: Brain activity; Side Effects	Questionnaires: MADRS; HAMD; HAMA; SF-36; WHO-BREF; YMRS; PSQI Neuropsychologic: Cogstate battery	Questionnaires: MADRS; CGI-S; HAMD; CGI-I; YMRS; C-SSRS; QIDS-SR; GAD-7; SDS; PRISE Neuropsychological Battery	HAMA
Sponsor/ Collaborators	Ruijin Hospital	Ruijin Hospital	Ruijin Hospital	Wayne Goodman MD Baylor College of Medicine	Beijing Pins Medical Co., Ltd
Gender	All	All	All	All	All
Age	18 to 65 Years (Adult, Older Adult)	18 to 65 Years (Adult, Older Adult)	18 to 65 Years (Adult, Older Adult)	21 to 70 Years (Adult, Older Adult)	18 to 70 Years (Adult, Older Adult)
Phases	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
Enrollment	6	13	2	6	6
Funded By	Other	Other	Other	Other	Industry
Study type	Interventional	Interventional	Interventional	Interventional	Interventional
Study designs	Allocation: N/D Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Allocation: N/D Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Allocation: N/D Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment	Allocation: Non-Randomized Intervention Model: Sequential Assignment Masking: Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor) Primary Purpose: Treatment	Allocation: N/D Intervention Model: Single Group Assignment Masking: None (Open Label) Primary Purpose: Treatment
Other study ID numbers	Habenula DBS for OCD	2018 DBS-Hb MDD	Habenula DBS	H40307 49593 277909 HSM#12-00467 GCO 12-1815	PINS-029
Start Date	March 1, 2018	November 13, 2017	July 24, 2017	February 2013	January 15, 2019
Primary completion date	February 28, 2020	July 30, 2020	November 30, 2018	August 2021	June 18, 2020
Completion date	February 28, 2020	September 30, 2020	August 30, 2019	August 2021	June 18, 2022
First posted	March 13, 2018	November 20, 2017	August 18, 2017	February 25, 2013	September 12, 2018
Results first posted	March 29, 2018	January 10, 2020	November 29, 2019	November 12, 2020	December 27, 2018
Location	Shanghai Ruijin Hospital Functional Neurosurgery, Shanghai, Shanghai, China	Shanghai Ruijin Hospital Functional Neurosurgery, Shanghai, Shanghai, China	Shanghai Ruijin Hospital Functional Neurosurgery, Shanghai, Shanghai, China	Baylor College of Medicine, Houston, Texas, United States	Shenzhen, Shenzhen, China

(Continued)

TABLE 4 | Continued

NCT number	NCT03463590	NCT03347487	NCT03254017	NCT01798407	NCT03667872
Investigator	N/D	N/D	N/D	Wayne Goodman MD	N/D
Responsible party	Bomin Sun, MD, PhD	Bomin Sun, MD, PhD	Bomin Sun, MD, PhD	Wayne Goodman MD	Beijing Pins Medical Co., Ltd

BDI, Beck Depression Inventory; CGI-I, Clinical Global Impression of Improvement; CGI-S, Clinical Global Impression of Severity; C-SSRS, Columbia Suicide Severity Rating Scale; DBS, Deep Brain Stimulation; GAD-7, Generalized Anxiety Disorder 7-item Scale; GAF, Global Assessment of Functioning Scale; Hb, Habenula; HAMA, Hamilton Anxiety Scale; HAMD, Hamilton Depression Scale; MADRS, Montgomery-Asberg Depression Rating Scale; N/D, not described; SF-36, MDD, Major Depressive Disorder; MOS item short from health survey; OCD, Obsessive Compulsive Disorder; PRISE, Patient Rated Inventory of Side Effects; PSQI, Pittsburgh Sleep Quality Index; QIDS-SR, Quick Inventory of Depressive Symptomatology; Q-LES-Q-SF, Quality of Life Enjoyment and Satisfaction Questionnaire-Short Form; SDS, Sheehan Disability Scale; TRD, treatment resistant major depressive disorder; WHO-BREF, World Health Organization Quality of Life-BREF; YMRS, Young Mania Rating Scale.

NCT01798407, NCT03667872) and OCD (NCT03463590). One trial (NCT01798407) is a non-randomized trial using quadruple masking, the other four are open label trials. Two of the publications identified in the literature search are associated with two of the clinical trials found: the study of Wang and colleagues (66) with NCT03667872 and the work of Zhang and colleagues (65) with NCT03254017. One trial is currently recruiting, one has been terminated, two are not yet recruiting and the status of the last is unknown. Bilateral Hb DBS is planned in all five trials and planned enrollment ranges from 2 to 13 patients, or 6 to 13 in the trials not terminated. China is the country of origin of four, the United states of one trial. All trials plan to recruit adults (from 18 or 21 years to 65 or 70 years of age) of both sexes.

DISCUSSION

This literature review highlights the encouraging preliminary outcomes of Hb DBS for a variety of treatment-resistant psychiatric conditions (schizophrenia, BD, OCD, and TRD) and tentatively suggests it could be a viable therapeutic option for these conditions in the future. However, more comprehensive cases series and sham-controlled clinical trials with larger enrollment are necessary to confirm the effectiveness of Hb DBS. The review of the clinical trial databases revealed that there are a number of such trials currently underway. These larger trials will allow for a better understanding of the patient characteristics associated with greater Hb DBS benefits. For example, the authors of the Hb DBS trial for schizophrenia speculate that decreased symptom severity and shorter disease duration might play a role in determining treatment response (63). As the Hb is involved in the major neurotransmitter systems, which are the target of the most common pharmacotherapies used in these patient populations (i.e., antidepressants and antipsychotics), detailed reporting of the medication regime of patients (both before and repeatedly during Hb DBS treatment) would provide valuable information to elucidate the treatment mechanism of action. Different medication regimes as well as differences in lead localization might play a role in the great variance of DBS stimulation parameters observed in the reported studies: stimulation voltage between 1.35 and 10.5 V and frequency between 60 and 160 Hz were reported. It remains unclear if high- or low-frequency stimulation is most beneficial. For example, while Zhang and colleagues (65) report a marked improvement when switching from high- to low- frequency stimulation, Wang and colleagues (66) report good clinical outcome over 3 months with high-frequency stimulation.

Beyond the need for more additional clinical evidence, there are several challenges that should be overcome if Hb DBS is to become a useful therapeutic tool. First, greater insight into the mechanism of action of this therapy is required. This is especially important given the variety of different rationales and potential mechanisms that have been proposed for Hb DBS (**Table 3**). Further work with preclinical models will be needed to robustly test these proposals in the context of the various neuropsychiatric diseases. Second, given the critical relationship between the precise location and nature of the electric field and clinical response to DBS (78), knowledge of how best to target this relatively small structure and subsequently select the optimal stimulation parameters is needed. This is particularly relevant for psychiatric DBS indications given that established clinical programming algorithms of the sort employed for movement disorder patients rely largely on immediate and objective clinical feedback following parameter adjustment (e.g., improved tremor or rigidity in PD patients) (79, 80). These strategies may not be suitable for Hb DBS, where authors such as Sartorius and colleagues have reported a delay of 4 months between initiating DBS treatment and observing antidepressant effect (47). Indeed, two of the five studies report that determining the optimal stimulation parameters was time-consuming [taking 9 months for Zhang et al. (65); 8 months for Wang et al. (63)]. As such, Hb DBS–like other types of psychiatric DBS (81)–will likely benefit greatly from the identification of robust electrophysiological or neuroimaging biomarkers of efficacious stimulation. Furthermore, while most preclinical work supporting the potential use of Hb DBS in neuropsychiatric disorders has specifically focused on the function and connections of the LHb, the small size and close proximity of LHb and medial Hb make it difficult to precisely target (or visualize) only LHb in humans. Future clinical studies should therefore report DBS targeting and electrical field modeling with the utmost precision in order to evaluate the relationship between efficacy and the precise locus of stimulation within the Hb.

An array of experimental techniques may be useful for elucidating the mechanism of action and optimal treatment parameters for Hb DBS. Animal model and *in vitro* research using microelectrode recording, microdialysis, and optogenetic approaches have previously uncovered important insights into the neuronal and synaptic mechanisms of DBS for movement disorders and could be similarly applied here. Prior preclinical microelectrode recording work, for example, has shown that high frequency subthalamic stimulation leads to decreased neuronal activity in interconnected deep motor nuclei (82, 83) and suppressed local activity within the target structure (84). Microdialysis studies, which permit measurement of local neurotransmitter levels, indicate that high frequency stimulation is also accompanied by increased extracellular levels of GABA (85, 86). Optogenetic work, meanwhile, has provided further insight into the action of DBS at the ion channel level (87–89) and allowed investigations of the precise neuronal circuits underlying DBS responses (90, 91). These research modalities can be complemented by functional and molecular neuroimaging techniques such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional magnetic resonance imaging (fMRI), which can be readily performed in patients and which capture information simultaneously from across the brain, thereby explicating the network-level effects of DBS (92–95). Indeed, recent fMRI studies have characterized brain-wide fingerprints of optimal subthalamic DBS for PD with respect to stimulation parameters such as contact, voltage, and frequency (96, 97). To this end, Zhang and colleagues used fMRI at various settings compared to a healthy control group (65) while Wang and colleagues conducted LFP recordings paired with a systematic assessment of the effects of stimulation (66).

Going forward, studies using these varying techniques will hopefully further elucidate the role of the Hb in psychiatric disease, advancing understanding of how best to modulate this structure and guiding optimization of DBS targeting and stimulation parameters. Ultimately, successful randomized, clinical trials with appropriate enrollment levels will be necessary to firmly establish the clinical benefit and optimal application of Hb DBS. Nevertheless, the reported studies (47, 63–66), albeit only reporting on a small number of patients, demonstrate that Hb DBS has potential for a multitude of clinical indications.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JG, MM, and AML: design of the work. JG, MM, and AT: acquisition of data. JG and MM: analysis of data and drafting the manuscript. JG, MM, GE, AL, FG, AB, and AML: interpretation of data. JG, MM, GE, AL, AT, FG, AB, and AML: critical revision of the manuscript. All authors approved the final version of the manuscript.

REFERENCES

1. Lozano AM, Lipsman N. Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron.* (2013) 77:406–24. doi: 10.1016/j.neuron.2013.01.020
2. Fox MD. Mapping symptoms to brain networks with the human connectome. *N Engl J Med.* (2018) 379:2237–45. doi: 10.1056/NEJMra1706158
3. Lozano AM, Lipsman N, Bergman H, Brown P, Chabardes S, Chang JW, et al. Deep brain stimulation: current challenges and future directions. *Nat Rev Neurol.* (2019) 15:148–60. doi: 10.1038/s41582-018-0128-2
4. Bonelli RM, Cummings JL. Frontal-subcortical circuitry and behavior. *Dialogues Clin Neurosci.* (2007) 9:141–51. doi: 10.31887/DCNS.2007.9.2/rbonelli
5. Hammond C, Ammari R, Bioulac B, Garcia L. Latest view on the mechanism of action of deep brain stimulation. *Mov Disord.* (2008) 23:2111–21. doi: 10.1002/mds.22120
6. Koeglsperger T, Palleis C, Hell F, Mehrkens JH, Bötzel K. Deep brain stimulation programming for movement disorders: current concepts and evidence-Based strategies. *Front Neurol.* (2019) 10:410. doi: 10.3389/fneur.2019.00410
7. Hauptman JS, DeSalles AAF, Espinoza R, Sedrak M, Ishida W. Potential surgical targets for deep brain stimulation in treatment-resistant depression. *Neurosurg Focus.* (2008) 25:E3. doi: 10.3171/FOC/2008/25/7/E3
8. Lee D, Dallapiazza R, De Vloo P, Lozano A. Current surgical treatments for parkinson's disease and potential therapeutic targets. *Neural Regeneration Res.* (2018) 13:1342. doi: 10.4103/1673-5374.235220
9. Honey CR, Hamani C, Kalia SK, Sankar T, Picillo M, Munhoz RP, et al. Deep brain stimulation target selection for parkinson's disease. *Can J Neurol Sci.* (2017) 44:3–8. doi: 10.1017/cjn.2016.22
10. Sullivan CRP, Olsen S, Widge AS. Deep brain stimulation for psychiatric disorders: from focal brain targets to cognitive networks. *Neuroimage.* (2021) 225:117515. doi: 10.1016/j.neuroimage.2020.117515
11. Holtzheimer PE, Mayberg HS. Deep brain stimulation for psychiatric disorders. *Annu Rev Neurosci.* (2011) 34:289–307. doi: 10.1146/annurev-neuro-061010-113638
12. Lee DJ, Lozano CS, Dallapiazza RF, Lozano AM. Current and future directions of deep brain stimulation for neurological and psychiatric disorders. *J Neurosurg.* (2019) 131:333–42. doi: 10.3171/2019.4.JNS181761
13. Sartorius A, Henn FA. Deep brain stimulation of the lateral habenula in treatment resistant major depression. *Med Hypotheses.* (2007) 69:1305–8. doi: 10.1016/j.mehy.2007.03.021
14. Mai JK, Majtanik M, Paxinos G. *Atlas of the Human Brain*. San Diego: Academic Press (2015).
15. Germann J, Gouveia FV, Martinez RCR, Zanetti MV, de Souza Duran FL, Chaim-Avancini TM, et al. Fully automated habenula segmentation provides robust and reliable volume estimation across large magnetic resonance imaging datasets, suggesting intriguing developmental trajectories in psychiatric disease. *Biol Psychiatry Cogn Neurosci Neuroimaging.* (2020) 5:923–9. doi: 10.1016/j.bpsc.2020.01.004
16. Fonov VS, Evans AC, McKinstry RC, Almli CR, Collins DL. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *Neuroimage.* (2009) 47:S102. doi: 10.1016/S1053-8119(09)70884-5
17. Hikosaka O. The habenula: from stress evasion to value-based decision-making. *Nat Rev Neurosci.* (2010) 11:503–13. doi: 10.1038/nrn2866

18. Hu H, Cui Y, Yang Y. Circuits and functions of the lateral habenula in health and in disease. *Nat Rev Neurosci.* (2020) 21:277–95. doi: 10.1038/s41583-020-0292-4
19. Mizumori SJY, Baker PM. The lateral habenula and adaptive behaviors. *Trends Neurosci.* (2017) 40:481–93. doi: 10.1016/j.tins.2017.06.001
20. Ely BA, Stern ER, Kim J-W, Gabbay V, Xu J. Detailed mapping of human habenula resting-state functional connectivity. *Neuroimage.* (2019) 200:621–34. doi: 10.1016/j.neuroimage.2019.06.015
21. Neudorfer C, Germann J, Elias GJB, Gramer R, Boutet A, Lozano AM. A high-resolution *in vivo* magnetic resonance imaging atlas of the human hypothalamic region. *Sci Data.* (2020) 7:305. doi: 10.1038/s41597-020-00644-6
22. Torrisi S, Nord CL, Balderston NL, Roiser JP, Grillon C, Ernst M. Resting state connectivity of the human habenula at ultra-high field. *Neuroimage.* (2017) 147:872–9. doi: 10.1016/j.neuroimage.2016.10.034
23. Aizawa H, Yanagihara S, Kobayashi M, Niisato K, Takekawa T, Harukuni R, et al. The synchronous activity of lateral habenular neurons is essential for regulating hippocampal theta oscillation. *J Neurosci.* (2013) 33:8909–21. doi: 10.1523/JNEUROSCI.4369-12.2013
24. Damborská A, Honzírková E, Barteček R, Horínková J, Fedorová S, Ondruš Š, et al. Altered directed functional connectivity of the right amygdala in depression: high-density eEG study. *Sci Rep.* (2020) 10:4398. doi: 10.1038/s41598-020-61264-z
25. Herkenham M, Nauta WJ. Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. *J Comp Neurol.* (1977) 173:123–46. doi: 10.1002/cne.901730107
26. Kim U, Lee T. Topography of descending projections from anterior insular and medial prefrontal regions to the lateral habenula of the epithalamus in the rat. *Eur J Neurosci.* (2012) 35:1253–69. doi: 10.1111/j.1460-9568.2012.08030.x
27. Herkenham M, Nauta WJ. Efferent connections of the habenular nuclei in the rat. *J Comp Neurol.* (1979) 187:19–47. doi: 10.1002/cne.901870103
28. Namboodiri VMK, Rodriguez-Romaguera J, Stuber GD. The habenula. *Curr Biol.* (2016) 26:R873–7. doi: 10.1016/j.cub.2016.08.051
29. Jhou TC, Geisler S, Marinelli M, Degarmo BA, Zahm DS. The mesopontine rostromedial tegmental nucleus: a structure targeted by the lateral habenula that projects to the ventral tegmental area of tsai and substantia nigra compacta. *J Comp Neurol.* (2009) 513:566–96. doi: 10.1002/cne.21891
30. Wang R, Aghajanian G. Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science.* (1977) 197:89–91. doi: 10.1126/science.194312
31. Stephenson-Jones M, Floros O, Robertson B, Grillner S. Evolutionary conservation of the habenular nuclei and their circuitry controlling the dopamine and 5-hydroxytryptophan (5-HT) systems. *Proc Natl Acad Sci USA.* (2012) 109:E164–73. doi: 10.1073/pnas.1119348109
32. Purvis EM, Klein AK, Ettenberg A. Lateral habenular norepinephrine contributes to states of arousal and anxiety in male rats. *Behav Brain Res.* (2018) 347:108–15. doi: 10.1016/j.bbr.2018.03.012
33. Fakhoury M. The habenula in psychiatric disorders: more than three decades of translational investigation. *Neurosci Biobehav Rev.* (2017) 83:721–35. doi: 10.1016/j.neubiorev.2017.02.010
34. McLaughlin I, Dani JA, De Biasi M. The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation. *J Neurochem.* (2017) 142 Suppl 2:130–43. doi: 10.1111/jnc.14008
35. Lipina TV, Khrapova MV, Serykh A, Dubrovina NI, Petrova ES, Mikhnevich N, et al. The increased density of the habenular neurons, high impulsivity, aggression and resistant fear memory in disc1-Q31l genetic mouse model of depression. *Behav Brain Res.* (2020) 392:112693. doi: 10.1016/j.bbr.2020.112693
36. Matsumoto M, Hikosaka O. Representation of negative motivational value in the primate lateral habenula. *Nat Neurosci.* (2009) 12:77–84. doi: 10.1038/nn.2233
37. Lecca S, Meye FJ, Trusel M, Tchenio A, Harris J, Schwarz MK, et al. Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior. *eLife.* (2017) 6:e30697. doi: 10.7554/eLife.30697
38. Lecca S, Namboodiri VMK, Restivo L, Gervasi N, Pillolla G, Stuber GD, et al. Heterogeneous habenular neuronal ensembles during selection of defensive behaviors. *Cell Rep.* (2020) 31:107752. doi: 10.1016/j.celrep.2020.107752
39. Nuno-Perez A, Trusel M, Lalive AL, Congiu M, Gastaldo D, Tchenio A, et al. Stress undermines reward-guided cognitive performance through synaptic depression in the lateral habenula. *Neuron.* (2021) 109:947–56.e5. doi: 10.1016/j.neuron.2021.01.008
40. Lecca S, Pelosi A, Tchenio A, Moutkine I, Lujan R, Hervé D, et al. Rescue of GABAB and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nat Med.* (2016) 22:254–61. doi: 10.1038/nm.4037
41. Li B, Piriz J, Mirrione M, Chung C, Proulx CD, Schulz D, et al. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature.* (2011) 470:535–9. doi: 10.1038/nature09742
42. Li K, Zhou T, Liao L, Yang Z, Wong C, Henn F, et al. βCaMKII in lateral habenula mediates core symptoms of depression. *Science.* (2013) 341:1016–20. doi: 10.1126/science.1240729
43. Baker PM, Jhou T, Li B, Matsumoto M, Mizumori SJY, Stephenson-Jones M, et al. The lateral habenula circuitry: reward processing and cognitive control. *J Neurosci.* (2016) 36:11482–88. doi: 10.1523/JNEUROSCI.2350-16.2016
44. Benekareddy M, Stachniak TJ, Bruns A, Knoflach F, von Kienlin M, Künnecke B, et al. Identification of a corticohabenular circuit regulating socially directed behavior. *Biol Psychiatry.* (2018) 83:607–17. doi: 10.1016/j.biopsych.2017.10.032
45. Boulos LJ, Darcq E, Kieffer BL. Translating the habenula—from rodents to humans. *Biol Psychiatry.* (2017) 81:296–305. doi: 10.1016/j.biopsych.2016.06.003
46. van Kerkhof LWM, Damsteegt R, Trezza V, Voorn P, Vanderschuren LJMJ. Functional integrity of the habenula is necessary for social play behaviour in rats. *Eur J Neurosci.* (2013) 38:3465–75. doi: 10.1111/ejn.12353
47. Sartorius A, Kiening KL, Kirsch P, von Gall CC, Haberkorn U, Unterberg AW, et al. Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biol Psychiatry.* (2010) 67:e9–11. doi: 10.1016/j.biopsych.2009.08.027
48. Bianciardi M, Strong C, Toschi N, Edlow BL, Fischl B, Brown EN, et al. A probabilistic template of human mesopontine tegmental nuclei from *in vivo* 7 t MRI. *Neuroimage.* (2018) 170:222–30. doi: 10.1016/j.neuroimage.2017.04.070
49. Horn A, Li N, Dembek TA, Kappel A, Boulay C, Ewert S, et al. Lead-DBS v2: towards a comprehensive pipeline for deep brain stimulation imaging. *Neuroimage.* (2019) 184:293–316. doi: 10.1016/j.neuroimage.2018.08.068
50. Tchenio A, Lecca S, Valentinova K, Mameli M. Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. *Nat Commun.* (2017) 8:1135. doi: 10.1038/s41467-017-01192-1
51. Friedman A, Lax E, Dikshtein Y, Abraham L, Flaumenhaft Y, Sudai E, et al. Electrical stimulation of the lateral habenula produces enduring inhibitory effect on cocaine seeking behavior. *Neuropharmacology.* (2010) 59:452–9. doi: 10.1016/j.neuropharm.2010.06.008
52. Friedman A, Lax E, Dikshtein Y, Abraham L, Flaumenhaft Y, Sudai E, et al. Electrical stimulation of the lateral habenula produces an inhibitory effect on sucrose self-administration. *Neuropharmacology.* (2011) 60:381–7. doi: 10.1016/j.neuropharm.2010.10.006
53. Lecca S, Meye FJ, Mameli M. The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview. *Eur J Neurosci.* (2014) 39:1170–8. doi: 10.1111/ejn.12480
54. Yadid G, Gispan I, Lax E. Lateral habenula deep brain stimulation for personalized treatment of drug addiction. *Front Hum Neurosci.* (2013) 7:806. doi: 10.3389/fnhum.2013.00806
55. Stopper CM, Floresco SB. Dopaminergic circuitry and risk/reward decision making: implications for schizophrenia. *Schizophr Bull.* (2015) 41:9–14. doi: 10.1093/schbul/sbu165
56. Shepard PD, Holcomb HH, Gold JM. Schizophrenia in translation: the presence of absence: habenular regulation of dopamine neurons and the encoding of negative outcomes. *Schizophr Bull.* (2006) 32:417–21. doi: 10.1093/schbul/sbj083

57. Loonen AJM, Kupka RW, Ivanova SA. Circuits regulating pleasure and happiness in bipolar disorder. *Front Neural Circuits.* (2017) 11:35. doi: 10.3389/fncir.2017.00035
58. Savitz JB, Nugent AC, Bogers W, Roiser JP, Bain EE, Neumeister A, et al. Habenula volume in bipolar disorder and major depressive disorder: a high-resolution magnetic resonance imaging study. *Biol Psychiatry.* (2011) 69:336–43. doi: 10.1016/j.biopsych.2010.09.027
59. Schafer M, Kim J-W, Joseph J, Xu J, Frangou S, Doucet GE. Imaging habenula volume in schizophrenia and bipolar disorder. *Front Psychiatry.* (2018) 9:456. doi: 10.3389/fpsyt.2018.00456
60. Loonen AJM, Ivanova SA. Consider role of glutamatergic habenula-projecting globus pallidus in OCD. *Pharmacopsychiatry.* (2019) 52:203–4. doi: 10.1055/a-0835-6447
61. Stein DJ, Costa DLC, Lochner C, Miguel EC, Reddy YCJ, Shavitt RG, et al. Obsessive-compulsive disorder. *Nat Rev Dis Primers.* (2019) 5:52. doi: 10.1038/s41572-019-0102-3
62. Germann J, Gouveia FV, Brentani H, Bedford SA, Tullo S, Chakravarty MM, et al. Involvement of the habenula in the pathophysiology of autism spectrum disorder. *medRxiv.* (2021) doi: 10.1101/2021.04.08.21255122
63. Wang Y, Zhang C, Zhang Y, Gong H, Li J, Jin H, et al. Habenula deep brain stimulation for intractable schizophrenia: a pilot study. *Neurosurg Focus.* (2020) 49:E9. doi: 10.3171/2020.4.FOCUS20174
64. Zhang C, Zhang Y, Li D, Deng Z, Nuttin B, Voon V, et al. Habenular stimulation for neurosurgery resistant obsessive-Compulsive disorder: a Case report. *Front Psychiatry.* (2020) 11:29. doi: 10.3389/fpsyt.2020.00029
65. Zhang C, Kim S-G, Li D, Zhang Y, Li Y, Husch A, et al. Habenula deep brain stimulation for refractory bipolar disorder. *Brain Stimul.* (2019) 12:1298–300. doi: 10.1016/j.brs.2019.05.010
66. Wang Z, Cai X, Qiu R, Yao C, Tian Y, Gong C, et al. Case report: lateral habenula deep brain stimulation for treatment-Resistant depression. *Front Psychiatry.* (2020) 11:616501. doi: 10.3389/fpsyt.2020.616501
67. Proulx CD, Hikosaka O, Malinow R. Reward processing by the lateral habenula in normal and depressive behaviors. *Nat Neurosci.* (2014) 17:1146–52. doi: 10.1038/nn.3779
68. Lawson RP, Nord CL, Seymour B, Thomas DL, Dolan RJ, Dayan P, et al. Habenula responses during appetitive and aversive conditioning in major depressive disorder. *J Neurol Neurosurg Psychiatry.* (2014) 85:e3. doi: 10.1136/jnnp-2014-308883.31
69. Ambrosi E, Arciniegas DB, Curtis KN, Patriquin MA, Spalletta G, Sani G, et al. Resting-state functional connectivity of the habenula in mood disorder patients with and without suicide-Related behaviors. *J Neuropsychiatry Clin Neurosci.* (2019) 31:49–56. doi: 10.1176/appi.neuropsych.17120351
70. Zhang L, Wang H, Luan S, Yang S, Wang Z, Wang J, et al. Altered volume and functional connectivity of the habenula in schizophrenia. *Front Hum Neurosci.* (2017) 11:636. doi: 10.3389/fnhum.2017.00636
71. Sandyk R. Pineal and habenula calcification in schizophrenia. *Int J Neurosci.* (1992) 67:19–30. doi: 10.3109/00207459208994773
72. Li J, Yang S, Liu X, Han Y, Li Y, Feng J, et al. Hypoactivity of the lateral habenula contributes to negative symptoms and cognitive dysfunction of schizophrenia in rats. *Exp Neurol.* (2019) 318:165–73. doi: 10.1016/j.expneurol.2019.05.005
73. Fineberg NA, Apergis-Schoute AM, Vaghi MM, Banca P, Gillan CM, Voon V, et al. Mapping compulsivity in the dSM-5 obsessive compulsive and related disorders: cognitive domains, neural circuitry, and treatment. *Int J Neuropsychopharmacol.* (2018) 21:42–58. doi: 10.1093/ijnp/pyx088
74. Isobe M, Vaghi M, Fineberg NA, Apergis-Schoute AM, Bullmore ET, Sahakian BJ, et al. Set-shifting-related basal ganglia deformation as a novel familial marker of obsessive–compulsive disorder. *Br J Psychiatry.* (2021) 17: 249–60. doi: 10.1192/bjp.2021.45
75. Goodwin GM. The overlap between anxiety, depression, and obsessive-compulsive disorder. *Dialogues Clin Neurosci.* (2015) 17:249–60. doi: 10.31887/DCNS.2015.17.3/ggoodwin
76. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: salience attribution revisited. *Schizophr Bull.* (2010) 36:472–85. doi: 10.1093/schbul/sbq031
77. Barr LC, Goodman WK, Price LH. The serotonin hypothesis of obsessive compulsive disorder. *Int Clin Psychopharmacol.* (1993) 8(Suppl. 2):79–82. doi: 10.1097/00004850-199311002-00011
78. Elias GJB, Boutet A, Joel SE, Germann J, Gwun D, Neudorfer C, et al. Probabilistic mapping of deep brain stimulation: insights from 15 years of therapy. *Ann Neurol.* (2020) 89: 426–43. doi: 10.1002/ana.25975
79. Rizzone M. Deep brain stimulation of the subthalamic nucleus in parkinson's disease: effects of variation in stimulation parameters. *J Neurol Neurosurg Psychiatry.* (2001) 71:215–9. doi: 10.1136/jnnp.71.2.215
80. Volkmann J, Herzog J, Kopper F, Deuschl G. Introduction to the programming of deep brain stimulators. *Mov Disord.* (2002) 17(Suppl. 3):S181–7. doi: 10.1002/mds.10162
81. Bari AA, Mikell CB, Abosch A, Ben-Haim S, Buchanan RJ, Burton AW, et al. Charting the road forward in psychiatric neurosurgery: proceedings of the 2016 American society for stereotactic and functional neurosurgery workshop on neuromodulation for psychiatric disorders. *J Neurol Neurosurg Psychiatry.* (2018) 89:886–96. doi: 10.1136/jnnp-2017-317082
82. Benazzouz A, Piallat B, Pollak P, Benabid AL. Responses of substantia nigra pars reticulata and globus pallidus complex to high frequency stimulation of the subthalamic nucleus in rats: electrophysiological data. *Neurosci Lett.* (1995) 189:77–80. doi: 10.1016/0304-3940(95)11455-6
83. Benazzouz A, Gao DM, Ni ZG, Piallat B, Bouali-Benazzouz R, Benabid AL. Effect of high-frequency stimulation of the subthalamic nucleus on the neuronal activities of the substantia nigra pars reticulata and ventrolateral nucleus of the thalamus in the rat. *Neuroscience.* (2000) 99:289–95. doi: 10.1016/S0306-4522(00)00199-8
84. Beurrier C, Bioulac B, Audin J, Hammond C. High-frequency stimulation produces a transient blockade of voltage-gated currents in subthalamic neurons. *J Neurophysiol.* (2001) 85:1351–6. doi: 10.1152/jn.2001.85.4.1351
85. Windels F, Bruet N, Poupard A, Feuerstein C, Bertrand A, Savasta M. Influence of the frequency parameter on extracellular glutamate and gamma-aminobutyric acid in substantia nigra and globus pallidus during electrical stimulation of subthalamic nucleus in rats. *J Neurosci Res.* (2003) 72:259–67. doi: 10.1002/jnr.10577
86. Moro E, Esselink RJA, Xie J, Hommel M, Benabid AL, Pollak P. The impact on Parkinson's disease of electrical parameter settings in STN stimulation. *Neurology.* (2002) 59:706–13. doi: 10.1212/WNL.59.5.706
87. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K. Optical deconstruction of parkinsonian neural circuitry. *Science.* (2009) 324:354–9. doi: 10.1126/science.1167093
88. Gunaydin LA, Yizhar O, Berndt A, Sohal VS, Deisseroth K, Hegemann P. Ultrafast optogenetic control. *Nat Neurosci.* (2010) 13:387–92. doi: 10.1038/nn.2495
89. Yu C, Cassar IR, Sambangi J, Grill WM. Frequency-specific optogenetic deep brain stimulation of subthalamic nucleus improves parkinsonian motor behaviors. *J Neurosci.* (2020) 40:4323–34. doi: 10.1523/JNEUROSCI.3071-19.2020
90. Mastro KJ, Zitelli KT, Willard AM, Leblanc KH, Kravitz AV, Gittis AH. Cell-specific pallidal intervention induces long-lasting motor recovery in dopamine-depleted mice. *Nat Neurosci.* (2017) 20:815–23. doi: 10.1038/nn.4559
91. Creed M, Pascoli VJ, Lüscher C. Addiction therapy. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. *Science.* (2015) 347:659–64. doi: 10.1126/science.1260776
92. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. *Nature.* (2001) 412:150–7. doi: 10.1038/35084005
93. Boutet A, Jain M, Gwun D, Rusjan P, Neudorfer C, Elias GJB, et al. "Modulation of CNS functions by deep brain stimulation: insights provided by molecular imaging. In: *PET SPECT in Neurology*. Dierckx RAJO, Ottee A, de Vries EFJ, van waarde A, Leenders KL, editors. Cham: Springer International Publishing (2021). p. 1177–244. doi: 10.1007/978-3-030-53168-3_46
94. Ko JH, Tang CC, Eidelberg D. Brain stimulation and functional imaging with fMRI and PET. *Handb Clin Neurol.* (2013) 116:77–95. doi: 10.1016/B978-0-444-53497-2.00008-5
95. Jech R. Functional imaging of deep brain stimulation: fMRI, SPECT, and PET. In: Tarsy D, Vitek JL, Starr PA, Okun MS, editors. *Deep Brain Stimulation in*

Neurological and Psychiatric Disorders. Totowa, NJ: Humana Press (2008). p. 179–201. doi: 10.1007/978-1-59745-360-8_9

96. Boutet A, Madhavan R, Elias GJB, Joel SE, Gramer R, Ranjan M, et al. Predicting optimal deep brain stimulation parameters for Parkinson's disease using functional mRI and machine learning. *Nat Commun.* (2021) 12:1–13. doi: 10.1038/s41467-021-23311-9
97. DiMarzio M, Madhavan R, Hancu I, Fiveland E, Prusik J, Joel S, et al. Use of functional MRI to assess effects of deep brain stimulation frequency changes on brain activation in Parkinson disease. *Neurosurgery.* (2021) 88:356–65. doi: 10.1093/neuros/nyaa397

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

MINI REVIEW
published: 26 November 2021
doi: 10.3389/fnbeh.2021.786011

Inhibition Within the Lateral Habenula—Implications for Affective Disorders

Jack F. Webster[1], *Salvatore Lecca*[2] *and Christian Wozny*[1,3]*

[1]Strathclyde Institute for Pharmacy and Biomedical Sciences, Strathclyde University, Glasgow, United Kingdom, [2]The Department of Fundamental Neurosciences, The University of Lausanne, Lausanne, Switzerland, [3]MSH Medical School Hamburg, IMM Institute for Molecular Medicine, Medical University, Hamburg, Germany

The lateral habenula (LHb) is a key brain region implicated in the pathology of major depressive disorder (MDD). Specifically, excitatory LHb neurons are known to be hyperactive in MDD, thus resulting in a greater excitatory output mainly to downstream inhibitory neurons in the rostromedial tegmental nucleus. This likely results in suppression of downstream dopaminergic ventral tegmental area neurons, therefore, resulting in an overall reduction in reward signalling. In line with this, increasing evidence implicates aberrant inhibitory signalling onto LHb neurons as a co-causative factor in MDD, likely as a result of disinhibition of excitatory neurons. Consistently, growing evidence now suggests that normalising inhibitory signalling within the LHb may be a potential therapeutic strategy for MDD. Despite these recent advances, however, the exact pharmacological and neural circuit mechanisms which control inhibitory signalling within the LHb are still incompletely understood. Thus, in this review article, we aim to provide an up-to-date summary of the current state of knowledge of the mechanisms by which inhibitory signalling is processed within the LHb, with a view of exploring how this may be targeted as a future therapy for MDD.

Keywords: lateral habenula, major depressive disorder, inhibition, local inhibitory interneurons, inhibitory afferents

OPEN ACCESS

Edited by:
Phillip Michael Baker, Seattle Pacific University, United States

Reviewed by:
Sheri Mizumori, University of Washington, United States
P. Leon Brown, University of Maryland, United States

***Correspondence:**
Christian Wozny
christian.wozny@medicalschool-hamburg.de

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *29 September 2021*
Accepted: *25 October 2021*
Published: *26 November 2021*

Citation:
Webster JF, Lecca S and Wozny C (2021) Inhibition Within the Lateral Habenula—Implications for Affective Disorders. Front. Behav. Neurosci. 15:786011. doi: 10.3389/fnbeh.2021.786011

INTRODUCTION

The lateral habenula (LHb) is a brain structure within the epithalamus which is now well established to have a causative role in the pathogenesis of major depressive disorder (MDD; Sartorius et al., 2010; Li et al., 2011; Yang et al., 2018a; Hu et al., 2020). Long-range excitatory neurons in the LHb project to and exert net inhibitory control over the ventral tegmental area (VTA; Ji and Shepard, 2007; Jhou et al., 2009), and the dorsal raphe nucleus (DRN; Wang and Aghajanian, 1977; Ferraro et al., 1996), thus acting as the "off switch" of the midbrain reward circuitry. From an evolutionary standpoint, this serves the important function of ensuring behaviours with negative outcomes are not reinforced (Hikosaka, 2010). However, this system becomes dysregulated in MDD and the LHb becomes hyperactive (Li et al., 2011; Lecca et al., 2016; Tchenio et al., 2017; Cui et al., 2018; Yang et al., 2018a), thus likely potentiating inhibitory modulation of the downstream reward circuitry and curtailing the associated positive emotions.

In addition to the wealth of information that now exists relating to how the LHb controls its' efferent targets, it is also known that the LHb receives afferent input from a variety of brain regions pivotal in emotional processing. These include the internal segment of the globus pallidus (Hong and Hikosaka, 2008), analogous to the rodent entopeduncular nucleus (Shabel et al., 2014; Meye et al., 2016; Wallace et al., 2017), the lateral hypothalamus (Stamatakis et al., 2016; Lecca et al., 2017; Lazaridis et al., 2019; Trusel et al., 2019) and the ventral pallidum (Knowland et al., 2017; Faget et al., 2018; Stephenson-Jones et al., 2020; Pribiag et al., 2021), as well as receiving reciprocal input from the VTA (Stamatakis et al., 2013; Root et al., 2014). These structures provide a combination of excitatory, inhibitory and GABA/glutamate co-releasing input to the LHb, in a very fine balance which has been shown to be altered in depressive states (Shabel et al., 2014; Knowland et al., 2017). In general, convergent evidence suggests that excitatory LHb afferents promote aversive states and depressive behaviour (Barker et al., 2017; Knowland et al., 2017; Lecca et al., 2017; Lazaridis et al., 2019), while conversely inhibitory afferents promote behavioural reinforcement (Faget et al., 2018; Stephenson-Jones et al., 2020) and reduce depressive behaviour (Winter et al., 2011; Huang et al., 2019). Taking this into account, we here review the current state of knowledge regarding the processing of inhibitory signalling within the LHb. We will discuss what is currently known about inhibitory input to the LHb, how this is altered in depressive states, and how this may eventually be exploited to develop novel therapies for MDD.

INHIBITORY AFFERENTS OF THE LHb

The Globus Pallidus Internal Segment/Entopeduncular Nucleus

The globus pallidus internal segment, or the analogous entopeduncular nucleus (EP) in rodents, is one of the primary afferent structures to the LHb. This structure is thought to be primarily GABAergic in nature (Oertel et al., 1984; Stephenson et al., 2005), although the EP-LHb pathway has a net excitatory effect on postsynaptic LHb neurons (Shabel et al., 2012; Stephenson-Jones et al., 2016). As such LHb-projecting EP neurons are thought to primarily encode aversion (Stephenson-Jones et al., 2016; Li H. et al., 2019). However, a striking feature of these neurons is that they co-release GABA and glutamate at LHb synapses (Shabel et al., 2012, 2014; Wallace et al., 2017; Lazaridis et al., 2019). This co-release phenomenon has been shown to be shifted in favour of reduced GABA in a rodent model of depression, and conversely towards increased GABA following antidepressant treatment (Shabel et al., 2014). Similarly, EP-LHb projections are known to be involved in cocaine withdrawal (Meye et al., 2016) and avoidance (Li H. et al., 2021), with a shift towards reduced GABA observed during cocaine withdrawal (Meye et al., 2016), thus consistent with the hypothesis that enhanced excitatory input to the LHb promotes aversive states. Consistently, potentiation of glutamatergic signalling at the EP to LHb synapse has also been observed in another rodent model of depression, with both an increase in presynaptic glutamate release probability and an increase in postsynaptic AMPA receptor expression thought to be causative mechanisms (Cerniauskas et al., 2019). Hence, the current evidence surrounding the functionality of LHb-projecting EP neurons appears to point to a scenario whereby the balance of glutamate and GABA release is in a fine balance which is shifted in favour of glutamate in aversive states, thus potentiating excitatory drive onto LHb neurons.

The Basal Forebrain

A basal forebrain is a group of heterogenous forebrain structures, composed of GABAergic, glutamatergic and cholinergic neurons (Zaborszky et al., 2012). Within the basal forebrain, the primarily GABAergic (Root et al., 2015) ventral pallidum (VP) is known to be another major LHb afferent. LHb-projecting VP neurons however are known to be distinct populations of both excitatory and inhibitory neurons (Faget et al., 2018; Stephenson-Jones et al., 2020), although recent work has also identified VP neurons that express both markers of GABAergic and glutamatergic neurons, and may co-release GABA and glutamate at LHb synapses (Pribiag et al., 2021). As with the EP, glutamatergic LHb-projecting VP neurons are activated by negative stimuli (Stephenson-Jones et al., 2020) and encode aversion (Faget et al., 2018; Tooley et al., 2018; Stephenson-Jones et al., 2020). Consistently, these neurons become more excitable in a rodent model of depression (Knowland et al., 2017), and ablating these glutamatergic neurons promotes reward-seeking behaviour (Tooley et al., 2018). Conversely, GABAergic LHb-projecting VP neurons are excited by reward-predicting stimuli and promote reward-seeking (Stephenson-Jones et al., 2020). These results seem to indicate that the VP-to-LHb projection exists as somewhat of a push-pull system, whereby inhibitory and excitatory LHb-projecting VP neurons oppositely promote reward and aversion, respectively.

However, while the VP-to-LHb projection is the most well-studied, other basal forebrain regions are believed to project to and modulate LHb activity. One study found that LHb-projecting GABAergic neurons within the basal forebrain as a whole promote aggressive behaviour, which is believed to be rewarding and is thus consistent with the hypothesis that GABAergic LHb afferents promote reward (Golden et al., 2016). Furthermore, another study identified a population of neurons in the portion of the basal forebrain ventral to the VP which projects to the LHb and expresses the GABAergic marker VGAT (Zhu et al., 2017), although the functionality of these neurons specifically at LHb synapses was not elucidated. Another work has identified a GABAergic projection to the LHb from the diagonal band of Broca which has a role in regulating hippocampal theta rhythm (Aizawa et al., 2013). Thus, it is becoming increasingly clear that the basal forebrain appears to be a major source of inhibitory input to the LHb, with various populations of LHb-projecting neurons encoding a variety of behavioural functionalities.

Other Inhibitory Afferents to the LHb

The LHb also receives GABAergic innervation from the VTA (Stamatakis et al., 2013; Root et al., 2014). Similarly to

LHb-projecting neurons within the EP, VTA neurons have intriguingly been shown to co-release GABA and glutamate at LHb synapses (Root et al., 2014). This pathway appears to be capable of bidirectionally controlling LHb activity (Root et al., 2014), possibly indicative of a feedback loop whereby hypo or hyperactivity within the LHb may be limited by reciprocal VTA connectivity. In addition to these co-releasing neurons, a further population of LHb-projecting VTA neurons has also been identified which unusually expresses the classical dopaminergic marker tyrosine hydroxylase, but exclusively releases GABA within the LHb (Stamatakis et al., 2013). While it remains unclear whether or not these neurons are indeed dopaminergic (Lammel et al., 2015), they are consistent with other GABAergic LHb afferents in that they encode reward (Stamatakis et al., 2013).

The lateral preoptic area (LPO) of the hypothalamus also contains distinct populations of excitatory and inhibitory neurons which provide convergent input to individual LHb neurons (Barker et al., 2017). These populations of neurons appear to control the LHb in a manner similar to the VP in that excitatory LPO neurons encode aversion, while inhibitory LPO neurons promote reward. Yet intriguingly, both of these populations respond to aversive stimuli (Barker et al., 2017). Additionally, GABAergic afferents from the lateral hypothalamus have also been identified (Stamatakis et al., 2016; Lecca et al., 2017), although these are fairly minimal in comparison to excitatory drive from this region.

Emerging evidence has also pointed to inhibitory LHb afferents arising from the thalamus. A projection to the LHb arising from the ventral lateral geniculate nucleus and intergeniculate leaflet of the thalamus has recently been shown to be critically involved in the antidepressant effects of light therapy (Huang et al., 2019). We have also recently described a population of neurons within the mediodorsal thalamus nucleus which inhibits the LHb (Webster et al., 2020), although the behavioural influence of this pathway remains to be clarified.

In summary, there is now much evidence indicating that the LHb receives afferent inhibitory innervation from a wide variety of sources (**Figure 1**). However, regardless of the source, there appears to be remarkable consistency within the literature; that is that inhibitory innervation of the LHb encodes reward, while excitatory innervation encodes aversion.

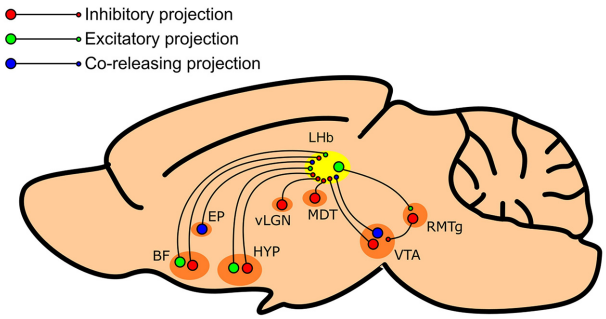

FIGURE 1 | Summary schematic of regions which provide inhibitory input to the LHb. The LHb (shown in yellow) receives inhibitory input from various regions such as the entopeduncular nucleus (EP), the basal forebrain (BF), the lateral preoptic area and lateral hypothalamus of the hypothalamus (HYP), the ventral lateral geniculate nucleus/intergeniculate leaflet complex of the thalamus (vLGN), the mediodorsal thalamus (MDT), and the ventral tegmental area (VTA). These afferent structures may feature both inhibitory (shown in red) and excitatory (shown in green) neuronal populations, primarily inhibitory populations, or GABA/glutamate co-releasing neurons (shown in blue).

LOCALLY-TARGETING INHIBITORY LHb NEURONS

It is well-accepted that the majority of LHb neurons form a physiologically homogenous, but morphologically diverse population (Weiss and Veh, 2011), of which most neurons are glutamatergic (Omelchenko et al., 2009; Brinschwitz et al., 2010). Yet mounting evidence now indicates the existence of locally-targeting inhibitory neurons (Zhang et al., 2018; Flanigan et al., 2020; Webster et al., 2020; Nakamura et al., 2021), which may form multiple distinct sub-classes. One such population express the inhibitory marker glutamic acid decarboxylase 2 (GAD-2) and are confined to the medial LHb (Flanigan et al., 2020). These neurons have been shown to inhibit other LHb neurons to promote aggressive behaviour in mice (Flanigan et al., 2020) which, as mentioned earlier, can be interpreted as a reward-seeking behaviour (Golden et al., 2016). We and others have recently shown that some LHb neurons which express the well-known cortical interneuron marker parvalbumin (Tremblay et al., 2016) are GABAergic and inhibit local LHb neurons (Webster et al., 2020; Nakamura et al., 2021). These neurons are likely distinct from the aforementioned population of GAD-2 expressing neurons in that they are confined to the lateral LHb and also express the vesicular GABA transporter (VGAT; Webster et al., 2020), another well-known marker of inhibitory neurons, but largely do not express GAD-2 (Nakamura et al., 2021). Note however that the expression patterns of GABAergic markers within the LHb appears to differ between mice and rats (Quina et al., 2020), with VGAT expression in rats observed in the medial LHb and GAD-2 expression largely absent (Zhang et al., 2018; Quina et al., 2020). Furthermore, early electrophysiological and morphological studies have proposed that a population of neurons akin to the very distinctive cortical neurogliaform interneuron exists within the LHb in rats (Weiss and Veh, 2011; Wagner et al., 2016), although these neurons appear to be different to their cortical counterparts at least in that they do not express similar molecular markers (Webster et al., 2021).

Interestingly, however, some studies have also shown that LHb neurons which express inhibitory markers also express the excitatory marker vesicular glutamate transporter 2 (VGLUT-2), and project to the midbrain (Zhang et al., 2018; Quina et al., 2020). Furthermore, these neurons appear to release exclusively glutamate in the tegmentum of the midbrain (Quina et al., 2020). Thus it may be the case that within the LHb, inhibitory neurons do not exist exclusively as the well-described populations of locally-targeting interneurons that they are known to form in other brain structures (Tremblay et al., 2016), but rather may be dual-functioning neurons which serve the additional purpose of exciting downstream regions (**Figure 2**).

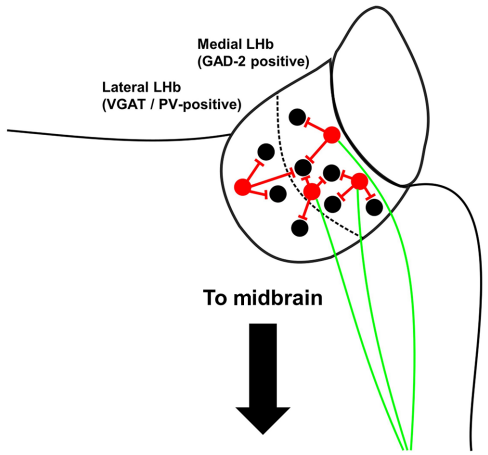

FIGURE 2 | Summary schematic of local inhibitory neurons within the mouse LHb. Locally-targeting inhibitory neurons (shown in red) in the medial LHb express glutamic acid decarboxylase 2 (GAD-2) but not vesicular GABA transporter (VGAT) and may also have excitatory projections to the tegmentum (shown in green), although note that Flanigan et al. (2020) found no evidence that inhibitory GAD-2-positive neurons project outside the LHb. Inhibitory neurons in the lateral LHb express VGAT and parvalbumin, but most do not express GAD-2.

MODULATION OF LHb INHIBITORY SIGNALLING BY OTHER NEUROTRANSMITTERS AND NEUROPEPTIDES

While the majority of work studying the role of the LHb in MDD and other psychiatric disorders has focused on alterations in direct excitatory and inhibitory inputs, there is an emerging body of evidence indicating that various other neurotransmitters and neuropeptides can influence the activity of the LHb, both directly and indirectly. These are discussed below.

Orexin

Interestingly, orexin receptor 2 expression within the LHb appears to be largely confined to GABAergic neurons (Zhang et al., 2018; Flanigan et al., 2020), hence suggesting that orexinergic modulation of the LHb may act *via* GABAergic neurons. At least some of these neurons are the same aforementioned GAD-2 expressing LHb neurons, which have been shown to be activated by orexinergic input from the lateral hypothalamus to promote aggressive behaviour (Flanigan et al., 2020). These neurons also express receptors for vasopressin, serotonin, and dopamine (Zhang et al., 2018), and as such may respond to a variety of transmitters. It is interesting to note that chronic social defeat stress (CSDS) also appears to activate orexinergic inputs to the LHb in socially defeated mice (Wang et al., 2021). Furthermore, direct infusion of orexin into the LHb alleviates the depressive phenotype induced by CSDS (Wang et al., 2021). Thus, current evidence seems to indicate that orexinergic signalling within the LHb is generally associated with social interactions between dominant and subordinate mice, but may be multifunctional; specifically, this can promote aggressive phenotypes in dominant mice, and bidirectionally control depressive behaviour in subordinate mice.

Endocannabinoids

Other recent work has pointed to the role of endocannabinoid signalling in modulating LHb function (Shepard and Nugent, 2021). CB1 receptor activation has been shown to reduce inhibitory synaptic input onto LHb neurons (Authement et al., 2018), while at the behavioural level, intra-LHb infusion of Δ9-tetrahydrocannabinol has been shown to induce a deficit in impulsivity control in rats (Zapata and Lupica, 2021).

Opioid Receptors

Opioid receptor signalling has also recently been shown to modulate neuronal excitability within the LHb (Simmons et al., 2020). Interestingly, Kappa opioid receptor activation bidirectionally excites and inhibits LHb neurons based on the size of the hyperpolarization-activated cation currents the neurons express. This is accompanied by a net decrease in both excitatory and inhibitory synaptic release onto LHb neurons (Simmons et al., 2020). Thus it may be the case that dynorphin/Kappa opioid receptor signalling has opposing effects on differing sub-populations of LHb neurons. However, the origin of preprodynorphin/dynorphin-positive fibres targeting LHb neurons remains unknown (Chen et al., 2020).

Cholinergic Signalling

While the role of cholinergic signalling within the adjacent medial habenula in nicotine withdrawal and drug addiction has been relatively well-studied in recent years (Lee et al., 2019), a specific role for cholinergic signalling within the LHb has also recently started to become apparent (Zapata et al., 2017; Wolfe et al., 2021). In a manner similar to Kappa opioid receptor activation, cholinergic receptor activation appears to differentially excite or inhibit different populations of LHb neurons, while also suppressing both excitatory and inhibitory synaptic inputs; although this effect appears to more strongly suppress excitatory input hence resulting in an overall net shift towards increased inhibition (Wolfe et al., 2021).

Neuropeptide Y

Neuropeptide Y (NPY) has recently been shown to modulate both excitatory and inhibitory synaptic transmission within the LHb (Cheon et al., 2019). Specifically, NPY receptor activation appears capable of bidirectionally modulating excitatory input in different groups of neurons, and has a net reduction of inhibitory input mediated by Y1 receptors (Cheon et al., 2019, 2020).

Serotonin and Dopamine

It has long been known that the LHb acts as a modulator of midbrain dopaminergic and serotonergic systems. However, some evidence indicates that these transmitters can also modulate the activity of the LHb. Serotonin has been shown to reduce both excitatory and inhibitory transmission at the entopeduncular nucleus to LHb synapses (Shabel et al., 2014).

Conversely, dopamine appears to excite a subset of LHb neurons in a D4 receptor-dependent manner, possibly indicative of an excitatory feedback loop connecting the LHb with the ventral tegmental area (Good et al., 2013).

Thus, an emerging image appears to be forming whereby other signalling systems are capable of modulating LHb activity. Although the behavioural relevance of these inputs remains largely unclear, it is interesting to note that many of these transmitters appear capable of both exciting and inhibiting different populations of LHb neurons. Therefore, it may be the case that these transmitters are targeting distinct neuronal sub-populations, and as such future work which differentiates these sub-populations based on the expression of receptors for such transmitters may serve to unveil much novel information regarding how the LHb encodes behaviour.

INHIBITION OF THE LHb AS A POTENTIAL THERAPY FOR DEPRESSION

To date, the majority of work focusing on the LHb as a potential therapeutic target for MDD has aimed to reduce the excitability of LHb neurons (for reviews, see Nuno-Perez et al., 2018; Yang et al., 2018b; Hu et al., 2020). Referring back to the original hypothesis that the LHb is hyperactive in depression (Li et al., 2011), there is now substantial evidence that reducing the excitability of LHb neurons *via* a variety of means has an antidepressant effect. Much work has focussed on normalising LHb activity by the use of deep brain stimulation (DBS), a technique whereby electrodes are implanted within the brain to modulate neuronal activity (Sartorius et al., 2010; Meng et al., 2011; Tchenio et al., 2017; Jakobs et al., 2019). This technique appears to hold great promise as a therapeutic intervention in that LHb-targeted DBS has been shown to induce full remission in a human patient (Sartorius et al., 2010). Interestingly, the antidepressant effect of DBS appears to be dependent on the stimulation frequency, in that high frequency stimulation (>100 Hz) has an antidepressant effect in rodents (Meng et al., 2011; Tchenio et al., 2017; Jakobs et al., 2019), while lower frequencies stimulation (5–20 Hz) appears to promote depressive behaviour (Elmer et al., 2019; Jakobs et al., 2019).

But what are the mechanisms by which high frequency DBS within the LHb elicits an antidepressant effect? Interestingly, in a recent investigation in rodents, Tchenio et al. (2017) showed that firing activity of LHb neurons recorded *in vivo* decreases during local LHb DBS. The authors, in line with a previous study (Li et al., 2011) also demonstrated that such high frequency stimulation dampens AMPA transmission onto LHb cells. It has also been proposed that DBS activates presynaptic GABAergic terminals to promote GABA release (Li et al., 2004), which may be a possible explanation given the extensive GABAergic innervation the LHb receives (Brinschwitz et al., 2010). However, whether DBS modulates GABAergic transmission in the LHb remains to be tested.

Modulating the electrical firing pattern and synaptic activity of LHb neurons *via* various pharmacological interventions has also been shown to have therapeutic benefits. For instance, blockade of NMDA receptors reduce bursting activity, and is at least partially responsible for the antidepressant actions of ketamine (Cui et al., 2018; Yang et al., 2018a), while AMPA receptor blockade has also been demonstrated to have antidepressant efficacy (Li et al., 2017; Zhang et al., 2019). Additionally, both local LHb chemogenetic inhibition (Nair et al., 2013; Tchenio et al., 2017), and direct pharmacological inhibition (Winter et al., 2011) and input-specific synaptic inhibition (Huang et al., 2019) have displayed efficacy as potential therapeutic options in rodent models of depression. The therapeutic benefits of direct inhibition of the LHb can be linked to both $GABA_A$and $GABA_B$ signalling. Direct injection of the $GABA_A$ agonist muscimol has been shown to alleviate depressive symptoms in both the classical learned helplessness rat model of depression (Winter et al., 2011) and interestingly in depression induced by a rat model of Parkinson's disease (Wang et al., 2017). Moreover, foot shock exposure in mice triggers a diminished functionality in $GABA_B$ transmission and has been observed in various rodent models of depression, such as acute learned helplessness (Lecca et al., 2016), maternal separation (Tchenio et al., 2017), and chronic social defeat (Li Z.-L. et al., 2021). Altogether the latter studies highlight the $GABA_B$ receptor in the LHb as a potential target to treat depressive symptoms. Mechanistically, a stressful event (i.e., foot shock exposure) in mice triggers internalisation of both $GABA_B$ receptors and G protein-gated inwardly rectifying potassium (GIRK) channels, along with a concurrent increase in protein phosphatase 2A activity, which is known to regulate the expression of these channels. Consistently, pharmacological inhibition of protein phosphatase 2A normalises $GABA_B$and GIRK expression and alleviates depressive symptoms (Lecca et al., 2016). It is also notable that glycinergic signalling within the LHb has been linked to depressive behaviour, in that direct injection of glycine has an anxiolytic effect in a rat model of alcohol withdrawal, while intra-LHb injection of the glycinergic antagonist strychnine is anxiogenic (Li W. et al., 2019).

CONCLUDING REMARKS

In summary, as the mechanisms by which inhibitory signalling within the LHb are processed become clearer, so too do the potential strategies by which this can be exploited as a therapeutic strategy in MDD. The consistency between pre- and postsynaptic modulation of LHb neurons in relation to depressive behaviour is striking- inhibitory afferents promote reward-like states, while excitatory afferents promote aversion, and consistently activation of postsynaptic inhibitory GABA and glycine receptors appears to have an antidepressant effect, as does blockade of excitatory AMPA and NMDA receptors. As such, novel therapies for MDD may attempt to capitalise on this knowledge by aiming to selectively modulate such pathways.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by an EPSRC Doctoral Prize to JFW, a NARSAD Young Investigator Award to either SL (Grant number 27089) or CW (Grant number 28217, named P&S Fund Investigator) and by the European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No. ICT-36-2020-101016787, DEEPER to CW.

REFERENCES

Aizawa, H., Yanagihara, S., Kobayashi, M., Niisato, K., Takekawa, T., Harukuni, R., et al. (2013). The synchronous activity of lateral habenular neurons is essential for regulating hippocampal theta oscillation. *J. Neurosci.* 33, 8909–8921. doi: 10.1523/JNEUROSCI.4369-12.2013

Authement, M. E., Langlois, L. D., Shepard, R. D., Browne, C. A., Lucki, I., Kassis, H., et al. (2018). A role for corticotropin-releasing factor signaling in the lateral habenula and its modulation by early-life stress. *Sci. Signal.* 11:eaan6480. doi: 10.1126/scisignal.aan6480

Barker, D. J., Miranda-Barrientos, J., Zhang, S., Root, D. H., Wang, H.-L., Liu, B., et al. (2017). Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission. *Cell Rep.* 21, 1757–1769. doi: 10.1016/j.celrep.2017.10.066

Brinschwitz, K., Dittgen, A., Madai, V. I., Lommel, R., Geisler, S., and Veh, R. W. (2010). Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain. *Neuroscience* 168, 463–476. doi: 10.1016/j.neuroscience.2010.03.050

Cerniauskas, I., Winterer, J., Jong, J. W. D., Lukacsovich, D., Yang, H., Khan, F., et al. (2019). Chronic stress induces activity, synaptic and transcriptional remodeling of the lateral habenula associated with deficits in motivated behaviors. *Neuron* 104, 899–915.e8. doi: 10.1016/j.neuron.2019.09.005

Chen, C., Willhouse, A. H., Huang, P., Ko, N., Wang, Y., Xu, B., et al. (2020). Characterization of a knock-in mouse line expressing a fusion protein of k opioid receptor conjugated with tdtomato: 3-dimensional brain imaging via clarity. *eNeuro* 7:ENEURO.0028-20.2020. doi: 10.1523/ENEURO.0028-20.2020

Cheon, M., Park, H., and Chung, C. H. (2020). Protein kinase C mediates neuropeptide Y-induced reduction in inhibitory neurotransmission in the lateral habenula. *Neuropharmacology* 180:108295. doi: 10.1016/j.neuropharm.2020.108295

Cheon, M., Park, H., Rhim, H., and Chung, C. H. (2019). Actions of neuropeptide Y on synaptic transmission in the lateral habenula. *Neuroscience* 410, 183–190. doi: 10.1016/j.neuroscience.2019.04.053

Cui, Y., Yang, Y., Ni, Z., Dong, Y., Cai, G., Foncelle, A., et al. (2018). Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression. *Nature* 554, 323–327. doi: 10.1038/nature25752

Elmer, G. I., Palacorolla, H., Mayo, C. L., Brown, P. L., Jhou, T. C., Brady, D., et al. (2019). The rostromedial tegmental nucleus modulates the development of stress-induced helpless behavior. *Behav. Brain Res.* 359, 950–957. doi: 10.1016/j.bbr.2018.06.014

Faget, L., Zell, V., Souter, E., McPherson, A., Ressler, R., Gutierrez-reed, N., et al. (2018). Opponent control of behavioral reinforcement by inhibitory and excitatory projections from the ventral pallidum. *Nat. Commun.* 9:849. doi: 10.1038/s41467-018-03125-y

Ferraro, G., Montalbano, M. E., Sardo, P., and La Grutta, V. (1996). Lateral habenular influence on dorsal raphe neurons. *Brain Res. Bull.* 41, 47–52. doi: 10.1016/0361-9230(96)00170-0

Flanigan, M. E., Aleyasin, H., Li, L., Burnett, C. J., Chan, K. L., LeClair, K. B., et al. (2020). Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice. *Nat. Neurosci.* 23, 638–650. doi: 10.1038/s41593-020-0617-7

Golden, S. A., Heshmati, M., Flanigan, M. E., Christoffel, D. J., Guise, K., Pfau, M. L., et al. (2016). Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature* 534, 688–692. doi: 10.1038/nature18601

Good, C. H., Wang, H., Chen, Y. H., Mejias-Aponte, C. A., Hoffman, A. F., and Lupica, C. R. (2013). Dopamine D4 receptor excitation of lateral habenula neurons *via* multiple cellular mechanisms. *J. Neurosci.* 33, 16853–16864. doi: 10.1523/JNEUROSCI.1844-13.2013

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.* 11, 503–513. doi: 10.1038/nrn2866

Hong, S., and Hikosaka, O. (2008). The globus pallidus sends reward-related signals to the lateral habenula. *Neuron* 60, 720–729. doi: 10.1016/j.neuron.2008.09.035

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Huang, L., Xi, Y., Peng, Y., Yang, Y., Huang, X., Fu, Y., et al. (2019). A visual circuit related to habenula underlies the antidepressive effects of light therapy. *Neuron* 102, 128–142.e8. doi: 10.1016/j.neuron.2019.01.037

Jakobs, M., Pitzer, C., Sartorius, A., Unterberg, A., and Kiening, K. (2019). Acute 5 Hz deep brain stimulation of the lateral habenula is associated with depressive-like behavior in male wild-type Wistar rats. *Brain Res.* 1721:146283. doi: 10.1016/j.brainres.2019.06.002

Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B., and Holland, P. C. (2009). The rostromedial tegmental nucleus (RMTg), a major GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron* 61, 786–800. doi: 10.1016/j.neuron.2009.02.001

Ji, H., and Shepard, P. D. (2007). Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *J. Neurosci.* 27, 6923–6930. doi: 10.1523/JNEUROSCI.0958-07.2007

Knowland, D., Lilascharoen, V., Pham Pacia, C., Shin, S., Hou-Jen Wang, E., Kook Lim, B., et al. (2017). Distinct ventral pallidal neural populations mediate separate symptoms of depression. *Cell* 170, 284–297.e18. doi: 10.1016/j.cell.2017.06.015

Lammel, S., Steinberg, E. E., Földy, C., Wall, N. R., Beier, K., Luo, L., et al. (2015). Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. *Neuron* 85, 429–438. doi: 10.1016/j.neuron.2014.12.036

Lazaridis, I., Tzortzi, O., Weglage, M., Märtin, A., Xuan, Y., Parent, M., et al. (2019). A hypothalamus-habenula circuit controls aversion. *Mol. Psychiatry* 24, 1351–1368. doi: 10.1038/s41380-019-0369-5

Lecca, S., Meye, F. J., Trusel, M., Tchenio, A., Harris, J., Schwarz, M. K., et al. (2017). Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior. *eLife* 6:e30697. doi: 10.7554/eLife.30697

Lecca, S., Pelosi, A., Tchenio, A., Moutkine, I., Lujan, R., Hervé, D., et al. (2016). Rescue of GABAB and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nat. Med.* 22, 254–261. doi: 10.1038/nm.4037

Lee, H. W., Yang, S. H., Kim, J. Y., and Kim, H. (2019). The role of the medial habenula cholinergic system in addiction and emotion-associated behaviors. *Front. Psychiatry* 10:100. doi: 10.3389/fpsyt.2019.00100

Li, H., Eid, M., Pullmann, D., Chao, Y. S., Thomas, A. A., and Jhou, T. C. (2021). Entopeduncular nucleus projections to the lateral habenula contribute to cocaine avoidance. *J. Neurosci.* 14, 298–306. doi: 10.1523/JNEUROSCI.0708-20.2020

Li, J., Kang, S., Fu, R., Wu, L., Wu, W., Liu, H., et al. (2017). Inhibition of AMPA receptor and CaMKII activity in the lateral habenula reduces depressive-like behavior and alcohol intake in rats. *Neuropharmacology* 126, 108–120. doi: 10.1016/j.neuropharm.2017.08.035

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C. D., Schulz, D., et al. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* 470, 535–539. doi: 10.1038/nature09742

Li, H., Pullmann, D., and Jhou, T. C. (2019). Valence-encoding in the lateral habenula arises from the entopeduncular region. *eLife* 8:e41223. doi: 10.7554/eLife.41223

Li, T., Qadri, F., and Moser, A. (2004). Neuronal electrical high frequency stimulation modulates presynaptic GABAergic physiology. *Neurosci. Lett.* 371, 117–121. doi: 10.1016/j.neulet.2004.08.050

Li, Z.-L., Wang, Y., Zou, H.-W., Jing, X.-Y., Liu, Y.-J., and Li, L.-F. (2021). GABA(B) receptors within the lateral habenula modulate stress resilience and

vulnerability in mice. *Physiol. Behav.* 230: 113311. doi: 10.1016/j.physbeh.2021.113311

Li, W., Zuo, W., Wu, W., Zuo, Q. K., Fu, R., Wu, L., et al. (2019). Activation of glycine receptors in the lateral habenula rescues anxiety- and depression-like behaviors associated with alcohol withdrawal and reduces alcohol intake in rats. *Neuropharmacology* 157:107688. doi: 10.1016/j.neuropharm.2019.107688

Meng, H., Wang, Y., Huang, M., Lin, W., Wang, S., and Zhang, B. (2011). Chronic deep brain stimulation of the lateral habenula nucleus in a rat model of depression. *Brain Res.* 1422, 32–38. doi: 10.1016/j.brainres.2011.08.041

Meye, F. J., Soiza-Reilly, M., Smit, T., Diana, M. A., Schwarz, M. K., and Mameli, M. (2016). Shifted pallidal co-release of GABA and glutamate in habenula drives cocaine withdrawal and relapse. *Nat. Neurosci.* 19, 1019–1024. doi: 10.1038/nn.4334

Nair, S. G., Strand, N. S., and Neumaier, J. F. (2013). DREADDing the lateral habenula: A review of methodological approaches for studying lateral habenula function. *Brain Res.* 1511, 93–101. doi: 10.1016/j.brainres.2012.10.011

Nakamura, T., Kurosaki, K., Kanemoto, M., Sasahara, M., and Ichijo, H. (2021). Early-life experiences altered the maturation of the lateral habenula in mouse models, resulting in behavioural disorders in adulthood. *J. Psychiatry Neurosci.* 46, E480–E489. doi: 10.1503/jpn.200226

Nuno-Perez, A., Tchenio, A., Mameli, M., and Lecca, S. (2018). Lateral habenula gone awry in depression: bridging cellular adaptations with therapeutics. *Front. Neurosci.* 12:485. doi: 10.3389/fnins.2018.00485

Oertel, W. H., Nitsch, C., and Mugnaini, E. (1984). Immunocytochemical demonstration of the GABA-ergic neurons in rat globus pallidus and nucleus entopeduncularis and their GABA-ergic innervation. *Adv. Neurol.* 40, 91–98.

Omelchenko, N., Bell, R., and Sesack, S. R. (2009). Lateral habenula projections to the rat ventral tegmental area: sparse synapses observed onto dopamine and GABA neurons. *Eur. J. Neurosci.* 30, 1239–1250. doi: 10.1111/j.1460-9568.2009.06924.x

Pribiag, H., Shin, S., Wang, E. H. J., Sun, F., Datta, P., Okamoto, A., et al. (2021). Ventral pallidum DRD3 potentiates a pallido-habenular circuit driving accumbal dopamine release and cocaine seeking. *Neuron* 109, 2165–2182.e10. doi: 10.1016/j.neuron.2021.05.002

Quina, L. A., Walker, A., Morton, G., Han, V., and Turner, E. E. (2020). GAD2-expression defines a class of excitatory lateral habenula neurons in mice that project to the raphe and pontine tegmentum. *eNeuro* 7:ENEURO.0527–19.2020. doi: 10.1523/ENEURO.0527-19.2020

Root, D. H., Mejias-Aponte, C. A., Zhang, S., Wang, H. L., Hoffman, A. F., Lupica, C. R., et al. (2014). Single rodent mesohabenular axons release glutamate and GABA. *Nat. Neurosci.* 17, 1543–1551. doi: 10.1038/nn.3823

Root, D. H., Melendez, R. I., Zaborszky, L., and Napier, T. C. (2015). The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors. *Prog. Neurobiol.* 130, 29–70. doi: 10.1016/j.pneurobio.2015.03.005

Sartorius, A., Kiening, K. L., Kirsch, P., von Gall, C. C., Haberkorn, U., Unterberg, A. W., et al. (2010). Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biol. Psychiatry* 67, e9–e11. doi: 10.1016/j.biopsych.2009.08.027

Shabel, S. J., Proulx, C. D., Piriz, J., and Manilow, R. (2014). Mood regulation. GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment. *Science* 345, 1494–1498. doi: 10.1126/science.1250469

Shabel, S. J., Proulx, C. D., Trias, A., Murphy, R. T., and Malinow, R. (2012). Input to the lateral habenula from the basal ganglia is excitatory, aversive and suppressed by serotonin. *Neuron* 74, 475–481. doi: 10.1016/j.neuron.2012.02.037

Shepard, R. D., and Nugent, F. S. (2021). Targeting endocannabinoid signaling in the lateral habenula as an intervention to prevent mental illnesses following early life stress: a perspective. *Front. Synaptic Neurosci.* 13:689518. doi: 10.3389/fnsyn.2021.689518

Simmons, S. C., Shepard, R. D., Gouty, S., Langlois, L. D., Flerlage, W. J., Cox, B. M., et al. (2020). Early life stress dysregulates kappa opioid receptor signaling within the lateral habenula. *Neurobiol. Stress* 13:100267. doi: 10.1016/j.ynstr.2020.100267

Stamatakis, A. M., Jennings, J. H., Ung, R. L., Blair, G. A., Weinberg, R. J., Neve, R. L., et al. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* 80, 1039–1053. doi: 10.1016/j.neuron.2013.08.023

Stamatakis, A. M., Van Swieten, M., Basiri, M. L., Blair, G. A., Kantak, P., and Stuber, G. D. (2016). Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward. *J. Neurosci.* 36, 302–311. doi: 10.1523/JNEUROSCI.1202-15.2016

Stephenson, D. T., Li, Q., Simmons, C., Connell, M. A., Meglasson, M. D., Merchant, K., et al. (2005). Expression of GAD65 and GAD67 immunoreactivity in MPTP-treated monkeys with or without L-DOPA administration. *Neurobiol. Dis.* 20, 347–359. doi: 10.1016/j.nbd.2005.03.016

Stephenson-Jones, M., Bravo-Rivera, C., Ahrens, S., Furlan, A., Xiao, X., Fernandes-Henriques, C., et al. (2020). Opposing contributions of GABAergic and glutamatergic ventral pallidal neurons to motivational behaviors. *Neuron* 105, 921–933.e5. doi: 10.1016/j.neuron.2019.12.006

Stephenson-Jones, M., Yu, K., Ahrens, S., Tucciarone, J. M., van Huijstee, A. N., Mejia, L. A., et al. (2016). A basal ganglia circuit for evaluating action outcomes. *Nature* 539, 289–293. doi: 10.1038/nature19845

Tchenio, A., Lecca, S., Valentinova, K., and Mameli, M. (2017). Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. *Nat. Commun.* 8:1135. doi: 10.1038/s41467-017-01192-1

Tooley, J., Marconi, L., Alipio, J. B., Matikainen-Ankney, B., Georgiou, P., Kravitz, A. V., et al. (2018). Glutamatergic ventral pallidal neurons modulate activity of the habenula-tegmental circuitry and constrain reward seeking. *Biol. Psychiatry* 83, 1012–1023. doi: 10.1016/j.biopsych.2018.01.003

Tremblay, R., Lee, S., and Rudy, B. (2016). GABAergic interneurons in the neocortex: from cellular properties to circuits. *Neuron* 91, 260–292. doi: 10.1016/j.neuron.2016.06.033

Trusel, M., Nuno-perez, A., Lecca, S., Harada, H., Lalive, A., Congui, M., et al. (2019). Punishment-predictive cues guide avoidance through potentiation of hypothalamus-to-habenula synapses. *Neuron* 102, 120–127.e4. doi: 10.1016/j.neuron.2019.01.025

Wagner, F., Bernard, R., Derst, C., French, L., and Veh, R. W. (2016). Microainvolvementrray analysis of transcripts with elevated expressions in the rat medial or lateral habenula suggest fast GABAergic excitation in the medial habenula and habenular in the regulation of feeding and energy balance. *Brain Struct. Funct.* 221, 4463–4489. doi: 10.1007/s00429-016-1195-z

Wallace, M. L., Saunders, A., Huang, K. W., Philson, A. C., Goldman, M., Macosko, E. Z., et al. (2017). Genetically distinct parallel pathways in the entopeduncular nucleus for limbic and sensorimotor output of the basal ganglia. *Neuron* 94, 138–152.e5. doi: 10.1016/j.neuron.2017.03.017

Wang, R. Y., and Aghajanian, G. K. (1977). Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science* 197, 89–91. doi: 10.1126/science.194312

Wang, D., Li, A., Dong, K., Li, H., Guo, Y., Zhang, X., et al. (2021). Lateral hypothalamus orexinergic inputs to lateral habenula modulate maladaptation after social defeat stress. *Neurobiol. Stress* 14:100298. doi: 10.1016/j.ynstr.2021.100298

Wang, T., Zhang, L., Zhang, Q. J., Wang, Y., Du, C.-X., Sun, Y.-N., et al. (2017). Involvement of lateral habenula α1 subunit-containing GABAA receptor-mediated inhibitory transmission in the regulation of depression-related behaviors in experimental Parkinson's disease. *Neuropharmacology* 116, 399–411. doi: 10.1016/j.neuropharm.2017.01.015

Webster, J. F., Vroman, R., Balueva, K., Wulff, P., Sakata, S., and Wozny, C. (2020). Disentangling neuronal inhibition and inhibitory pathways in the lateral habenula. *Sci. Rep.* 10:8490. doi: 10.1038/s41598-020-65349-7

Webster, J. F., Vroman, R., Beerens, S., Sakata, S., and Wozny, C. (2021). NDNF is selectively expressed by neocortical, but not habenular neurogliaform cells. *Eur. J. Neurosci.* 53, 3561–3575. doi: 10.1111/ejn.15237

Weiss, T., and Veh, R. W. (2011). Morphological and electrophysiological characteristics of neurons within identified subnuclei of the lateral habenula in rat brain slices. *Neuroscience* 172, 74–93. doi: 10.1016/j.neuroscience.2010.10.047

Winter, C., Vollmayr, B., Djodari-Irani, A., Klein, J., and Sartorius, A. (2011). Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. *Behav. Brain Res.* 216, 463–465. doi: 10.1016/j.bbr.2010.07.034

Wolfe, C. I. C., Hwang, E.-K., Zapata, A., Hoffman, A. F., and Lupica, C. R. (2021). Lateral habenula M2 muscarinic receptor control of neuronal activity and cocaine seeking behavior. *bioRxiv* [Preprint]. doi: 10.1101/2021.07.24.453572

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., et al. (2018a). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature* 554, 317–322. doi: 10.1038/nature25509

Yang, Y., Wang, H., Hu, J., and Hu, H. (2018b). Lateral habenula in the pathophysiology of depression. *Curr. Opin. Neurobiol.* 48, 90–96. doi: 10.1016/j.conb.2017.10.024

Zaborszky, L., van den Pol, A. N., and Gyengesi, E. (2012). "The basal forebrain cholinergic projection system in mice," in *The Mouse Nervous System*, eds C. Watson, G. Paxinos, and L. Puelles (USA: Academic Press Elsevier), 684–717. doi: 10.1016/B978-0-12-369497-3.10028-7

Zapata, A., Hwang, E. K., and Lupica, C. R. (2017). Lateral habenula involvement in impulsive cocaine seeking. *Neuropsychopharmacology* 42, 1103–1112. doi: 10.1038/npp.2016.286

Zapata, A., and Lupica, C. R. (2021). Lateral habenula cannabinoid CB1 receptor involvement in drug-associated impulsive behavior. *Neuropharmacology* 192:108604. doi: 10.1016/j.neuropharm.2021.108604

Zhang, L., Hernández, V. S., Swinny, J. D., Verma, A. K., Giesecke, T., Emery, A. C., et al. (2018). A GABAergic cell type in the lateral habenula links hypothalamic homeostatic and midbrain motivation circuits with sex steroid signaling. *Transl. Psychiatry* 8: 50. doi: 10.1038/s41398-018-0099-5

Zhang, J., Wang, Y., Sun, Y. N., Li, L. B., Zhang, L., Guo, Y., et al. (2019). Blockade of calcium-permeable AMPA receptors in the lateral habenula produces increased antidepressant-like effects in unilateral 6-hydroxydopamine-lesioned rats compared to sham-lesioned rats. *Neuropharmacology* 157:107687. doi: 10.1016/j.neuropharm.2019.107687

Zhu, C., Yao, Y., Xiong, Y., Cheng, M., Chen, J., Zhao, R., et al. (2017). Somatostatin neurons in the basal forebrain promote high-calorie food intake. *Cell Rep.* 20, 112–123. doi: 10.1016/j.celrep.2017.06.007

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Behavioral Neuroscience

SYSTEMATIC REVIEW
published: 28 January 2022
doi: 10.3389/fnbeh.2021.815700

Circadian Influences on the Habenula and Their Potential Contribution to Neuropsychiatric Disorders

*Callum J. Young, David Lyons and Hugh D. Piggins**

School of Physiology, Pharmacology, and Neuroscience, University of Bristol, Bristol, United Kingdom

The neural circadian system consists of the master circadian clock in the hypothalamic suprachiasmatic nuclei (SCN) communicating time of day cues to the rest of the body including other brain areas that also rhythmically express circadian clock genes. Over the past 16 years, evidence has emerged to indicate that the habenula of the epithalamus is a candidate extra-SCN circadian oscillator. When isolated from the SCN, the habenula sustains rhythms in clock gene expression and neuronal activity, with the lateral habenula expressing more robust rhythms than the adjacent medial habenula. The lateral habenula is responsive to putative SCN output factors as well as light information conveyed to the perihabenula area. Neuronal activity in the lateral habenula is altered in depression and intriguingly disruptions in circadian rhythms can elevate risk of developing mental health disorders including depression. In this review, we will principally focus on how circadian and light signals affect the lateral habenula and evaluate the possibility that alteration in these influences contribute to mental health disorders.

Keywords: circadian, depression, clock gene, burst firing, epithalamus

OPEN ACCESS

Edited by:
Jurgen Germann, University Health Network (UHN), Canada

Reviewed by:
Timothy Brown, The University of Manchester, United Kingdom
Ilia N. Karatsoreos, University of Massachusetts Amherst, United States

****Correspondence:***
Hugh D. Piggins
hugh.piggins@bristol.ac.uk

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *15 November 2021*
Accepted: *27 December 2021*
Published: *28 January 2022*

Citation:
Young CJ, Lyons D and Piggins HD (2022) Circadian Influences on the Habenula and Their Potential Contribution to Neuropsychiatric Disorders. Front. Behav. Neurosci. 15:815700. doi: 10.3389/fnbeh.2021.815700

INTRODUCTION

It takes a mere cursory glance at social media to deduce that the mental-health conversation is as critical today as it has ever been. We live in a world where the public feel that mental illness remains an unsolved dilemma. They express dissatisfaction with their acute and long-term suffering, as well as the pharmacotherapies available to alleviate this (Priest et al., 1996; Hergerl et al., 2003; Partridge et al., 2012; Horowitz and Graf, 2019). Exemplifying this discontent, depression is a mood disorder characterised by persistent low mood and anhedonia (Tolles-Correia et al., 2018). This mental illness is the leading cause of disability globally (Charlson et al., 2019) and affects over 264 million people (GBD 2017 Disease Injury Incidence Prevalence Collaborators, 2018). Despite this, a universally effective treatment for depression is unavailable, and approximately one third of patients fail to respond to conventional antidepressant drugs (Corriger and Pickering, 2019). Moreover, there is an absence of consensus on how to define depression (Tolles-Correia et al., 2018), making its diagnosis and treatment one of the greatest challenges in modern psychiatry.

As such, a surge of neuroscience research continues to dissect the pathophysiology that underpins depression. At the turn of the previous decade, the brain's epithalamus was identified as a potential new therapeutic target for treatment resistant depression. Specifically, deep brain stimulation (DBS) of the lateral habenula (LHb) was shown to alleviate depressive symptoms in a therapy-refractive patient (Sartorius et al., 2010). Further, when DBS was discontinued, there was an immediate and profound relapse of these depressive symptoms. This highlights the potential importance of the epithalamus in mood regulation (Shabel et al., 2014).

The LHb is an evolutionarily conserved epithalamic structure (Bianco and Wilson, 2009; Hikosaka, 2010), most investigated for its role as an "anti-reward" centre (Shabel et al., 2012) with anatomical connections allowing it to exert inhibitory control over midbrain monoaminergic centres (Hu et al., 2020). In part, it is these network connections which make the LHb an interesting candidate for depression research (Sartorius and Henn, 2007). Recently, rodent studies have further implicated the epithalamus in depression (Hu et al., 2020). More specifically, elevated neural activity in the rodent LHb has been associated with various depression models including learned helplessness (Li et al., 2011; Cui et al., 2018), chronic stress (Cerniauskas et al., 2019) and chronic pain (Zhuo et al., 2019). A landmark paper has also implicated the LHb in the antidepressant mechanism of ketamine (Yang et al., 2018a), although this utilised supra-clinical doses. In addition, human fMRI studies show differing LHb activity, and functional connectivity, between depressed and healthy individuals (Lawson et al., 2017; Zhu et al., 2019; Rivas-Garajales et al., 2021). From this evidence, it is implied that the LHb is an epicentre for depression's mechanistic underpinnings.

In addition to this, the LHb is situated within an extended neural circadian circuit (Bano-Otalora and Piggins, 2017). Immunohistochemical evidence indicates that the rodent LHb receives regulation from the master circadian pacemaker in the suprachiasmatic nucleus (SCN; Hastings et al., 2018). Further, the LHb receives input from other hypothalamic circadian oscillators (Guilding and Piggins, 2007; Guilding et al., 2009; Poller et al., 2013; Stamatkis et al., 2016) and the retina (Qu et al., 1996). Indeed, rodent LHb neurones show diurnal variation in their electrophysiological properties and are responsive to retinal illumination (Zhao and Rusak, 2005; Sakhi et al., 2014b).

Interestingly, circadian disruptions are commonly reported in patients suffering from depression (Jagannath et al., 2013; Difrancesco et al., 2019). Moreover, the efficacy of certain antidepressant therapies has been hypothesised to be time of day dependent (Swanson et al., 2017). Given its endogenous circadian properties, and neural connections with brain structures associated with circadian timing and mood, the LHb is a likely candidate as a locus of interaction between depression and circadian disruptions. In this review, we evaluate the current state of understanding regarding the LHb's anatomical and molecular organisation, and its functional involvement in the pathology of depression. We also consider the intrinsic and extrinsic factors shaping the daily variation in this structure's properties, and explore the potential links between circadian rhythms, depression, and the LHb.

ORGANISATION OF THE HABENULA COMPLEX

The habenula is a cellularly diverse structure located in the posterior-dorsal-medial region of the thalamus (Hu et al., 2020) and can be divided into anatomically distinct lateral and medial nuclei [the LHb and the medial habenula (MHb); Diaz et al., 2011]. The LHb and MHb differ in their afferent and efferent connections (Aizawa et al., 2011, 2012) and play largely different roles in their respective neural circuits. Unlike the predominantly glutamatergic LHb, the MHb contains substance P expressing neurones and cholinergic cells in its dorsal and ventral division, respectively (Aizawa et al., 2012). Although both structures are becoming increasingly implicated in the pathophysiology of various psychiatric conditions (Metzger et al., 2021), the nature of these differ. The MHb is predominantly implicated in nicotine withdrawal (Fowler et al., 2011; Hsu et al., 2013), drug addiction (Glick et al., 2006; Lopez et al., 2018) and anxiety (Zhang et al., 2016), while functional studies suggest that aberrant LHb activity is more closely associated with depression (Li et al., 2013; Seo et al., 2018; Yang et al., 2018b). However, recent research has highlighted an increasingly complex role for the LHb in various reward pathologies, including drug seeking (Li et al., 2017; Nair et al., 2021).

The LHb shows considerable intranuclear heterogeneity in morphology and cytochemistry (Diaz et al., 2011; Hu et al., 2020). As such, it can be subdivided into medial and lateral divisions (LHbM and LHbL; Aizawa et al., 2012) and further apportioned into nine subnuclei based on immunocytochemical, topographical, and morphological criteria (**Figure 1**; Wagner et al., 2014). Subsequent transcriptomic profiling suggests though that LHb cell types are heterogeneous and do not map closely with these subnuclear boundaries (Wagner et al., 2016). Instead, topographically and transcriptionally distinct cell clusters have been identified as a more useful approach to divide the LHb (Hashikawa et al., 2020). Not only does this approach provide a more accurate reflection of cell type-distribution, but these transcriptionally distinct neurones are differentially recruited by aversive stimuli (Cerniauskas et al., 2019), illustrating a heterogeneous response to stress within the LHb.

As well as defining LHb cell types according to their transcriptome, neurones can be distinguished by their mode of firing (Weiss and Veh, 2011; Sakhi et al., 2014b). Three modes of firing have been identified in the LHb: silent, tonic firing, and burst firing (Yang et al., 2018a). Another study distinguished between depolarised and hyperpolarised silent states and described an additional bistable state in which neurones oscillate between firing and quiescence (Sakhi et al., 2014b). Cells exhibiting different firing modes have different resting membrane potentials (RMP), with bursting neurones more hyperpolarised than their tonic, silent, and bistable counterparts. Firing mode is not dependent on cellular morphology or subnuclear location, but is acutely sensitive to perturbations in membrane potential (Kim and Chang, 2005; Weiss and Veh, 2011). Indeed, LHb neurones show extensive firing mode plasticity, with depolarising stimuli driving a shift in firing mode from burst to tonic firing (Cui et al., 2018), and injections of negative current causing rebound bursts (Kim and Chang, 2005; Yang et al., 2018a).

The necessity for hyperpolarisation in burst firing neurones is explained by two principal bursting mechanisms in the LHb (Yang et al., 2018a). The first—requiring the most pronounced hyperpolarisation—is cell autonomous and driven by sequential recruitment of intrinsic ionic conductances. These are hyperpolarisation-activated cyclic nucleotide–gated channels and T-type calcium channels (TTCCs), which are activated

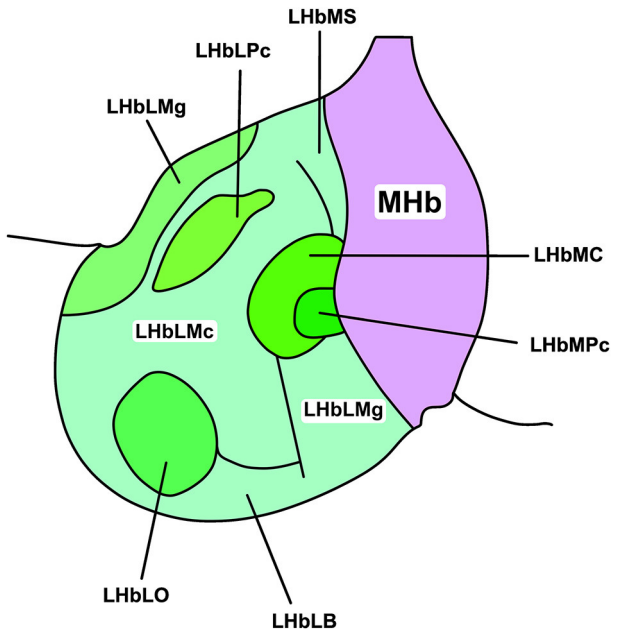

FIGURE 1 | Subnuclear organisation of the rat LHb. The habenular complex can be divided into lateral and medial subdivisions (LHb and MHb, respectively). The LHb can be further divided into medial and lateral regions (LHbL and LHbM, respectively), and thus into nine cytochemically, topographically, and morphologically distinct subnuclei. These include, in the LHbL: the marginal subnucleus of the LHbL (LHbLMg), the magnocellular subnucleus of the LHbL (LHbLMc), the oval subnucleus of the LHbL (LHbLO), the basal subnucleus of the LHbL (LHbLB), and the parvocellular subnucleus of the LHbL (LHbLPc). In the LHbM: the superior subnucleus of the LHbM (LHbMS), the central subnucleus of the LHbM (LHbMC), the parvocellular subnucleus of the LHbM (LHbMPc), and the marginal subnucleus of the LHbM (LHbMMg). Redrawn and adapted from Wagner et al. (2014).

and de-inactivated by membrane hyperpolarisation, respectively. The second occurs within a specific membrane potential range (−55 to −65 mV), and is network dependent, utilising the interplay between NMDA receptors and TTCCs. Understanding the electrophysiological mechanisms underpinning burst firing is critical to elucidating the function of this activity profile (**Figure 2**).

As well as these firing modes, LHb function is characterised by its status as a highly interconnected relay structure mediating communication between the limbic forebrain and midbrain (**Figure 3**; Herkenham and Nauta, 1977, 1979; Sutherland, 1982). Most simplistically, the LHb recieves input from the hypothalamus and basal forebrain, integrates these signals, and exerts inhibitory control on monoaminergic centres in the midbrain. In reality, the nature of this connectivity is more complex, and is subject to extensive regulation. Potentially, the most influential factors driving daily variation in the LHb arises from its afferent connectivity with the SCN (Buijs, 1978; Zhang et al., 2009; Bano-Otalora and Piggins, 2017), the paraventricular nuclei (PVN; Hernandez et al., 2015), the dorsomedial hypothalamus (DMH; Ter Horst and Luiten, 1986; Morin, 2013), the lateral hypothalamic area (LH; Poller et al., 2013; Stamatkis et al., 2016) and the retina (Qu et al., 1996). Moreover, reciprocal connections with the Globus pallidus internus (GPi; Entopenduncular nucleus/EP in rodents; Shabel et al., 2012), ventral tegmental area (VTA; Omelchenko et al., 2009; Cerniauskas et al., 2019), the dorsal and medial raphe (DR, MR; Lima et al., 2017; Szonyi et al., 2019), and the rostromedial tegmental area (RMTg; Laurent et al., 2017; Tooley et al., 2018) are critical for the LHb to exert its role in the aetiology of depression. These connections will be explored more fully throughout this article (for a full review of LHb network connections, please see Hu et al., 2020).

INTRINSIC REGULATION OF LATERAL HABENULA CIRCADIAN ACTIVITY

Expression of the immediate early gene *c-fos* is conventionally used as a proxy for neuronal activity, and immunohistochemical detection of c-Fos protein facilitates assessment of such activity *ex vivo* (He et al., 2019). This tool is useful when determining if neuronal activity shows circadian variation and associating this with an animal's active behavioural state. In the rodent LHbM, c-Fos immunoreactivity (-ir) is positively correlated with activity levels, showing elevation during the behaviourally active night (Paul et al., 2011).

The potential explanations for this observation are myriad. It could mean that LHbM activity responds to behavioural feedback, that increased late day/nocturnal activity in the LHbM is regulated by the same networks which drive these temporal behavioural patterns, or that the LHbM possesses its own intrinsic rhythmicity. In support of the latter of these hypotheses, *ex vivo* electrophysiological studies revealed sustained day-night/circadian variation of neuronal activity in rodent LHb brain slices (Zhao and Rusak, 2005; Sakhi et al., 2014b). Since these LHb explants are isolated from extra-habenular circadian input, this suggests that these 24 h oscillations in LHb neurones are, at least in part, intrinsically generated.

In the SCN, and other circadian oscillators, intrinsic rhythmicity is generated by a "molecular clock." This molecular clock is driven by a series of transcription/translation feedback loops (TTFL), featuring "clock genes" which encode transcription factors controlling the expression of highly diverse gene suites (for full review see Partch et al., 2014). The protein products of four integral clock genes drive the core TTFL: two activators (CLOCK and BMAL1) and two repressors (PER and CRY). Two homologues of *Per (Per1 and Per2)* and *Cry (Cry1 and Cry2)* exist and exhibit different distributions of expression across the mammalian brain (Shieh, 2003; Christiansen et al., 2016), and specifically within the habenula complex (Olejniczak et al., 2021).

Due to the rhythmic pattern of their expression, and their role in generating cell intrinsic rhythmicity, these clock genes can be used to investigate the independence of LHb oscillations. If, in *ex vivo* LHb explants, clock genes continue to show rhythmic expression then it suggests that oscillations in LHb activity undergo intrinsic regulation *via* the molecular clock. Assessments of clock gene expression, measuring bioluminescence from the PER2:Luciferase reporter construct, showed the maintenance of rhythmicity for up to

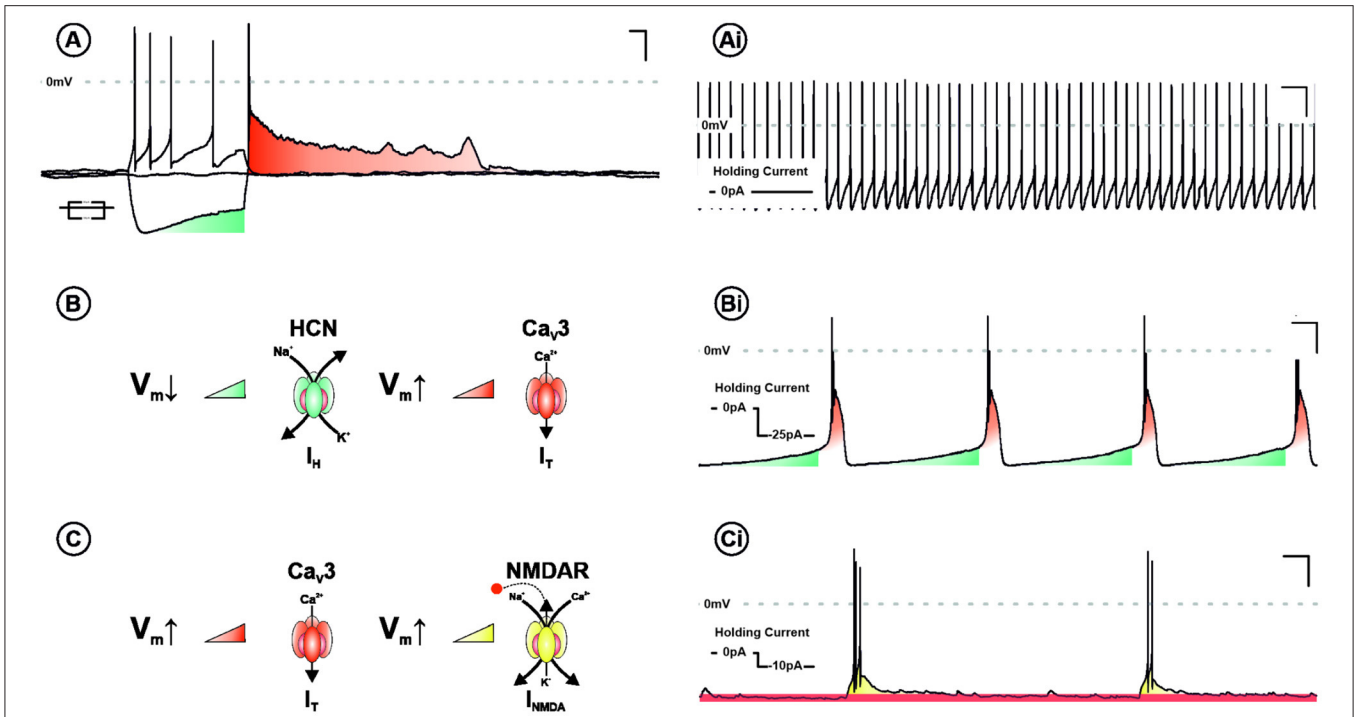

FIGURE 2 | Ionic mechanisms underpinning burst firing in the LHb. **(A)** Voltage response of a LHb neurone to negative and positive square form current injection. Note the depolarising sag (blue) and subsequent rebound excitation (red). In addition to tonic discharge patterns **(Ai)**, LHb neurones are capable of two mechanistically distinct modes of burst firing. Both require membrane hyperpolarisation and are characterised by rhythmic periods of depolarisation, capped by high frequency action potentials. One mechanism, requiring more pronounced depolarisation, is cell autonomous and recurrent **(B)**. This cycle relies on the sequential activation and de-inactivation of hyperpolarisation-activated cyclic nucleotide–gated channels (HCN—blue) and T-type calcium channels (Ca_V3—red), respectively **(Bi)**. The other is network dependent **(C)** and occurs within a specific membrane potential range (−55 to −65 mv). This depends on the interplay between Ca_V3 and NMDA receptors (NMDARs—Yellow). Activation of T-type removes Mg^{2+}-dependent blockade of NMDARs, synergistically driving a burst of action potentials. With membrane potential repolarisation Ca_V3 channels de-inactivate permitting the cycle to begin again **(Ci)**.

48–72 h in the *ex vivo* LHb brain slice (Guilding et al., 2010, 2013), while less robust, low amplitude rhythms are visualised in the adjacent MHb. Rhythms in PER2::LUC were most prominent in the LHbM, with a less distinctive signal detected in the LHbL. Similar to the SCN, the period of PER2::LUC rhythms in the LHb is elongated by the *Afterhours* mutation (Guilding et al., 2013), indicating that the molecular basis of circadian oscillations is conserved between the SCN and LHb. Indeed, *Per1*:Luciferase oscillations in the LHb explant are also observed (Sakhi et al., 2014b) and are lost in LHb slices from mice lacking a functional molecular clock.

There are some inconsistencies in the reporting of habenular clock gene expression, likely due to different approaches and experimental settings. When measured using *in-situ* hybridisation, *Per1* and *Per2* expression was observed to be more prominent in the MHb than the LHb (Olejniczak et al., 2021), while an earlier study suggested that clock gene expression was absent from the LHb (Shieh, 2003). Since Olejiniczak and colleagues combined regional markers in conjunction with real time PCR, when measuring *Per1* and *Per2* expression in the LHb, the most parsimonious conclusion is that expression of clock genes in the LHb is at a low level and may be localised to the LHbM which is adjacent to the lateral border of the MHb.

Daily changes in neurophysiological activity of LHb explants are also abrogated in $Cry1^{-/-}Cry2^{-/-}$ mice (Sakhi et al., 2014b). Since these mice do not have functional molecular clocks, the most likely explanation is that daily functional variation in LHb neuronal activity arises from intrinsic regulation by clock genes. However, it is also possible that other epithalamic/thalamic structures can entrain LHb activity and that it is the absence of rhythmic clock gene expression in these structures that underpins the loss in daily fluctuations in LHb neuronal activity. Interestingly, similar ablations of neural oscillations occur in the MHb of $Cry1^{-/-}Cry2^{-/-}$ mice (Sakhi et al., 2014a). It is therefore possible that functional circadian variations in the LHb are dependent on information flow from the MHb. Such an intrahabenular circadian circuit is evidenced by the existence of a unidirectional projection from the MHb to LHb (Kim and Chang, 2005), but the relevance of this requires further study.

EXTRINSIC REGULATION OF LATERAL HABENULA CIRCADIAN ACTIVITY

As well as manifesting intrinsic regulation, *via* the molecular clock, the LHb sits within a diffuse neural circadian network

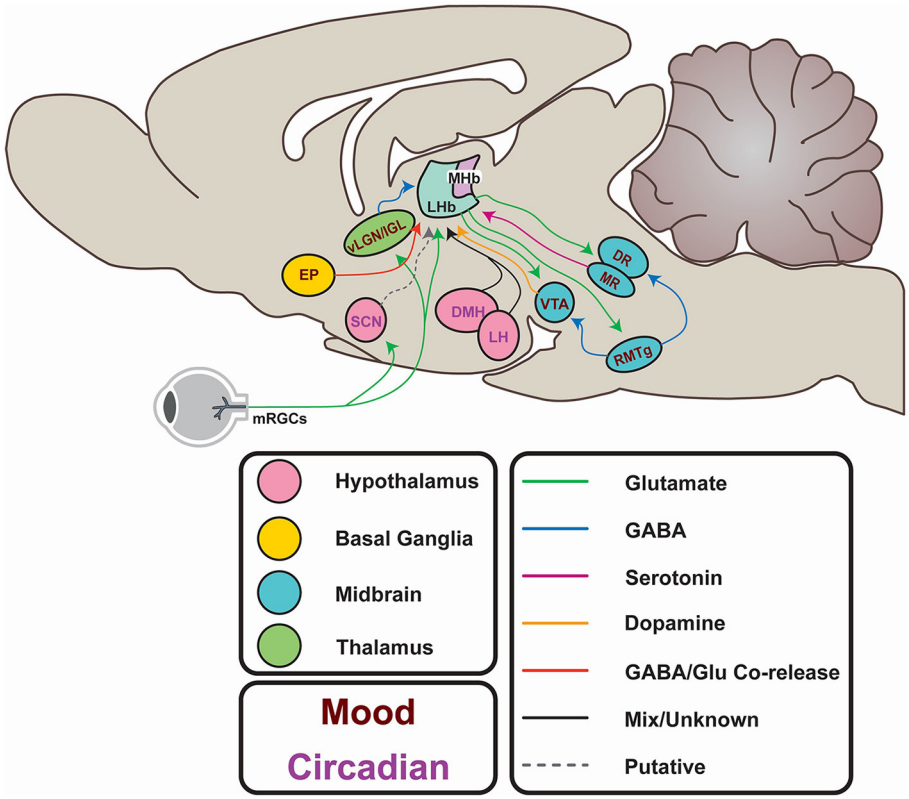

FIGURE 3 | Afferents and efferents of the LHb. From the hypothalamus, the lateral hypothalamic area (LH) and dorsomedial hypothalamus (DMH) potentially entrain the LHb to circadian rhythms. These areas receive input from the suprachiasmatic nucleus (SCN), which in turn may entrain the LHb, either directly (*via* putative Prokineticin 2-containing efferents) or indirectly *via* SCN innervation of the LH and DMH. The SCN itself is entrained to the external light-dark cycle through photic information directly conveyed by non-image forming retinal projections. Similar projections from melanopsin-expressing retinal ganglion cells (mRGCs) terminate in areas immediately adjacent to the LHb. Additionally, the LHb may receive photic information *via* the ventral lateral geniculate or Intergeniculate nucleus (vlGN/IGL). Input from the entopeduncular nucleus (EP) encodes anti-reward, and activity in this pathway is elevated in depression. Excitatory projections to the rostromedial tegmental area (RMTg) provides feedforward inhibition to the ventral tegmental area (VTA) and the raphe nuclei (DR and MR) which regulates mood and reward learning. The VTA provides reciprocal GABAergic projections to the LHb to provide feedback inhibition and encode reward. The DR provides serotonergic projections which inhibit excitatory innervation of the LHb from the EP, and itself generates excitatory postsynaptic currents.

featuring the SCN and various secondary oscillators (Bano-Otalora and Piggins, 2017). A large body of evidence suggests that the LHb receives either direct or indirect regulation from these timekeeping components. The presence of AVP-ir terminals in the LHb provides evidence for a putative projection from the SCN, as this neuropeptide is a highly expressed output molecule for the master clock (Buijs, 1978; Cagampang et al., 1994). Similarly, prokineticin 2 (PK2) projections from the SCN terminate in the LHb (Zhang et al., 2009); the LHb expresses the PK2 receptor (Zhou and Cheng, 2005), and PK2 evokes gabazine sensitive inhibitory currents to inhibit LHb neurones (Sakhi et al., 2014b). However, this PK2 projection is yet to be independently replicated, and AVP terminals in the LHb have since been traced to magnocellular neurones in the PVN (Hernandez et al., 2015). As such, the question of whether the SCN directly projects to LHb remains unresolved.

Nonetheless, the LHb likely receives indirect regulation from the SCN *via* other oscillators in the forebrain, thalamus and brainstem (Morin, 2013). For example, the SCN directly innervates the DMH and LH, which both show daily changes in neuronal activity (Marston et al., 2008; Guilding et al., 2009), and innervate the LHb (Poller et al., 2013; Stamatkis et al., 2016). This highlights polysynaptic pathways through which the SCN could indirectly regulate the LHb.

Alongside PK2 and AVP, LHb neurones are responsive to orexin (Flanigan et al., 2020; Wang et al., 2021). Orexin is a major output molecule of the LH (Aston-Jones et al., 2009; Richardson and Aston-Jones, 2012; Ferrari et al., 2018), and orexinergic neurones undergo circadian regulation from the SCN (Deboer et al., 2004; Marston et al., 2008; Kalsbeek et al., 2011). It is therefore possible that LHb dependent behaviours, driven by orexin, are under circadian control and that orexin is another mechanism by which daily variations in the LHb are extrinsically regulated. This is particularly relevant when considering the role of LH orexinergic neurones in driving appetitive drug seeking, a behaviour associated with reward dysfunction typical of aberrant LHb activity (James et al., 2019; Yeoh et al., 2019).

To complement this evidence for extrinsic circadian regulation of LHb activity, presynaptic release probability of LHb afferents varies throughout the day (Park et al., 2017). This peaks during late afternoon, coincident with increasing neuronal activity in the LHb (Zhao and Rusak, 2005; Sakhi et al., 2014b). This suggests that circadian variation in LHb activity is driven by an interaction between intrinsic and extrinsic regulation.

In addition to entrainment from the SCN and other secondary oscillators, evidence suggests that LHb activity could be influenced by light. An early tracing study evidenced a direct retinal projection to the LHb (Qu et al., 1996), suggesting that this structure could integrate photic information. Indeed, *in vivo* electrophysiological recordings show that LHb neurones respond to light (Zhao and Rusak, 2005; Sakhi et al., 2014b; Huang et al., 2019). However, later tract-tracing indicates that the retina innervates areas just external to the anatomical borders of the LHb (Sakhi et al., 2014b). Further, melanopsin expressing retinal ganglion cells (mRGCs), responsible for light entrainment of the SCN and other oscillators, do not directly target the LHb and instead innervate the adjacent perihabenula (PHb; Hattar et al., 2006; Morin and Studholme, 2014). Moreover, the delay in LHb response to retinal illumination is too slow to reflect a direct retina-LHb connection (Sakhi et al., 2014b), instead indicating that light information is conveyed to the LHb *via* polysynaptic connections.

Candidates for intermediate structures in this pathway include the ventral lateral geniculate and intergeniculate leaflet (vLGN-IGN; Huang et al., 2019). Viral tracing shows that these structures receive direct input from mRGCs, and optogenetic interrogation reveals that activation of this pathway drives inhibitory postsynaptic currents in LHb neurones. Moreover, *in vivo* electrophysiology reveals that retinal illumination decreases the action potential frequency, and burst frequency, of LHb neurones in an intensity dependent manner. Another investigation found varying levels of light intensities to increase mouse LHb neuronal activity (Sakhi et al., 2014b). These data highlight the uncertainty surrounding the predominant effect of light in regulating LHb activity. Light's role in entraining the LHb to daily rhythms has yet to receive extensive investigation.

Finally, it is worth considering a role for social cues in the regulation of this daily activity. The LHb is implicated in reward processing, and it is embedded within many pathways which regulate reward-based feeding (Stamatkis et al., 2016), drug seeking (Nair et al., 2021), and social behaviour (Valentinova et al., 2019; Rigney et al., 2020). It is possible that the LHb may receive feedback from the drivers of these behaviours—which accordingly entrain neural activity. Moreover, peak LHb neural activity *in vivo* is coincident with heightened physical activity levels during a rodent's subjective night (Paul et al., 2011), raising the possibility of a feedback loop entraining the LHb to physical exercise. Behavioural and SCN entrainment to such arousal and other non-photic cues is widely documented (Rosenwasser et al., 1984; Gillman et al., 2013; Crosby et al., 2019; Hughes et al., 2021; Robbers et al., 2021). As the LHb plays such a vital role in integrating this information and coordinating an animal's reward response (Lammel et al., 2012), it is possible that it also responds to variation in this information throughout the day. Interestingly, rhythmic expression of clock genes in the LHb can be dampened by voluntary consumption of a high-fat high-sugar diet, illustrating how food intake may regulate circadian rhythms in this structure (Blancas-Velazquez et al., 2017). However, contrasting research suggests that while this effect occurs throughout the brain, it is absent in the LHb (Blancas-Velazquez et al., 2018). Clearly, more research is required to resolve how hedonic eating, and other social cues, affect daily variation of molecular activity and function in the LHb.

IMPLICATIONS FOR PSYCHIATRIC ILLNESS

As previously discussed, the LHb is implicated in the pathophysiology of multiple psychiatric illnesses (Hu et al., 2020). Numerous depressive phenotypes, in various animal models, are associated with increased LHb activity. For example, elevated LHb spike frequency is correlated with depressive behaviour for mice in the tail suspension and sucrose preference test, following chronic mild stress (Cerniauskas et al., 2019). Conversely, increasing $GABA_{B1}$ receptor function suppresses LHb hyperactivity and ameliorates depressive phenotypes (Lecca et al., 2016). This illustrates how bidirectional modulation of LHb activity can drive a two-way regulation of depressive behaviours.

This is likely due to the inverse correlation between LHb activity and the activity of its midbrain targets. The VTA receives direct innervation from the LHb and activation of this pathway promotes behavioural despair and place avoidance (Cerniauskas et al., 2019). Similarly, there is reciprocal connectivity between the LHb and the raphe nuclei (Sego et al., 2014); with stimulation of the LHb attenuating activity in the raphe (Stern et al., 1979). Paradoxically, though, almost all projections from the LHb are glutamatergic (Aizawa et al., 2012). Excitatory glutamatergic transmission to VTA and raphe neurones, predominantly dopaminergic and serotonergic, respectively, would be predicted to promote antidepressant-like effects. To reconcile this, the role of the RMTg must be considered (Barrot and Thome, 2011; Jhou, 2021).

The RMTg, also called the tail of the VTA, is a predominantly GABAergic midbrain centre which exerts feedforward inhibition onto various monoaminergic structures including the VTA, MR, and DR (Metzger et al., 2021). The LhB innervates the RMTg (Li et al., 2019), and c-Fos-ir in this structure is elevated following LHb stimulation (Lammel et al., 2012). Correspondingly, activation of the RMTg induces pro-depressive behavioural consequences (Stamatakis and Stuber, 2012; Smith et al., 2019), and reduces activity levels in the VTA and raphe nuclei.

The activity of the LHb, and thus feedforward inhibition of midbrain monoaminergic centres, is tightly regulated. Firstly, the VTA is reciprocally connected to the LHb, innervating the

LHb with gabazine sensitive inhibitory synapses (Stamatakis et al., 2013). Activation of this pathway disinhibits the VTA *via* the suppression of the LHb, and thus the RMTg. This is one mechanism whereby a LHb-VTA circuit may mediate reward prediction error (Stopper et al., 2014). Elevated LHb activity is correlated with reward omission (Matsumoto and Hikosaka, 2007), and pathological LHb hyperactivity can therefore encode anhedonia typical of depression sufferers (Yang et al., 2018a).

The LHb also receives extensive innervation from the GPi (or rodent EP; Shabel et al., 2012). This glutamatergic projection promotes aversion learning, and optogenetic stimulation of the EP-LHb pathway conditions avoidance in the place preference test. Activity in this pathway is bidirectionally regulated by expected reward outcome, increasing and decreasing upon worse and better outcomes, respectively (Stephenson-Jones et al., 2016). Intriguingly, GABA and Glutamate can be co-released at the EP-LHb synapse, implicating release balance of these neurotransmitters in encoding mood and reward valence (Shabel et al., 2014).

Similar to the VTA, there is reciprocal connectivity between the LHb and the raphe nuclei (Metzger et al., 2021). Interestingly, despite the well-established role for serotonin signalling in the mechanism of traditional antidepressant drugs (Blier and Montigny, 1998), activation of 5-HT_{2C} receptors in the LHb causes depolarisation and increased spike frequency (Zuo et al., 2016). This is accompanied by the expression of an anhedonic and passive coping phenotype (Han et al., 2015). However, serotonin also decreases release probability of glutamate at the EP-LHb synapse, decreasing EPSP amplitude and potentially reducing excitatory input to the LHb (Shabel et al., 2012). By attenuating excitatory drive to the LHb, hyperactivity is suppressed, thus elucidating a potential mechanism whereby serotonin exerts its antidepressant effects.

There is accumulating evidence that increased LHb burst firing plays a role in the aetiology of depression (Cui et al., 2018; Yang et al., 2018a). For example, the proportion of bursting LHb neurones is elevated in congenitally learned helpless mice, compared to control animals. Indeed, optogenetic induction of LHb rebound bursting is sufficient to induce depressive-like behavioural performance in the sucrose preference and tail suspension tests (Yang et al., 2018a). This is demonstrative of a potentially causal relationship between this discharge pattern and depression.

This increase in LHb bursting is possibly associated with enhanced astrocytic expression of the inwardly rectifying potassium channel, Kir4.1 (Cui et al., 2018). Historically, glial expression of Kir4.1 has been colocalised with tripartite synapses (Newman, 1993), where its K^+ buffering properties controls neuronal membrane potential according to the Nernst equation (Amédée et al., 1998; Neusch et al., 2006; Cui et al., 2018). Dysfunction of Kir4.1 results in local hyperactive neuronal states associated with various pathologies such as Parkinson's and epilepsy (Haj-Yasein et al., 2011; Tong et al., 2014; Nwaobi et al., 2016).

In the LHb, these channels sit within the membrane of astrocytic processes that surround neuronal somata. In this context, Kir 4.1 drives the hyperpolarisation of neighbouring neurones by decreasing the extracellular concentration of K^+ (Tong et al., 2014). LHb neurones require membrane hyperpolarisation to exhibit burst firing. As such, astrocytic overexpression of Kir4.1 promotes LHb burst firing, and consequent depressive phenotypes (**Figure 4**).

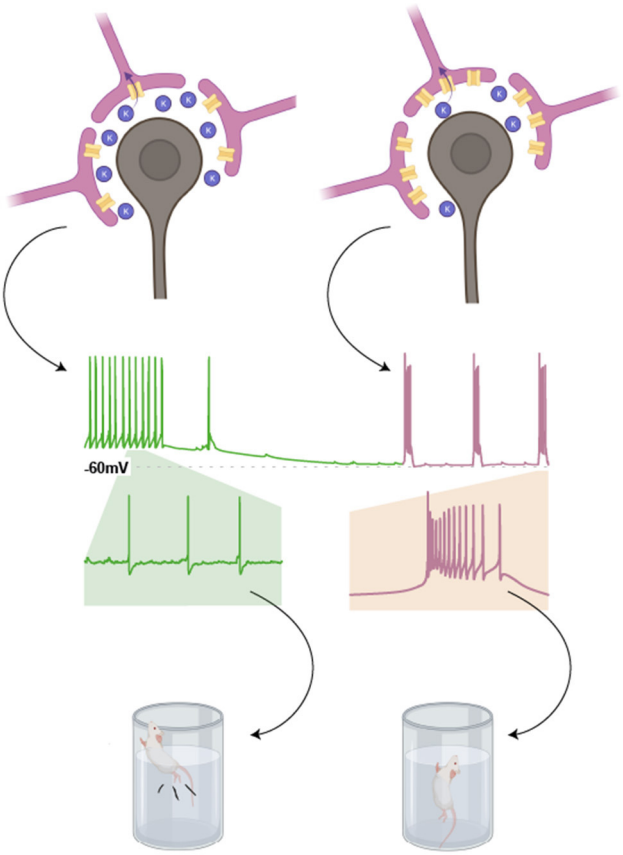

FIGURE 4 | Astrocytic expression of Kir4.1 promotes burst firing in neighbouring LHb neurones. Expression of the inwardly rectifying potassium channel, Kir4.1, in astrocytic processes surrounding the somata of LHb neurones, lowers the concentration of K^+ in the extracellular space. This is because K^+ is taken up by these astrocytes. According to the Nernst equation, this results in a more negative resting membrane potential for neighbouring LHb neurones (as indicated in the cartoon trace). This in turn promotes burst firing in LHb neurones, a phenomenon associated with depressive phenotypes in rodent models (Drawn with BioRender).

This link between depression and the voltage dependence of burst firing may explain the paradoxical nature of serotonin's depolarising effects in the lateral habenula (Zuo et al., 2016), and its well-characterised involvement in antidepressant pharmacotherapies (Blier and Montigny, 1998). By causing depolarisation in the LHb, serotonin may shift membrane potential out of a range in which burst firing occurs. In a similar fashion, knockdown of Kir4.1 causes depolarisation in LHb neurones (Cui et al., 2018). This is accompanied by attenuation of burst firing and alleviation of passive

coping and anhedonic phenotypes in chronically learned helpless mice.

LINKING CIRCADIAN VARIABILITY WITH DEPRESSION, *VIA* THE LATERAL HABENULA

Circadian disruptions are common in bipolar disorder and animal models of bipolar disorder (Wulff et al., 2010; Timothy et al., 2018) as well as in people suffering from depression (Jagannath et al., 2013; Mendoza, 2019). Many sufferers struggle to initiate or maintain sleep, and experience aberrant hormonal daily rhythms (e.g., cortisol; Difrancesco et al., 2019; Hoyos et al., 2020). Unpicking the cause-and-effect relationship of this comorbidity is complex, but the circadian and pro-depressive properties of the LHb make it a possible candidate to mediate this interaction.

Circadian variations in LHb activity are a good place to start when considering this interaction. LHb activity levels are closely linked to depression, therefore it would be predicted that peak exhibition of depressive symptoms to be coincident with elevated LHb firing rate. Mood does show diurnal variation, with negative affect showing more pronounced circadian rhythms in depression sufferers than healthy individuals (Wirz-Justice, 2008). Interestingly, the patterns of mood oscillations are different in healthy individuals compared to those exhibiting depressive mood (Rusting and Larsen, 1998). Low mood sufferers tend to experience an evening-worse pattern of mood, in which low mood is exacerbated in late day. However, some research indicates that, in diagnosed depressives, this peak in low mood occurs in early morning (Zerssen et al., 1985). This is coincident with the lowest behavioural activity in depressed adults over age 30 (Smagula et al., 2021). It is pertinent to note, though, that most recordings which illustrate circadian oscillations in the LHb were made in or from tissue prepared from nocturnal rodents. To fully appreciate how changes in LHb firing rate associate with daily variation in human mood, studies investigating neural activity variations in the LHb of diurnal rodents or indeed humans are necessary.

While circadian variation in spike rate has been independently investigated, it is unknown if the occurrence of burst firing or intraburst frequency alters with time of day/circadian cycles. This could have implications for symptoms of depression, as passive coping and anhedonia are shown to positively correlate with both of these variables (Yang et al., 2018a; Cerniauskas et al., 2019). Although circadian variation in the proportion of bursting LHb cells is not reported, Kir4.1 shows similar oscillatory expression to the clock gene *Bmal1* in retinal Müller cells (Luo et al., 2019). As astrocytic Kir4.1 expression is implicated in LHb bursting (**Figure 3**; Cui et al., 2018), it is possible that circadian oscillations in Kir4.1 drive a corresponding rhythm in burst firing and resultant depressive behaviours. Moreover, Kir4.1 expression is additionally regulated by insulin receptor substrate-1 (IRS-1; Luo et al., 2019), with increased IRS-1 signalling causing an upregulation of Kir4.1. Interestingly, the habenula complex is an enriched site for IRS-1 (Baskin et al., 1993). This suggests a potential mechanism whereby LHb activity is entrained by diet, with post-feeding surges in insulin causing increased IRS-1 signalling and thus changes in Kir4.1 expression (Crosby et al., 2019). Such a process could also explain how high-sugar diets may exacerbate depression (Vermeulen et al., 2017).

Circadian oscillations in LHb activity may entrain 24 h oscillations in the activity and function of neural structures that are targeted by LHb efferents (Mendoza, 2017). Some VTA neurones exhibit circadian variation in electrophysiological properties (Luo et al., 2008), and disruptions in these normal oscillations are associated with mood switching, typical of bipolar disorder (Sidor et al., 2016). The VTA shows circadian oscillations in clock gene expression (Webb et al., 2009), and is directly innervated by the SCN (Luo and Aston-Jones, 2009), both of which may entrain VTA function to its daily rhythms. However, LHb-VTA connectivity is another mechanism through which circadian variation in the VTA may occur. This particular pathway may be responsible for circadian oscillations in motivation and reward valency (Webb et al., 2009; Acosta et al., 2020). Aberrations in this process could explain the dysregulated reward seeking behaviour typical of addiction, or the anhedonia described in depression sufferers (Fox and Lobo, 2019). This is reinforced by the observation that arrhythmic animals show an overall decrease in motivation (Acosta et al., 2020).

Similar to the VTA, serotonergic raphe neurones appear to be under circadian control from the LHb (Mendoza, 2017). Serotonin release, throughout the brain, is heightened during the active night of nocturnal rodents (Dudley et al., 1998). There are some direct glutamatergic projections from the LHb to the DR, and electrical stimulation of these increase serotonin release (Kalen et al., 1989). Further, *in vivo* recordings of LHb neurones projecting to the DR show an elevated spike rate at night (Liu et al., 2021). This may explain the coincident peaks in LHb neural activity and serotonin release.

This circadian variation was blunted in stress susceptible mice, with an overall increase in activity at both day and night (Liu et al., 2021). Such stress susceptibility may be encoded by reciprocal serotonergic projections from the DR to the LHb, which thus increase LHb spike rate (Zuo et al., 2016). Alternatively, these recordings may have been taken from inhibitory pathways which inhibit serotonergic activity. To resolve these possibilities, recordings in postsynaptic raphe neurones are required.

Entrainment of LHb activity, by light, potentially underpins resistance to depressive mood. Bright light therapy (BLT) is an effective chronotherapy when administered in the early morning (Olejniczak et al., 2021), and is especially effective at treating seasonal affective disorder (Pjerk et al., 2020). In the forced swim test, passive coping behaviour is attenuated by transient light pulses during late night (Olejniczak et al., 2021). This suggests that light itself does not provide the antidepressant effect, but instead causes a circadian phase

shift (Menculini et al., 2018). A putative pathway, connecting the retina to the vLGN-IGN and onward to the LHb, is thought to mediate the anti-depressive effect of BLT (Huang et al., 2019). Optogenetic activation of this pathway elicits an antidepressant effect in the sucrose preference and forced swim test, and these effects are replicated following retinal illumination. However, light suppresses active behaviour raising the possibility that observed changes are the consequence of a non-circadian "masking" effect (Redlin, 2001). Moreover, projections from the retina to the PHb are associated with mood regulation (Fernandez et al., 2018). Mice exposed to aberrant light conditions exhibit anhedonic and passive coping behaviours, and this is thought to be mediated *via* PHb neurones. This is another example of non-circadian response to light, near the habenula complex, which could explains light's regulation of affect.

An alternative explanation for the antidepressant-like effects of BLT is its capacity to induce *Per1* expression. This occurs in the SCN, and the LHb (Yan and Silver, 2002; Kuhlman et al., 2003; Olejniczak et al., 2021). More importantly, the capacity for BLT to elicit antidepressant-like effects appears to depend on *Per1* expression in the LHb and is abrogated by *Per1* knockdown in this structure. However, this may not be demonstrative of a causal relationship between *Per1* induction and antidepressant-like effects of BLT. *Per1* controls the transcription of various ion channels (Gumz et al., 2010; Stow et al., 2012; Alli et al., 2019) which regulate neuronal excitability (Carr et al., 2001; Lang et al., 2003; Amin et al., 2005). Upon *Per1* knockdown, these proteins become unregulated and the LHb could default to a state of hyperactivity. Interestingly, in the $Cry1^{-/-}Cry2^{-/-}$ mouse in which the molecular clock does not function, LHb spiking activity is around an intermediate level (Sakhi et al., 2014b), indicating that more investigation is necessary to determine if and how altered clock gene expression influences neuronal excitability.

Other studies support the contention that the molecular clock is involved in depression. For example, *Per2* knockdown in the LHb results in a passive coping phenotype during night-time (Li et al., 2020). Moreover, the antidepressant effects of ketamine have been associated with its impact on the molecular clock. Ketamine administration can dampen clock gene oscillations by interfering with the CLOCK:Bmal1 heterodimer (Bellet et al., 2011), which occurs in a GSK3β dependent manner. Ketamine's antidepressant effects are also dependent on GSK3β (Zanos and Gould, 2018), thus linking the molecular clock to depression. However, no behaviour experiments have yet shown a correlation between ketamine's effect on CLOCK:Bmal1 and depressive phenotypes.

If the exact nature of clock gene function in depression is questionable, their relevance in the entrainment of circadian oscillators is not. However, other cues are also important and may contribute to the link between circadian disruptions and depression. One example of this may be stress. Exposure to stressful stimuli can profoundly interrupt normal reward processing, a behavioural effect triggered by synaptic depression in the LHb (Nuno-Perez et al., 2021). Alongside this, acute stress is able to transform LHb reward responses into punishment-like signals, and this occurs synchronously with onset of anhedonic behaviour (Shabel et al., 2019). These stress signals can also cause circadian disruptions (Kalmbach et al., 2018). In fact, there has been an unprecedented rise in sleep disturbances as a consequence of the COVID-19 pandemic (Morin et al., 2020), an event which is concomitant with elevated depression and anxiety levels (Luo et al., 2020). The susceptibility of the LHb to stress, and its role as a neural circadian oscillator, places it well to mediate this link between stress dependent circadian disruptions and affective disorders.

In addition to stress, arousal-promoting stimuli such as physical exercise entrain circadian rhythms (Mistlberger and Antle, 2011; Hughes and Piggins, 2012). Scheduled exercise can entrain the SCN and behavioural rhythms in animals with otherwise disrupted activity patterns (Power et al., 2010; Hughes et al., 2021). There is also evidence of a feedback loop coordinating LHb activity with exercise (Paul et al., 2011), and animals with LHb Per1 knockdown exhibit blunted behavioural rhythms (Li et al., 2020). Lower levels of exercise are associated with depression (Mcmahon et al., 2017), and exercise-based interventions can alleviate symptoms in sufferers (Cramer et al., 2013; Paolucci et al., 2018; Schuch and Stubbs, 2019). It is possible that there is a circadian association between depression and exercise, and that timed exercise intervention may benefit depression sufferers by entraining their otherwise disrupted diurnal rhythms (Hughes et al., 2021). If this this proves to be the case, the LHb may be a critical component in the neural circuitry which underpins it.

CONCLUSION

The LHb varies its activity over 24 h, and this epithalamic area is ideally situated to mediate the links between depression and circadian disruption. It is a highly heterogeneous structure that resides within an anatomically diffuse neural circadian network, potentially receiving circadian entrainment signals from other neural oscillators, arousal, and social cues, as well as light input. The LHb's role in the regulation of reward processing, and the function this has in entraining rhythms according to motivation, evidences a connections between these rhythms and mood. Aberrant and dysregulated reward seeking is characteristic of various psychiatric disorders, and disruptions in LHb signalling may underpin this.

Extensive further research is necessary before firm conclusions can be drawn regarding the importance of changes in the circadian function of the LHb in the aetiology of depression. To date, most of the evidence supporting this conjecture is of a correlational or associative nature and here we have highlighted potential avenues for future investigation. Indeed, preliminary work is emerging which focusses on the role of circadian oscillations in LHb-dependent depression and antidepressant treatments. While important to understanding depression, such approaches need to be carefully critiqued to fully elucidate the complexity of the LHb's role in both depression and circadian timekeeping in general.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CY and HP wrote and edited the manuscript. DL edited the manuscript. CY and DL composed the figures. All authors contributed to the article and approved the submitted version.

FUNDING

CY was supported by a studentship from the Biotechnology and Biological Sciences Research Council (BBSRC; UK) South West Biosciences Doctoral Training Programme (BB/T008741/1). Research in HP's lab was supported by project grants from the BBSRC (BB/R019223 and BB/W000865).

ACKNOWLEDGMENTS

We thank Eleanor Bell and Dr. Adriana Basnakova for stimulating discussions.

REFERENCES

Acosta, J., Bussi, I. L., Esquivel, M., Hocht, C., Golombek, D. A., and Agostino, P. V. (2020). Circadian modulation of motivation in mice. *Behav. Brain Res.* 382:112471. doi: 10.1016/j.bbr.2020.112471

Aizawa, H., Amo, R., and Okamoto, H. (2011). Phylogeny and ontogeny of the habenular structure. *Front. Neurosci.* 5:138. doi: 10.3389/fnins.2011.00138

Aizawa, H., Kobayashi, M., Tanaka, S., Fukai, T., and Okamoto, H. (2012). Molecular characterization of the subnuclei in rat habenula. *J. Comp. Neurol.* 520, 4051–4066. doi: 10.1002/cne.23167

Alli, A., Yu, L., Holzworth, M., Richards, J., Cheng, K.-Y., Lynch, I. J., et al. (2019). Direct and indirect inhibition of the circadian clock protein Per1: effects on ENaC and blood pressure. *Am. J. Physiol. Renal Physiol.* 316, F807–F813. doi: 10.1152/ajprenal.00408.2018

Amédée, T., Robert, A., and Coles, J. A. (1998). Potassium homeostasis and glial energy metabolism. *Glia* 21, 46–55. doi: 10.1002/(SICI)1098-1136(199709)21:1<46::AID-GLIA5>3.0.CO;2-#

Amin, M. S., Wang, H.-W., Reza, E., Whitman, S. C., Tuana, B. S., and Lenen, F. H. H. (2005). Distribution of epithelial sodium channels and mineralocorticoid receptors in cardiovascular regulatory centers in rat brain. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R1787–R1797. doi: 10.1152/ajpregu.00063.2005

Aston-Jones, G., Smith, R. J., Moorman, D. E., and Richardson, K. A. (2009). Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology* 56, 112–121. doi: 10.1016/j.neuropharm.2008.06.060

Bano-Otalora, B., and Piggins, H. D. (2017). Contributions of the lateral habenula to circadian timekeeping. *Pharmacol. Biochem. Behav.* 162, 46–54. doi: 10.1016/j.pbb.2017.06.007

Barrot, M., and Thome, J. (2011). Discovering a new anatomical structure in the brain: implications for neuropsychiatry and therapy. *World J. Biol. Psychiatry* 12, 19–22. doi: 10.3109/15622975.2011.598386

Baskin, D. G., Sipols, A. J., Schwartz, M. W., and White, M. F. (1993). Immunocytochemical detection of insulin receptor substrate-1 (IRS-1) in rat brain: colocalization with phosphotyrosine. *Regul. Peptides* 48, 257–266. doi: 10.1016/0167-0115(93)90355-C

Bellet, M. M., Vawter, M. P., Bunney, B. G., Bunney, W. E., and Sassone-Corsi, P. (2011). Ketamine influences CLOCK:BMAL1 function leading to altered circadian gene expression. *PLoS ONE* 6:e23982. doi: 10.1371/journal.pone.0023982

Bianco, I. H., and Wilson, S. W. (2009). The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain. *Philos. Trans. R. Soc. B* 364, 1005–1020. doi: 10.1098/rstb.2008.0213

Blancas-Velazquez, A., La Fleur, S. E., and Mendoza, J. (2017). Effects of a free-choice high-fat high-sugar diet on brain PER2 and BMAL1 protein expression in mice. *Appetite* 117, 263–269. doi: 10.1016/j.appet.2017.07.002

Blancas-Velazquez, A. S., Unmehopa, U. A., Eggels, L., Koekkoek, L., Kalsbeek, A., Mendoza, J., et al. (2018). A free-choice high-fat high-sugar diet alters day-night Per2 gene expression in reward-related brain areas in rats. *Front. Endocrinol.* 9:154. doi: 10.3389/fendo.2018.00154

Blier, P., and Montigny, C. D. (1998). Possible serotonergic mechanisms underlying antidepressant and anti-obsessive-compulsive disorder responses. *Biol. Psychiatry* 44, 313–323. doi: 10.1016/S0006-3223(98)00114-0

Buijs, R. M. (1978). Intra - and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell Tissue Res.* 192, 423–435. doi: 10.1007/BF00224932

Cagampang, F. R. A., Yang, J., Nakayama, Y., Fukuhara, C., and Inouye, S.-I. T. (1994). Circadian variation of arginine-vasopressin messenger RNA in the rat suprachiasmatic nucleus. *Mol. Brain Res.* 24, 179–184. doi: 10.1016/0169-328X(94)90130-9

Carr, M. J., Gover, T. D., Weinreich, D., and Undem, B. J. (2001). Inhibition of mechanical activation of guinea-pig airway afferent neurons by amiloride analogues. *Br. J. Pharmacol.* 133, 1255–1262. doi: 10.1038/sj.bjp.0704197

Cerniauskas, I., Winterer, J., De Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., et al. (2019). Chronic stress induces activity, synaptic, and transcriptional remodeling of the lateral habenula associated with deficits in motivated behaviors. *Neuron* 104, 899–915. doi: 10.1016/j.neuron.2019.09.005

Charlson, F., Ommeren, M. V., Flaxman, A., Cornett, J., and Whiteford, H. And Saxena, S. (2019). New WHO prevalence estimates of mental disorders in conflict settings: a systematic review and meta-analysis. *Lancet* 394, 240–248. doi: 10.1016/S0140-6736(19)30934-1

Christiansen, S. L., Bouzinova, E. V., Fahrenkrug, J., and Wiborg, O. (2016). Altered expression pattern of clock genes in a rat model of depression. *Int. J. Neuropsychopharmacol.* 19:61. doi: 10.1093/ijnp/pyw061

Corriger, A., and Pickering, G. (2019). KEtamine and depression: a narrative review. *Drug Design Dev. Ther.* 13, 3051–3067. doi: 10.2147/DDDT.S221437

Cramer, H., Lauche, R., Langhorst, J., and Dobos, G. (2013). Yoga for depression: a systematic review and meta-analysis. *Depr. Anxiety* 30, 1068–1083. doi: 10.1002/da.22166

Crosby, P., Hamnett, R., Putker, M., Hoyle, N. P., Reed, M., Karam, C. J., et al. (2019). Insulin/IGF-1 drives PERIOD synthesis to entrain circadian rhythms with feeding time. *Cell* 177, 896–909. doi: 10.1016/j.cell.2019.02.017

Cui, Y., Yang, Y., Ni, Z., Dong, Y., Cai, G., Foncelle, A., et al. (2018). Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression. *Nature* 14, 323–327. doi: 10.1038/nature25752

Deboer, T., Overseem, S., Visser, N. A. H., Duindam, H., Frolich, M., Lammers, G. J., et al. (2004). Convergence of circadian and sleep regulatory mechanisms on hypocretin-1. *Neuroscience* 129, 727–732. doi: 10.1016/j.neuroscience.2004.07.049

Diaz, E., Bravo, D., Rojas, X., and Concha, M. L. (2011). Morphologic and immunohistochemical organization of the human habenular complex. *J. Comp. Neurol.* 519, 3727–3747. doi: 10.1002/cne.22687

Difrancesco, S., Lemers, F., Riese, H., Merikangas, K. R., Beekman, A. T. F., Van Hemert, A. M., et al. (2019). Sleep, circadian rhythm, and physical activity patterns in depressive and anxiety disorders: A 2-week ambulatory assessment study. *Depres. Anxiety* 36, 975–986. doi: 10.1002/da.22949

Dudley, T. E., Dinardo, L. A., and Glass, J. D. (1998). Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus. *J. Neurosci.* 18, 5045–5052. doi: 10.1523/JNEUROSCI.18-13-05045.1998

Fernandez, D. C., Fogerson, P. M., Ospri, L. L., Thomsen, M. B., Layned, R. M., Severin, D., et al. (2018). Light affects mood and learning through distinct retina-brain pathways. *Cell* 175, 71–84 doi: 10.1016/j.cell.2018.08.004

Ferrari, L. L., Park, D., Zhu, L., Palmer, M. R., Broadhurst, R. Y., and Arrigoni, E. (2018). Regulation of lateral hypothalamic orexin activity by local GABAergic neurons. *J. Neurosci.* 36, 1588–1599. doi: 10.1523/JNEUROSCI.1925-17.2017

Flanigan, M. E., Aleyasin, H., Li, L., Burnett, C. J., Chan, K. L., Leclair, K. B., et al. (2020). Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice. *Nat. Neurosci.* 23, 638–650. doi: 10.1038/s41593-020-0617-7

Fowler, C. D., Lu, Q., Johnson, P. M., Marks, M. J., and Kenny, P. J. (2011). Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* 471, 597–601. doi: 10.1038/nature09797

Fox, M. E., and Lobo, M. K. (2019). The molecular and cellular mechanisms of depression: a focus on reward circuitry. *Mol. Psychiatry* 24, 1798–1815. doi: 10.1038/s41380-019-0415-3

GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 392, 1789–1858. doi: 10.1016/S0140-6736(18)32279-7

Gillman, A. G., Leffel, J. K., Kosobud, A. E. K., and Timberlake, W. (2013). Behavioral characteristics and pharmacological manipulations of a nicotine-entrainable circadian oscillator. *Chronobiol. Int.* 30, 855–869. doi: 10.3109/07420528.2013.782314

Glick, S. D., Ramirez, R. L., Livi, J. M., and Maisonneuve, I. M. (2006). 18-Methoxycoronaridine acts in the medial habenula and/or interpeduncular nucleus to decrease morphine self-administration in rats. *Euro. J. Pharmacol.* 537, 94–98. doi: 10.1016/j.ejphar.2006.03.045

Guilding, C., Hughes, A. T., Brown, T. M., Namvar, S., and Piggins, H. D. (2009). A riot of rhythms: neuronal and glial circadian oscillators in the mediobasal hypothalamus. *Mol. Brain* 2:28. doi: 10.1186/1756-6606-2-28

Guilding, C., Hughes, A. T. L., and Piggins, H. D. (2010). Circadian oscillators in the epithalamus. *Neuroscience* 169, 1630–1639. doi: 10.1016/j.neuroscience.2010.06.015

Guilding, C., and Piggins, H. D. (2007). Challenging the omnipotence of the suprachiasmatic timekeeper: are circadian oscillators present throughout the mammalian brain? *Euro. J. Neurosci.* 25, 3195–3216. doi: 10.1111/j.1460-9568.2007.05581.x

Guilding, C., Scott, F., Bechtold, D. A., Brown, T. M., Wegner, S., and Piggins, H. D. (2013). Suppressed cellular oscillations in after-hours mutant mice are associated with enhanced circadian phase-resetting. *J. Physiol.* 591, 1063–1080. doi: 10.1113/jphysiol.2012.242198

Gumz, M. L., Cheng, K.-Y., Lynch, I. J., Stow, L. R., Greenlee, M. M., Cain, B. D., et al. (2010). Regulation of αENaC expression by the circadian clock protein Period 1 in mpkCCD(c14) cells. *Biochim. Biophys. Acta* 1799, 622–629. doi: 10.1016/j.bbagrm.2010.09.003

Haj-Yasein, N. N., Jensen, V., Vindedal, G. F., Gundersen, G. A., Klungland, A., Ottersen, O. P., et al. (2011). Evidence that compromised K+ spatial buffering contributes to the epileptogenic effect of mutations in the human kir4.1 gene (KCNJ10). *Glia* 59, 1635–1642. doi: 10.1002/glia.21205

Han, L.-N., Zhang, L., Li, L.-B., Sun, Y.-N., Wang, Y., Chen, L., et al. (2015). Activation of 5-Ht2c receptors in the LHb nucleus increases the expression of depression-related behaviours in the hemiparkinsonian rat. *Neuropharmacology* 93, 68–79. doi: 10.1016/j.neuropharm.2015.01.024

Hashikawa, Y., Hashikawa, K., Rosi, M. A., Basiri, M. L., Liu, Y., Johnstor, N. L., et al. (2020). Transcriptional and spatial resolution of cell types in the mammalian habenula. *Neuron* 106, 743–758. doi: 10.1016/j.neuron.2020.03.011

Hastings, M. H., Maywood, E. S., and Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. *Nat. Rev. Neurosci.* 19, 453–469. doi: 10.1038/s41583-018-0026-z

Hattar, S., Kumar, M., Park, A., Tong, P., Tung, J., Yau, K. W., et al. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J. Comp. Neurol.* 497, 326–340. doi: 10.1002/cne.20970

He, Q., Wang, J., and Hu, H. (2019). Illuminating the activated brain: emerging activity-dependent tools to capture and control functional neural circuits. *Neurosci. Bull.* 35, 369–377. doi: 10.1007/s12264-018-0291-x

Hergerl, U., Althaus, D., and Stefanek, J. (2003). Public attitudes toward treatment of depression: effects of an information campaign. *Pharmacopsychiatry* 36, 288–291. doi: 10.1055/s-2003-45115

Herkenham, M., and Nauta, W. J. H. (1977). *Afferent Connections of the Habenular Nuclei in the Rat. A Horseradish Peroxidase Study, with a Note on the Fiber-of-Passage Problem. Neuroanatomy. Contemporary Neuroscientists (Selected Papers of Leaders in Brain Research)*. Boston, MA: Birkhäuser. doi: 10.1007/978-1-4684-7920-1_16

Herkenham, M., and Nauta, W. J. H. (1979). Efferent connections of the habenular nuclei in the rat. *J. Comp. Neurol.* 187, 19–47. doi: 10.1002/cne.901870103

Hernandez, V. S., Vazquez-Juarez, E., Marquez, M. M., Jauregui-Huerta, F., Barrio, R. A., and Zhang, L. (2015). Extra-neurohypophyseal axonal projections from individual vasopressin-containing magnocellular neurons in rat hypothalamus. *Front. Neuroanat.* 9:130. doi: 10.3389/fnana.2015.00130

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.* 11, 503–513. doi: 10.1038/nrn2866

Horowitz, J. M., and Graf, N. (2019). *Most U.S. Teens See Anxiety and Depression as a Major Problem Among their Peers*. Washington, DC: Pew Research Center.

Hoyos, C. M., Gordon, C., Terpening, Z., Norrie, L., Lewis, S. J. G., Hickie, I. B., et al. (2020). Circadian rhythm and sleep alterations in older people with lifetime depression: a case-control study. *BMC Psychiatry* 20:192. doi: 10.1186/s12888-020-02606-z

Hsu, Y.-W. A., Tempest, L., Quina, L. A., Wei, A. D., Zeng, H., and Turner, E. E. (2013). Medial habenula output circuit mediated by α5 nicotinic receptor-expressing GABAergic neurons in the interpeduncular nucleus. *J. Neurosci.* 33, 18022–18035. doi: 10.1523/JNEUROSCI.2927-13.2013

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Huang, L., Xi, Y., Peng, Y., Yang, Y., Huang, X., Fu, Y., et al. (2019). A visual circuit related to habenula underlies the antidepressive effects of light therapy. *Neuron* 102, 128–142. doi: 10.1016/j.neuron.2019.01.037

Hughes, A. T. L., and Piggins, H. D. (2012). "Chapter 18 - feedback actions of locomotor activity to the circadian clock," in *The Neurobiology of Circadian Timing,* eds A. Kalsbeek, M. Merrow, T. Roenneberg, and R. G. Foster (Amsterdam; Oxford: Elsevier). doi: 10.1016/B978-0-444-59427-3.00018-6

Hughes, A. T. L., Samuels, R. E., Bano-Otalora, B., Belle, M. D. C., Wegner, S., Guilding, C., et al. (2021). Timed daily exercise remodels circadian rhythms in mice. *Commun. Biol.* 4:761. doi: 10.1038/s42003-021-02239-2

Jagannath, A., Peirson, S. N., and Foster, R. G. (2013). Sleep and circadian rhythm disruption in neuropsychiatric illness. *Curr. Opin. Neurobiol.* 23, 888–894. doi: 10.1016/j.conb.2013.03.008

James, M. H., Stopper, C. M., Zimmer, B. A., Koll, N. E., Bowrey, H. E., and Aston-Jones, G. (2019). Increased number and activity of a lateral subpopulation of hypothalamic orexin/hypocretin neurons underlies the expression of an addicted state in rats. *Biol. Psychiatry* 85, 925–935. doi: 10.1016/j.biopsych.2018.07.022

Jhou, T. C. (2021). The rostromedial tegmental (RMTg) "brake" on dopamine and behavior: a decade of progress but also much unfinished work. *Neuropharmacology* 198:108763. doi: 10.1016/j.neuropharm.2021.108763

Kalen, P., Strecker, R. E., Rosengren, E., and Bjorklund, A. (1989). Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by *in vivo* microdialysis: role of excitatory amino acids and GABA. *Brain Res.* 492, 187–202. doi: 10.1016/0006-8993(89)90901-3

Kalmbach, D. A., Anderson, J. R., and Drake, C. L. (2018). The impact of stress on sleep: pathogenic sleep reactivity as a vulnerability to insomnia and circadian disorders. *J. Sleep Res.* 27:e12710. doi: 10.1111/jsr.12710

Kalsbeek, A., Scheer, F. A., Perreau-Lenz, S., La Fleur, S. E., Yi, C.-X., Fliers, E., et al. (2011). Circadian disruption and SCN control of energy metabolism. *FEBS Lett.* 585, 1412–1426. doi: 10.1016/j.febslet.2011.03.021

Kim, U., and Chang, S.-Y. (2005). Dendritic morphology, local circuitry, and intrinsic electrophysiology of neurons in the rat medial and lateral habenular nuclei of the epithalamus. *J. Comp. Neurol.* 483, 236–250. doi: 10.1002/cne.20410

Kuhlman, S. J., Silver, R., Le Sauter, J., Bult-Ito, A., and Mcmahon, D. G. (2003). Phase resetting light pulses induce Per1 and persistent spike activity in a subpopulation of biological clock neurons. *J. Neurosci.* 23, 1441–1450. doi: 10.1523/JNEUROSCI.23-04-01441.2003

Lammel, S., Lim, B. K., Ran, C., Huang, K. W., Betley, M. J., Tye, K. M., et al. (2012). Input-specific control of reward and aversion in the ventral tegmental area. *Nature* 49, 212–217. doi: 10.1038/nature11527

Lang, F., Henke, G., Embark, H. M., Waldegger, S., Palmada, M., Bohmer, C., et al. (2003). Regulation of channels by the serum and glucocorticoid-inducible

kinase - implications for transport, excitability and cell proliferation. *Cell Physiol. Biochem.* 13, 41–50. doi: 10.1159/000070248

Laurent, V., Wong, F. L., and Balleine, B. W. (2017). The lateral habenula and its input to the rostromedial tegmental nucleus mediates outcome-specific conditioned inhibition. *J. Neurosci.* 37, 10932–10942. doi: 10.1523/JNEUROSCI.3415-16.2017

Lawson, R. P., Nord, C. L., Seymour, B., Thomas, D. L., Dayan, P., Pilling, S., et al. (2017). Disrupted habenula function in major depression. *Mol. Psychiatry* 22, 202–208. doi: 10.1038/mp.2016.81

Lecca, S., Pelosi, A., Tchenio, A., Moutkine, I., Lujan, R., Herve, D., et al. (2016). Rescue of GABAB and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nat. Med.* 22, 254–261. doi: 10.1038/nm.4037

Li, B., Piriz, J., Mirrione, M., Chung, C. H., Proulx, C. D., Shultz, D., et al. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature*. 470, 535–539. doi: 10.1038/nature09742

Li, H., Pullman, D., and Jhou, T. C. (2019). Valence-encoding in the lateral habenula arises from the entopeduncular region. *Elife* 8:e41223. doi: 10.7554/eLife.41223

Li, J., Kang, S., Fu, R., Wu, L., Wu, W., Liu, H., et al. (2017). Inhibition of AMPA receptor and CaMKII activity in the lateral habenula reduces depressive-like behavior and alcohol intake in rats. *Neuropharmacology* 126, 108–120. doi: 10.1016/j.neuropharm.2017.08.035

Li, K., Liao, L., Yang, Z., Wong, C., Henn, F., Malinow, R., et al. (2013). βCaMKII in lateral habenula mediates core symptoms of depression. *Science* 30, 1016–1020. doi: 10.1126/science.1240729

Li, Y., Li, G., Li, J., Cai, X., Sun, Y., Zhang, B., et al. (2020). Depression-like behavior is associated with lower Per2 mRNA expression in the lateral habenula of rats. *Genes Brain Behav.* 20:e12702. doi: 10.1111/gbb.12702

Lima, L. B., Bueno, D., Leite, F., Souza, S., Goncalves, L., Furigo, I. C., et al. (2017). Afferent and efferent connections of the interpeduncular nucleus with special reference to circuits involving the habenula and raphe nuclei. *J. Comp. Neurol.* 525, 2411–2442. doi: 10.1002/cne.24217

Liu, H., Rastogi, A., Narain, P., Xu, Q., Sabanovic, M., Alhammadi, A. D., et al. (2021). Blunted diurnal firing in lateral habenula projections to dorsal raphe nucleus and delayed photoentrainment in stress-susceptible mice. *PLoS Biol.* 19:e30000709. doi: 10.1371/journal.pbio.3000709

Lopez, A. J., Jia, Y., White, A. O., Kwapis, J. L., Espinoza, M., Hwang, P., et al. (2018). Medial habenula cholinergic signaling regulates cocaine-associated relapse-like behavior. *Addict. Biol.* 24, 403–413. doi: 10.1111/adb.12605

Luo, A. H., and Aston-Jones, G. (2009). Circuit projections from suprachiasmatic nucleus to ventral tegmental area: a novel circadian output pathway. *Euro. J. Neurosci.* 29, 748–760. doi: 10.1111/j.1460-9568.2008.06606.x

Luo, A. H., Georges, F. E., and Aston-Jones, G. S. (2008). Novel neurons in ventral tegmental area fire selectively during the active phase of the diurnal cycle. *Euro. J. Neurosci.* 27, 408–422. doi: 10.1111/j.1460-9568.2007.05985.x

Luo, M., Guo, L., Yu, M., Jiang, W., and Wang, H. (2020). The psychological and mental impact of coronavirus disease 2019 (COVID-19) on medical staff and general public - a systematic review and meta-analysis. *Psychiatry Res.* 291:113190. doi: 10.1016/j.psychres.2020.113190

Luo, Q., Xiao, Y., Alex, A., Cummins, T. R., and Bhatwadekar, A. D. (2019). Diurnal rhythm of insulin receptor substrate-1 (IRS-1) and Kir4.1 in diabetes: implications for a Clock Gene BMAL1. *Retinal Cell Biol.* 60, 1928–1936. doi: 10.1167/iovs.18-26045

Marston, O. J., Williams, R. H., Canal, M. M., Samuels, R. E., Upton, N., and Piggins, H. D. (2008). Circadian and dark-pulse activation of orexin/hypocretin neurons. *Mol. Brain* 1:19. doi: 10.1186/1756-6606-1-19

Matsumoto, M., and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447, 1111–1115. doi: 10.1038/nature05860

Mcmahon, E. M., Corcoran, P., O'regan, G., Keeley, H., Cannon, M., Carli, V., Wasserman, C., et al. (2017). Physical activity in European adolescents and associations with anxiety, depression and well-being. *Euro. Child Adolesc. Psychiatry* 26, 111–122. doi: 10.1007/s00787-016-0875-9

Menculini, G., Verdolini, N., Murru, A., Pacchiarotti, I., Volpe, U., Cervino, A., et al. (2018). Depressive mood and circadian rhythms disturbances as outcomes of seasonal affective disorder treatment: a systematic review. *J. Affect. Disord.* 241, 608–626. doi: 10.1016/j.jad.2018.08.071

Mendoza, J. (2017). Circadian neurons in the lateral habenula: clocking motivated behaviors. *Pharmacol. Biochem. Behav.* 162, 55–61. doi: 10.1016/j.pbb.2017.06.013

Mendoza, J. (2019). Circadian insights into the biology of depression: symptoms, treatments and animal models. *Behav. Brain Res.* 376:112186. doi: 10.1016/j.bbr.2019.112186

Metzger, M., Souza, R., Lima, L. B., Bueno, D., Goncalbes, L., Sego, C., et al. (2021). Habenular connections with the dopaminergic and serotonergic system and their role in stress-related psychiatric disorders. *Euro. J. Neurosci.* 53, 65–88. doi: 10.1111/ejn.14647

Mistlberger, R. E., and Antle, M. C. (2011). Entrainment of circadian clocks in mammals by arousal and food. *Essays Biochem.* 49, 119–136. doi: 10.1042/bse0490119

Morin, C. M., Carrier, J., Bastien, C., and Godbout, R. (2020). Sleep and circadian rhythm in response to the COVID-19 pandemic. *Canad. J. Public Health* 111, 654–657. doi: 10.17269/s41997-020-00382-7

Morin, L. P. (2013). Neuroanatomy of the extended circadian rhythm system. *Exp. Neurol.* 243, 4–20. doi: 10.1016/j.expneurol.2012.06.026

Morin, L. P., and Studholme, K. M. (2014). Retinofugal projections in the mouse. *J. Comp. Neurol.* 522, 3733–3753. doi: 10.1002/cne.23635

Nair, S. G., Smirnov, D. S., Estrabrook, M. M., Chisholm, A. D., Silva, P. R., and Neumaier, J. F. (2021). Effect of chemogenetic inhibition of lateral habenula neuronal activity on cocaine- and food- seeking behaviours in the rat. *Addict. Biol.* 26:e12865. doi: 10.1111/adb.12865

Neusch, C., Papadopoulos, N., Müller, M., Maletzki, I., Winter, S. M., Hirrlinger, J., et al. (2006). Lack of the Kir4.1 channel subunit abolishes K+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K+ regulation. *J. Neurophysiol.* 95, 1843–1852. doi: 10.1152/jn.00996.2005

Newman, E. A. (1993). Inward-rectifying potassium channels in retinal glial (Muller) cells. *J. Neurosci.* 13, 3333–3345. doi: 10.1523/JNEUROSCI.13-08-03333.1993

Nuno-Perez, A., Trusel, M., Lalive, A. L., Congiu, M., Gastaldo, D., Tchenio, A., et al. (2021). Stress undermines reward-guided cognitive performance through synaptic depression in the lateral habenula. *Neuron* 109, 947–956. doi: 10.1016/j.neuron.2021.01.008

Nwaobi, S. E., Cuddapah, V. A., Patterson, K. C., Randolph, A. C., and Olsen, M. L. (2016). The role of glial-specific Kir4.1 in normal and pathological states of the CNS. *Acta Neuropathl.* 132, 1–21. doi: 10.1007/s00401-016-1553-1

Olejniczak, I., Ripperger, J. A., Sandrelli, F., Schnell, A., Mansencal-Strittmatter, L., Wendrich, K., et al. (2021). Light affects behavioral despair involving the clock gene Period 1. *PLOS Genetics* 17:e1009625. doi: 10.1371/journal.pgen.1009625

Omelchenko, N., Bell, R., and Sesack, S. R. (2009). Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area. *Euro. J. Neurosci.* 30, 1239–1250. doi: 10.1111/j.1460-9568.2009.06924.x

Paolucci, E. M., Loukov, D., Bowdish, D. M. E., and Heisz, J. J. (2018). Exercise reduces depression and inflammation but intensity matters. *Biol. Psychiatry* 133, 79–84. doi: 10.1016/j.biopsycho.2018.01.015

Park, H., Cheon, M., Kim, S., and Chung, C. (2017). Temporal variations in presynaptic release probability in the lateral habenula. *Sci. Rep.* 7:40866. doi: 10.1038/srep40866

Partch, C. L., Green, C. B., and Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends Cell Biol.* 24, 90–99. doi: 10.1016/j.tcb.2013.07.002

Partridge, B., Lucke, J., and Hall, W. (2012). Public attitudes towards the acceptability of using drugs to treat depression and ADHD. *Austr. N. Zeal. J. Psychiatry* 46, 958–965. doi: 10.1177/0004867412450755

Paul, M. J., Indic, P., and Schwartz, W. J. (2011). A role for the habenula in the regulation of locomotor activity cycles. *Euro. J. Neurosci.* 34, 478–488. doi: 10.1111/j.1460-9568.2011.07762.x

Pjerk, E., Friedrich, M.-E., Cambioli, L., Dold, M., Jager, F., Komorowski, A., et al. (2020). The efficacy of light therapy in the treatment of seasonal affective disorder: a meta-analysis of randomized controlled trials. *Psychother. Psychosom.* 89, 17–24. doi: 10.1159/000502891

Poller, W. C., Madai, V. I., Bernard, R., Laube, G., and Veh, R. W. (2013). A glutamatergic projection from the lateral hypothalamus targets VTA-projecting neurons in the lateral habenula of the rat. *Brain Res.* 1507, 45–60. doi: 10.1016/j.brainres.2013.01.029

Power, A., Hughes, A. T. L., Samuels, R. E., and Piggins, H. D. (2010). Rhythm-promoting actions of exercise in mice with deficient neuropeptide signaling. *J. Biol. Rhythms* 25, 235–246. doi: 10.1177/0748730410374446

Priest, R. G., Vize, C., Roberts, A., Roberts, M., and Tylee, A. (1996). Lay people's attitudes to treatment of depression: results of opinion poll for Defeat Depression Campaign just before its launch. *BMJ* 313, 858–859. doi: 10.1136/bmj.313.7061.858

Qu, T., Dong, K., Sugioka, K., and Yamadori, T. (1996). Demonstration of direct input from the retina to the lateral habenular nucleus in the albino rat. *Brain Res.* 709, 251–258. doi: 10.1016/0006-8993(95)01306-7

Redlin, U. (2001). Neural basis and biological function of masking by light in mammals: suppression of melatonin and locomotor activity. *Chronobiol. Int.* 18, 737–758. doi: 10.1081/CBI-100107511

Richardson, K. A., and Aston-Jones, G. (2012). Lateral hypothalamic orexin/hypocretin neurons that project to ventral tegmental area are differentially activated with morphine preference. *J. Neurosci.* 32, 3809–3817. doi: 10.1523/JNEUROSCI.3917-11.2012

Rigney, N., Beaumont, R., and Petrulis, A. (2020). Sex differences in vasopressin 1a receptor regulation of social communication within the lateral habenula and dorsal raphe of mice. *Hormones Behav.* 121:104715. doi: 10.1016/j.yhbeh.2020.104715

Rivas-Garajales, A. M., Salas, R., Robinson, M. E., Qi, K., Murrough, J. W., and Mathew, S. J. (2021). Habenula connectivity and intravenous ketamine in treatment-resistant depression. *Int. J. Neuropsychopharmacol.* 24, 383–391. doi: 10.1093/ijnp/pyaa089

Robbers, Y., Tersteeg, M. M. H., Meijer, J. H., and Coomans, C. P. (2021). Group housing and social dominance hierarchy affect circadian activity patterns in mice. *R. Soc. Open Sci.* 8:201985. doi: 10.1098/rsos.201985

Rosenwasser, A. M., Pelchat, R. J., and Adler, N. T. (1984). Memory for feeding time: Possible dependence on coupled circadian oscillators. *Physiol. Behav.* 32, 25–30. doi: 10.1016/0031-9384(84)90064-7

Rusting, C. L., and Larsen, R. J. (1998). Diurnal patterns of unpleasant mood: associations with neuroticism, depression, and anxiety. *J. Person.* 66, 85–103. doi: 10.1111/1467-6494.00004

Sakhi, K., Belle, M. D. C., Gossan, N., Delagrange, P., and Piggins, H. D. (2014a). Daily variation in the electrophysiological activity of mouse medial habenula neurones. *J. Physiol.* 592, 587–603. doi: 10.1113/jphysiol.2013.263319

Sakhi, K., Wegner, S., Belle, M. D. C., Howarth, M., Delagrange, P., Brown, T. M., et al. (2014b). Intrinsic and extrinsic cues regulate the daily profile of mouse lateral habenula neuronal activity. *J. Physiol.* 592, 5025–5045. doi: 10.1113/jphysiol.2014.280065

Sartorius, A., and Henn, F. A. (2007). Deep brain stimulation of the lateral habenula in treatment resistant major depression. *Med. Hypotheses* 69, 1305–1308. doi: 10.1016/j.mehy.2007.03.021

Sartorius, A., Kiening, K. L., Kirsch, P., Von Gall, C. C., Haberkorn, U., Unterberg, A. W., et al. (2010). Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biol. Psychiatry* 67, e9–e11. doi: 10.1016/j.biopsych.2009.08.027

Schuch, F. B., and Stubbs, B. (2019). The role of exercise in preventing and treating depression. *Curr. Sports Med. Rep.* 18, 299–304. doi: 10.1249/JSR.0000000000000620

Sego, C., Goncalves, L., Lima, L., Furigo, I. C., Donato Jr, J., and Metzger, M. (2014). Lateral habenula and the rostromedial tegmental nucleus innervate neurochemically distinct subdivisions of the dorsal raphe nucleus in the rat. *J. Comp. Neurol.* 522, 1454–1484. doi: 10.1002/cne.23533

Seo, J.-S., Zhong, P., Liu, A., Yan, Z., and Greengard, P. (2018). Elevation of p11 in lateral habenula mediates depression-like behavior. *Mol. Psychiatry* 23, 1113–1119. doi: 10.1038/mp.2017.96

Shabel, S. J., Proulx, C. D., Piriz, J., and Malinow, R. (2014). GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment. *Science* 345, 1494–1498. doi: 10.1126/science.1250469

Shabel, S. J., Proulx, C. D., Trias, A., Murphy, R. T., and Malinow, R. (2012). Input to the lateral habenula from the basal ganglia is excitatory, aversive and suppressed by serotonin. *Neuron* 74, 475–481. doi: 10.1016/j.neuron.2012.02.037

Shabel, S. J., Wang, C., Monk, B., Aronson, S., and Malinow, R. (2019). Stress transforms lateral habenula reward responses into punishment signals. *Proc. Natl. Acad. Sci. U.S.A.* 116, 12488–12493. doi: 10.1073/pnas.1903334116

Shieh, K. R. (2003). Distribution of the rhythm-related genes rPERIOD1, rPERIOD2 and rCLOCK in the rat brain. *Neuroscience* 118, 831–843. doi: 10.1016/S0306-4522(03)00004-6

Sidor, M. M., Spencer, S. M., Dzirasa, K., Parekh, P. K., Tye, K. M., Warden, M. R., et al. (2016). Daytime spikes in dopaminergic activity drive rapid mood-cycling in mice. *Mol. Psychiatry* 20, 1406–1419. doi: 10.1038/mp.2014.167

Smagula, S. F., Capps, C. S., and Krafty, R. T. (2021). Evaluating the timing of differences in activity related to depression symptoms across adulthood in the United States. *J. Affect. Disord.* 284, 64–68. doi: 10.1016/j.jad.2021.01.069

Smith, R. J., Vento, P. J., Chao, Y. S., Good, C. H., and Jhou, T. C. (2019). Gene expression and neurochemical characterization of the rostromedial tegmental nucleus in rats and mice. *Brain Struct. Funct.* 224, 219–238. doi: 10.1007/s00429-018-1761-7

Stamatakis, A. M., Jennings, J. H., Ung, R. L., Blair, G. A., Weinberg, R. J., Neve, R. L., et al. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* 80, 1039–1053. doi: 10.1016/j.neuron.2013.08.023

Stamatakis, A. M., and Stuber, G. D. (2012). Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. *Nat. Neurosci.* 24, 1105–1107. doi: 10.1038/nn.3145

Stamatkis, A. M., Swieten, M. V., Basiri, M. L., Blair, G. A., Kanatak, P., and Stuber, G. D. (2016). Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward. *J. Neurosci.* 36, 301–311. doi: 10.1523/JNEUROSCI.1202-15.2016

Stephenson-Jones, M., Yu, K., Ahrens, S., Tucciarone, J. M., Van Huijstee, A. N., Mejia, L. A., et al. (2016). A basal ganglia circuit for evaluating action outcomes. *Nature* 539, 289–293. doi: 10.1038/nature19845

Stern, W. C., Johnson, A., Bonzino, J. D., and Morgane, P. J. (1979). Effects of electrical stimulation of the lateral habenula on single-unit activity of raphe neurons. *Exp. Neurol.* 65, 326–342. doi: 10.1016/0014-4886(79)90102-X

Stopper, C. M., Tse, M. T. L., Montes, D. R., Wiedman, C. R., and Floresco, S. B. (2014). Overriding phasic dopamine signals redirects action selection during risk/reward decision making. *Neuron* 84, 177–189. doi: 10.1016/j.neuron.2014.08.033

Stow, L. R., Richards, J., Cheng, K.-Y., Lynch, I. J., Jeffers, L. A., Greenlee, M. M., et al. (2012). The circadian protein period 1 contributes to blood pressure control and coordinately regulates renal sodium transport genes. *Hypertension* 59, 1151–1156. doi: 10.1161/HYPERTENSIONAHA.112.190892

Sutherland, R. J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neurosci. Biobehav. Rev.* 6, 1–13. doi: 10.1016/0149-7634(82)90003-3

Swanson, L. M., Burgess, H. J., Huntley, E. D., Bertram, H., Mooney, A., Zollars, J., et al. (2017). Relationships between circadian measures, depression, and response to antidepressant treatment: a preliminary investigation. *Psychiatry Res.* 252, 262–269. doi: 10.1016/j.psychres.2017.03.010

Szonyi, A., Zicho, K., Barth, A. M., Gonczi, R. T., Schlingloff, D., Torok, B., et al. (2019). Median raphe controls acquisition of negative experience in the mouse. *Science* 366:aay8746. doi: 10.1126/science.aay8746

Ter Horst, G. J., and Luiten, P. G. M. (1986). The projections of the dorsomedial hypothalamic nucleus in the rat. *Brain Res. Bull.* 16, 231–248. doi: 10.1016/0361-9230(86)90038-9

Timothy, J. W. S., Klas, N., Sanghani, H. R., Al-Mansouri, T., Hughes, A. T. L., Kirshenbaum, G. S., et al. (2018). Circadian disruptions in the myshkin mouse model of mania are independent of deficits in suprachiasmatic molecular clock function. *Biol. Psychiatry* 84, 827–837. doi: 10.1016/j.biopsych.2017.04.018

Tolles-Correia, D., Saravia, S., and Goncalves, J. (2018). Mental disorder—the need for an accurate definition. *Front. Psychiatry* 9:64. doi: 10.3389/fpsyt.2018.00064

Tong, X., Ao, Y., Faas, G. C., Nwaobi, S. E., Xu, J., Haustein, M. D., et al. (2014). Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nat. Neurosci.* 17, 694–703. doi: 10.1038/nn.3691

Tooley, J., Marconi, L., Alipio, J. B., Matikainen-Ankey, B., Georgiou, P., Kravitz, A., et al. (2018). Glutamatergic ventral pallidal neurons modulate activity of the habenula-tegmental circuitry and constrain reward seeking. *Biol. Psychiatry* 83, 1012–1023. doi: 10.1016/j.biopsych.2018.01.003

Valentinova, K., Tchenio, A., Trusel, M., Clerke, J. A., Lalive, A. L., Tzanoulinou, S., et al. (2019). Morphine withdrawal recruits lateral habenula cytokine signaling to reduce synaptic excitation and sociability. *Nat. Neurosci.* 22, 1053–1056. doi: 10.1038/s41593-019-0421-4

Vermeulen, E., Stronks, K., Snijder, M. B., Schene, A. H., Lok, A., De Vries, J. H., et al. (2017). A combined high-sugar and high-saturated-fat dietary pattern is associated with more depressive symptoms in a multi-ethnic population: the HELIUS (Healthy Life in an Urban Setting) study. *Public Health Nutr.* 20, 2374–2382. doi: 10.1017/S1368980017001550

Wagner, F., French, L., and Veh, R. W. (2016). Transcriptomic-anatomic analysis of the mouse habenula uncovers a high molecular heterogeneity among neurons in the lateral complex, while gene expression in the medial complex largely obeys subnuclear boundaries. *Brain Struct. Funct.* 221, 39–58. doi: 10.1007/s00429-014-0891-9

Wagner, F., Stroh, T., and Veh, R. W. (2014). Correlating habenular subnuclei in rat and mouse by using topographic, morphological and cytochemical criteria. *J. Comp. Neurol.* 522, 2650–2662. doi: 10.1002/cne.23554

Wang, D., Li, A., Dong, K., Li, H., Guo, Y., Zhang, X., et al. (2021). Lateral hypothalamus orexinergic inputs to lateral habenula modulate maladaptation after social defeat stress. *Neurobiol. Stress* 14:100298. doi: 10.1016/j.ynstr.2021.100298

Webb, I. C., Baltazar, R. M., Wang, X., Pitchers, K. K., Coolen, L. M., and Lehman, M. N. (2009). Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. *J. Biol. Rhythms* 24, 465–476. doi: 10.1177/0748730409346657

Weiss, T., and Veh, R. W. (2011). Morphological and electrophysiological characteristics of neurons within identified subnuclei of the lateral habenula in rat brain slices. *Neuroscience* 172, 74–93. doi: 10.1016/j.neuroscience.2010.10.047

Wirz-Justice, A. (2008). Diurnal variation of depressive symptoms. *Dial. Clin. Neurosci.* 10, 337–343. doi: 10.31887/DCNS.2008.10.3/awjustice

Wulff, K., Gatti, S., Wettstein, J. G., and Foster, R. G. (2010). Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nat. Rev. Neurosci.* 11, 589–599. doi: 10.1038/nrn2868

Yan, L., and Silver, R. (2002). Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. *Euro. J. Neurosci.* 16, 1531–1540. doi: 10.1046/j.1460-9568.2002.02224.x

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., et al. (2018a). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature* 554, 317–322. doi: 10.1038/nature25509

Yang, Y., Wang, H., Hu, J., and Hailan, H. (2018b). Lateral habenula in the pathophysiology of depression. *Curr. Opin. Neurobiol.* 48, 90–96. doi: 10.1016/j.conb.2017.10.024

Yeoh, J. W., James, M. H., Adams, C. D., Bains, J. S., Sakuri, T., Aston-Jones, G., et al. (2019). Activation of lateral hypothalamic group III metabotropic glutamate receptors suppresses cocaine-seeking following abstinence and normalizes drug-associated increases in excitatory drive to orexin/hypocretin cells. *Neuropharmacology* 154, 22–33. doi: 10.1016/j.neuropharm.2018.09.033

Zanos, P., and Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Mol. Psychiatry* 23, 801–811. doi: 10.1038/mp.2017.255

Zerssen, D. V., Bathelmes, H., Dirlich, G., Doerr, P., Emrich, H. M., Von Lindern, L., et al. (1985). Circadian rhythms in endogenous depression. *Psychiatry Res.* 16, 51–63. doi: 10.1016/0165-1781(85)90028-9

Zhang, C., Truong, K. K., and Zhou, Q.-Y. (2009). Efferent projections of prokineticin 2 expressing neurones in the mouse suprachiasmatic nucleus. *PLoS ONE* 4:e7151. doi: 10.1371/journal.pone.0007151

Zhang, J., Tan, L., Ren, Y., Liang, J., Lin, R., Feng, Q., et al. (2016). Presynaptic excitation via GABAB receptors in habenula cholinergic neurons regulates fear memory expression. *Cell* 166, 716–728. doi: 10.1016/j.cell.2016.06.026

Zhao, H., and Rusak, B. (2005). Circadian firing-rat rhythms and light responses of rat habenular nuclei *in vivo* and *in vitro*. *Neuroscience* 132, 519–528. doi: 10.1016/j.neuroscience.2005.01.012

Zhou, Q.-Y., and Cheng, M. Y. (2005). Prokineticin 2 and circadian clock output. *FEBS J.* 272, 5703–5709. doi: 10.1111/j.1742-4658.2005.04984.x

Zhu, Y., Qi, S., Zhang, B., He, D., and Teng, Y. (2019). Connectome-based biomarkers predict subclinical depression and identify abnormal brain connections with the lateral habenula and thalamus. *Front. Psychiatry* 10:371. doi: 10.3389/fpsyt.2019.00371

Zhuo, C., Tian, H., Li, G., Chen, M., Jiang, D., Lin, X., et al. (2019). Effects of ketamine on circadian rhythm and synaptic homeostasis in patients with treatment-resistant depression: a protocol for mechanistic studies of its rapid and sustained antidepressant actions in humans. *Brain Behav.* 9:e01423. doi: 10.1002/brb3.1423

Zuo, W., Zhang, Y., Xie, G., Gregor, D., Bekker, A., and Ye, J.-H. (2016). Serotonin stimulates lateral habenula via activation of the post-synaptic serotonin 2/3 receptors and transient receptor potential channels *Neuropharmacology* 101, 449–459. doi: 10.1016/j.neuropharm.2015.10.012

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Psychiatry

BRIEF RESEARCH REPORT
published: 04 February 2022
doi: 10.3389/fpsyt.2022.810777

Habenular Involvement in Response to Subcallosal Cingulate Deep Brain Stimulation for Depression

Gavin J. B. Elias[1†], *Jürgen Germann*[1†], *Aaron Loh*[1], *Alexandre Boutet*[1,2], *Aditya Pancholi*[1], *Michelle E. Beyn*[1], *Venkat Bhat*[3], *D. Blake Woodside*[4], *Peter Giacobbe*[5], *Sidney H. Kennedy*[4,6] *and Andres M. Lozano*[1,6*]

[1] Division of Neurosurgery, Department of Surgery, University Health Network and University of Toronto, Toronto, ON, Canada, [2] Joint Department of Medical Imaging, University of Toronto, Toronto, ON, Canada, [3] Centre for Mental Health and Krembil Research Centre, University Health Network, Toronto, ON, Canada, [4] Centre for Mental Health, University Health Network, Toronto, ON, Canada, [5] Department of Psychiatry, Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, Toronto, ON, Canada, [6] Krembil Research Institute, University of Toronto, Toronto, ON, Canada

OPEN ACCESS

Edited by:
André Schmidt,
University of Basel, Switzerland

Reviewed by:
Chencheng Zhang,
Shanghai Jiao Tong University, China
Hiroyuki Ichijo,
University of Toyama, Japan
Hua Zhao,
Jilin University, China

***Correspondence:**
Andres M. Lozano
lozano@uhnresearch.ca

[†]*These authors have contributed equally to this work*

Specialty section:
This article was submitted to Neuroimaging and Stimulation, a section of the journal Frontiers in Psychiatry

Received: *07 November 2021*
Accepted: *03 January 2022*
Published: *04 February 2022*

Citation:
Elias GJB, Germann J, Loh A, Boutet A, Pancholi A, Beyn ME, Bhat V, Woodside DB, Giacobbe P, Kennedy SH and Lozano AM (2022) Habenular Involvement in Response to Subcallosal Cingulate Deep Brain Stimulation for Depression. Front. Psychiatry 13:810777. doi: 10.3389/fpsyt.2022.810777

The habenula (Hb) is a small, evolutionarily conserved epithalamic structure implicated in functions such as reward and mood regulation. Prior imaging work suggests that Hb's structural and functional properties may relate to treatment response in depression and other mood disorders. We used multimodal MRI techniques to investigate the potential involvement of Hb in response to subcallosal cingulate area deep brain stimulation (SCC-DBS) for treatment-resistant mood disorders. Using an automated segmentation technique, we compared Hb volume at baseline and at a subsequent post-operative timepoint (4.4 ± 3.0 years after surgery) in a cohort of 32 patients who received SCC-DBS. Clinical response to treatment (≥50% decrease in HAMD-17 from baseline to 12 months post-operation) was significantly associated with longitudinal Hb volume change: responders tended to have increased Hb volume over time, while non-responders showed decreased Hb volume ($t = 2.4$, $p = 0.021$). We additionally used functional MRI (fMRI) in a subcohort of SCC-DBS patients ($n = 12$) to investigate immediate within-patient changes in Hb functional connectivity associated with SCC-DBS stimulation. Active DBS was significantly associated with increased Hb connectivity to several prefrontal and corticolimbic regions (TFCE-adjusted $p_{Bonferroni} < 0.0001$), many of which have been previously implicated in the neurocircuitry of depression. Taken together, our results suggest that Hb may play an important role in the antidepressant effect of SCC-DBS.

Keywords: habenula, depression, deep brain stimulation, neuroimaging, neuromodulation, treatment biomarker

INTRODUCTION

The habenula (Hb) is a small, bilateral, and highly evolutionarily preserved structure situated in the epithalamus (1, 2). Known to play a key role in the regulation of brainstem monoaminergic systems, Hb is broadly implicated in reward processing, social interaction, behavioral adaptation, circadian rhythm, and sensory integration (2–11). It has also been linked to several neuropsychiatric disorders, particularly depression and other mood disorders (3, 7, 9, 12–19). Animal studies have shown that both Hb activity and metrics of reward processing and motivational behavior

are altered in models of depression (2, 5, 20, 21). Volumetric studies in humans have likewise detected altered habenula volume in individuals with depression, bipolar disorder, schizophrenia, and autism spectrum disorder (12, 19, 22, 23).

There is also evidence to suggest that Hb plays a role in subserving treatment response in mood disorders. For instance, Hb volume changes have been observed in bipolar disorder (BD) and major depressive disorder (MDD) patients who received pharmacotherapy but not in medication-naïve patients (23). Other studies report response-related changes in Hb functional connectivity in MDD, patients following treatment with electroconvulsive therapy (24) or ketamine (24, 25). Baseline structural and functional Hb connectivity patterns have moreover been shown to predict response (75% sensitivity; 72% specificity) to inpatient treatment in a large MDD cohort (26).

Deep brain stimulation (DBS) is a neuromodulatory technique that employs surgically implanted electrodes to deliver carefully titrated electrical pulses to a specific brain region to modulate brain activity (27, 28). DBS targeting the subcallosal cingulate area (SCC-DBS) is a promising treatment for a variety of psychiatric disorders, including MDD, BD, and anorexia nervosa (AN) (29–39). Evidence from positron emission tomography studies indicates that SCC-DBS not only affects the focal target region but alters activity across distributed circuits in the brain (29, 30, 36). In this study, we employed multimodal MRI techniques to investigate Hb involvement in clinical response to SCC-DBS. Given the putative involvement of Hb in response to antidepressant pharmacotherapy and ECT, we looked at baseline and longitudinal Hb volume in a SCC-DBS cohort, exploring how these variables might relate to clinical outcome. Additionally, in a subcohort of SCC-DBS patients with post-operative functional imaging, we explored how Hb functional connectivity is acutely modified by stimulation.

METHODS

Design and Patients

This study involved analysis of both retrospectively and prospectively acquired imaging data in psychiatric patients—diagnosed with either major depressive disorder (MDD), bipolar disorder (BD), or anorexia nervosa (AN)—who underwent SCC-DBS therapy for management of depressive symptoms. The eligibility criteria, electrode implantation methods, and post-operative device programming procedure have been previously described (30, 31, 35, 36). All patients received high frequency (130 Hz) stimulation with conventional pulse width settings (60–90 μs). The amplitude/voltage of stimulation and configuration of active electrode contacts were individualized for each patient.

The retrospective component of this study, conducted following institutional research ethics board approval (University Health Network ID: #15-9777), involved review of clinical charts and available structural MR imaging. In keeping with prior retrospective DBS imaging work conducted at our institution (40), we included all patients for whom sufficient clinical data [baseline pre-operative and 12-month follow-up scores on the 17-item Hamilton rating scale for depression (HAMD-17)] and structural MR imaging (pre-operative high-quality scans with complete brain coverage) were available, as long as they had not previously undergone other neurosurgical interventions or received confounding pre- or post-DBS neurological diagnoses. Immediate and—where available—later post-operative structural MR images meeting the aforementioned standards were also collected. This permitted longitudinal analysis of post-DBS brain volume changes in patients for whom ≥2 post-operative scans were available.

The prospective component of this study was conducted with institutional REB approval (University Health Network ID: #14-8255) as part of a publicly registered clinical trial (ClinicalTrials.gov ID: NCT03153670). Here, SCC-DBS patients who were implanted with specific hardware and who were actively using their devices were recruited for functional MRI (fMRI) scanning.

HAMD-17 scores were employed as an index of depression symptom severity. For each patient, percentage improvement from baseline at the 12-month timepoint was computed. In accordance with prior studies, patients with ≥ 50% HAMD-17 reduction were categorized as "responders" (29, 30, 35, 41).

MRI Acquisition

T1-weighted structural MR imaging (**Supplementary Table 1**) was obtained in all SCC-DBS patients prior to and immediately following DBS implantation to guide surgical planning and confirm electrode placement, respectively (**Figure 1A**). Additional post-operative structural images were obtained at various later timepoints for clinical purposes or as part of prospective fMRI scanning (see below).

Building on extensive institutional safety testing (43, 44) and prior fMRI scanning in a large cohort of DBS patients (45, 46), we also prospectively obtained 3 Tesla fMRI scans in a subset of SCC-DBS patients following electrode implantation (1.5–13.5 years post-implantation). Only patients who were fully implanted with specific Medtronic DBS hardware (3387 quadripolar leads, 37601 Activa PC, or 37612 Activa RC implantable pulse generators, and 37086 or 7482 extension wires) were considered to be eligible for scanning. The full fMRI paradigm employed here has been described previously (47). Briefly, resting state fMRI (rsfMRI) sequences (**Supplementary Table 2**) were acquired for each patient while their DBS device was turned on at clinically defined "optimal" settings (DBS-ON) and while it was turned off (DBS-OFF). These sequences, along with a structural scan to facilitate image registration, were acquired in a single MRI session. After changing an individual's DBS settings, we observed a 5-min washout period before beginning the rsfMRI scan.

Habenular Segmentation and Volumetric Analysis

Using the minc-bpipe preprocessing pipeline (https://github.com/CoBrALab/minc-bpipe-library), all structural MR images were iteratively corrected for non-uniformity, skull-stripped, and rigidly aligned to Montreal Neurological Institute space (MNI 152 ICBM 2009b NLIN asymmetric) without resampling. Subsequently, the bilateral Hb (**Figure 1B**) was segmented on every image using the automated Multiple Automatically Generated Templates (MAGeT) brain segmentation algorithm

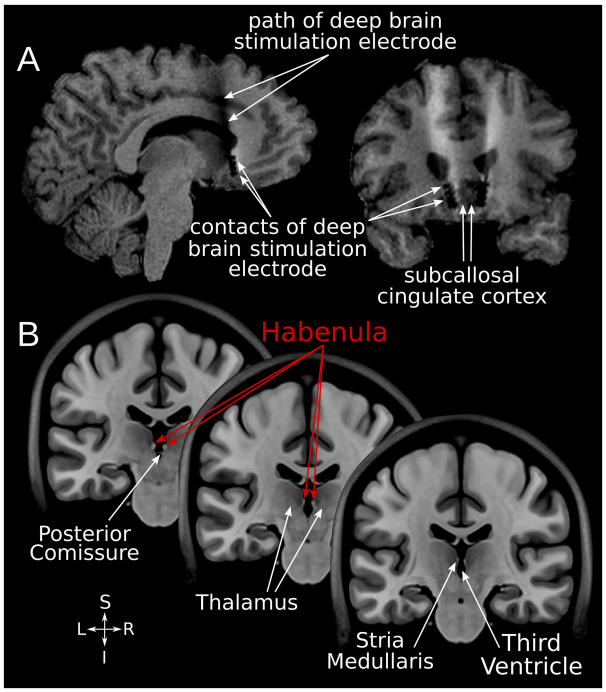

FIGURE 1 | SCC-DBS and the habenula. **(A)** Sagittal and coronal structural T1-weighted MRI slices from an exemplar patient showing the position of the implanted bilateral electrodes. These are placed in the white matter bordering the subcallosal cingulate cortex. **(B)** Coronal slices of the asymmetrical MNI152 template (42) illustrating the location of the habenula and surrounding structures. The habenula appears bright (hyperintense) on T1-weighted MRI. *DBS*, deep brain stimulation; *MNI*, Montreal Neurological Institute; *SCC*, subcallosal cingulate area.

(https://github.com/CoBrALab/MAGeTbrain) (12, 48). The hippocampus and its subfields were also segmented bilaterally using MAGeT in order to assess the specificity of any volumetric findings. MAGeT segments structures of interest on individual input images in a contrast-driven fashion *via* registration-based label propagation. It has been shown to be robust for various anatomical structures and subject populations in prior work (12, 19, 48, 49). Specifically, five manually segmented high-resolution atlases are propagated using 21 template images selected from the input dataset, yielding a large number ($5 \times 21 = 105$) of candidate segmentations; these are then fused using a majority vote approach to generate final individualized segmentations. The use of the template library helps to reduce atlas bias and also diminishes registration errors by averaging (49). For quality assurance, each Hb segmentation label was overlaid on the corresponding structural image with DISPLAY (https://www.mcgill.ca/bic/software/minc/minctoolkit) and visually inspected by two raters (JG and GJBE).

FMRI Functional Connectivity Analysis

Preprocessing of rsfMRI data was conducted with the BRANT toolbox (http://brant.brainnetome.org/) (50). After removing the first 10 volumes, each fMRI sequence was (i) corrected for head motion using volume realignment; (ii) nonlinearly normalized to MNI152 space *via* coregistration to the corresponding structural scan; (iii) resampled to $3 \times 3 \times 3$ mm^3; (iv) denoised for nuisance variables using a multiple regression model (51); (v) filtered with a temporal bandpass filter (0.01–0.08 Hz); (vi) smoothed with a 6 mm full width at half maximum gaussian kernel. In order to account for the artifact created by the DBS leads and extension wire (45), affected voxels in each individual fMRI image were manually segmented with DISPLAY (https://github.com/BIC-MNI/minc-tools). A group summation map incorporating these individual artifact masks was then created in MNI space; any voxels overlapping this summation map were excluded from further analysis. Finally, the brain-wide functional connectivity of the Hb was examined in each preprocessed rsfMRI image. To do so, we computed Pearson correlations between the average blood oxygen level-dependent (BOLD) time course within a bilateral Hb seed (**Supplementary Figure 2**) and the BOLD time course of all other voxels in the brain. The resulting connectivity r-maps were then Fisher transformed to z-maps, in which each voxel's value reflected the strength of connectedness between that voxel and the Hb.

Statistics

The relationship between Hb volume (as derived from MAGeT segmentations) and clinical response status (responder vs. non-responder) was investigated both at pre-operative baseline and longitudinally following DBS implantation. Baseline Hb volume was compared between responders and non-responders using a linear model that controlled for each patient's whole-brain volume (Hb volume $\sim$ response status + whole-brain volume). The interaction between patient-specific Hb volume change over time and response status was investigated *via* a linear mixed-effect model with patient as random intercept [Hb volume $\sim$ time $\times$ response status + (1 | patient)]. For the fMRI analysis, changes in Hb functional connectivity between each patient's DBS-ON and DBS-OFF states were examined using voxel-wise paired-tests. Threshold-free cluster enhancement (TFCE) (52) and Bonferroni correction ($p_{\text{Bonferroni}} < 0.0001$) were applied to the resulting *t*-map to correct for multiple corrections across the brain. All statistical analyses were performed using R [version 3.6.1; https://www.r-project.org, including the lme4 (version 1.1-21) and lmerTest (version 3.1.1) packages] and RMINC (https://github.com/Mouse-Imaging-Centre/RMINC).

RESULTS

Overall, 86 SCC-DBS patients, 54 (62.8%) of whom were responders, were included for retrospective analysis of baseline pre-operative Hb volume (**Table 1**). From this number, 32 patients with serial post-operative structural MR images (80 images overall), 22 (68.8%) of whom were responders, were included for retrospective volumetric analysis (mean duration between baseline and latest post-operative images = 4.4 $\pm$ 3.0 years). Twelve of these patients, nine (75.0%) of whom were responders, were also prospectively scanned with 3T fMRI in both DBS-ON and DBS-OFF conditions (mean duration between

TABLE 1 | Demographics, baseline clinical characteristics, and clinical outcome.

Cohort	Age at surgery, mean (SD), years	Sex	Baseline HAMD-17 score, mean (SD)	Disease duration at surgery, mean (SD), years	Number of patients by diagnosis (%)	Number of responders (%)	HAMD-17 percentage reduction from baseline, mean (SD)
Baseline volume analysis ($n = 86$)	43.8 (10.4)	61 f, 25 m	24.5 (4.9)	22.2 (9.5)	*MDD*: 65 (75.6) *BD*: 4 (4.7) *AN*: 17 (19.8)	54 (62.8)	53.6 (27.1)
Longitudinal volume analysis ($n = 32$)	40.2 (10.7)	26 f, 6 m	23.3 (5.9)	19.7 (7.2)	*MDD*: 16 (50.0) *BD*: 2 (6.3) *AN*: 14 (43.8)	22 (68.8)	54.4 (30.4)
fMRI analysis ($n = 12$)	34.6 (10.1)	11 f, 1 m	23.4 (5.4)	15.7 (7.0)	*MDD*: 3 (25.0) *BD*: 1 (8.3) *AN*: 8 (66.7)	9 (75.0)	61.1 (26.3)

Responder status and HAMD-17 reduction reported as of 12 months post-operative follow-up. AN, anorexia nervosa; BD, bipolar disorder; HAMD-17, 17-item Hamilton rating scale for depression; MDD, major depressive disorder.

surgery and fMRI acquisition = 5.6 ± 3.2 years). A study flowchart is provided in the **Supplementary Figure 1**.

No significant difference in baseline pre-operative Hb volume was apparent between eventual responders (mean = 30.5 ± 3.4 mm^3) and non-responders (mean = 30.9 ± 4.9 mm^3) ($t = -0.8$, $p = 0.940$). However, analysis of longitudinal Hb volume change revealed a significant interaction effect between volume change and response status, with bilateral Hb volume increasing following SCC-DBS surgery in responders but decreasing in non-responders ($t = 2.4$, $p = 0.021$; **Figure 2**). There was no significant difference between longitudinal cohort responders and non-responders in terms of age (responders: mean = 40.0 ± 10.3; non-responders: mean = 40.7 ± 12.1) or proportion of females (responders: 81.8%; non-responders: 80.0%). No response-related differences in hippocampal volume were detected, either at baseline or longitudinally.

Comparison of fMRI-derived functional connectivity maps between DBS-ON and DBS-OFF states uncovered a number of brain regions whose connectedness to the Hb was significantly (TFCE-adjusted $p_{\text{Bonferroni}} < 0.0001$) altered by SCC-DBS stimulation. Specifically, active stimulation appeared to increase Hb functional connectivity with several prefrontal and corticolimbic regions, including rostral and dorsal anterior cingulate cortex (ACC), posterior cingulate cortex (PCC), medial prefrontal cortex, and dorsolateral prefrontal cortex (dlPFC). In addition, increased and decreased Hb connectivity was noted with superior temporal gyrus and fusiform gyrus, respectively (**Figure 3**). Due to the small number of patients included in the prospective fMRI analysis ($n = 12$), we were unable to conduct a comparison of habenular connectivity changes between responders and non-responders.

DISCUSSION

The present study employed multimodal MRI techniques to specifically investigate the involvement of the habenula (Hb) in clinical response to SCC-DBS. Evidence supporting such an involvement was observed in two ways: (i) longitudinal Hb volume change following DBS treatment differed in SCC-DBS responders compared to non-responders; (ii) active SCC-DBS stimulation acutely modulated Hb's functional connectivity to a number of regions that are implicated in brain-wide depression networks (54–57). Building on existing ECT, ketamine, and standard pharmacotherapy imaging work (23, 25), these results strengthen the notion that Hb plays an important role in subserving clinical response to a variety of antidepressant therapies. They also fit with preliminary evidence that DBS directly targeting Hb itself may be a useful therapy for depression (58, 59) and various other refractory neuropsychiatric disorders (60).

Our MRI-based volumetric analysis demonstrated that the trajectory of Hb volume change following SCC-DBS differed according to individual treatment response. Specifically, clinical responders (patients who experienced ≥ 50% symptom reduction) tended to exhibit increased habenular volume over time, while non-responders showed the opposite trend. Long-term volume alterations have been previously demonstrated in patients receiving DBS for various indications including Parkinson's and Alzheimer's disease (61, 62), suggesting that part of this intervention's therapeutic effect is mediated by neuroplastic changes. Indeed, a prior data-driven study—using a different method for volumetric analysis—in this same SCC-DBS patient population (55) identified other (mostly cortical) regions whose trajectories of volume change over time corresponded to patient outcome. The phenomenon of Hb volume changes in the context of psychiatric interventions is also supported by existing evidence. Work by Savitz et al. (23) indicates that antidepressant and/or mood stabilizing pharmacotherapy can also lead to Hb volume increases, suggesting that this may be a common marker of clinical response.

Using 3T fMRI, we additionally observed that acute SCC-DBS was associated with immediate changes in Hb functional connectivity. These changes occurred within minutes of switching between DBS-ON and DBS-OFF states, and

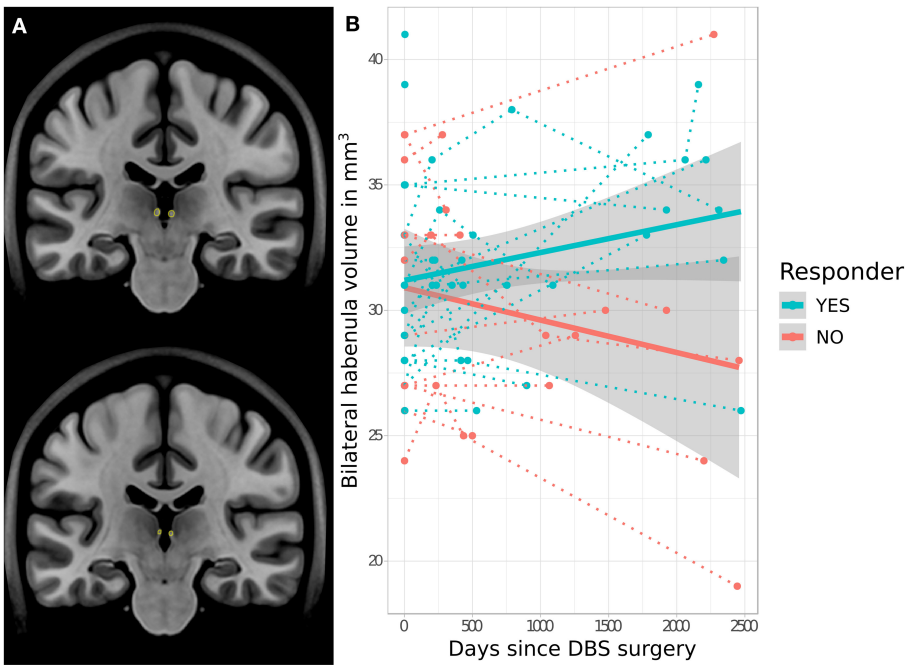

FIGURE 2 | Response-related trajectories of change in habenular volume over time following SCC-DBS. **(A)** Outline of the habenula—as per automated MAGeT segmentation—shown on two different coronal slices of the MNI152 template brain for illustrative purposes. **(B)** Changes in bilateral habenula volume over time following SCC-DBS surgery are shown for each patient (follow-up is cut off at 2,500 days post-surgery for visualization purposes, although some post-operative scans were acquired at later timepoints). The dotted lines indicate the change in habenular volume over time in individual patients, while the thick solid lines indicate the estimated change in habenular volume over time for each cohort overall. The gray shaded zones surrounding the solid lines denote the 95% confidence interval. A significant difference in trajectory of change was found in responders (≥50% HAMD improvement) compared to non-responders: habenula volume decreased over time in non-responders but increased in responders ($t = 2.4$, $p < 0.021$ for interaction of time and response status). *DBS*: deep brain stimulation; *HAMD-17*: 17-item Hamilton rating scale for depression; *MNI*, Montreal Neurological Institute; *SCC*, subcallosal cingulate area.

might be mediated by direct projections between Hb and the cingulate cortex and medial prefrontal region, which have been demonstrated in rodent tracer studies (63). Active SCC stimulation generally increased Hb connectivity with a number of depression-implicated brain areas such as the anterior (specifically rostral and dorsal anterior cingulate cortex, lying outside of the direct stimulation target area) and posterior cingulate cortices, medial prefrontal cortex, dorsolateral prefrontal cortex, superior temporal gyrus, and fusiform gyrus. Many of these, including posterior cingulate cortex (64), superior and middle temporal gyrus (65–68), medial prefrontal cortex (69, 70), and fusiform gyrus (71), are heavily implicated in depression and mood regulation. The rostral and dorsal anterior cingulate activity in particular have been identified as key predictors of pharmacotherapy success (72, 73). Moreover, previous voxel-wise, data-driven fMRI analyses of an overlapping SCC-DBS cohort detected significant amplitude of low frequency fluctuations (ALFF; a measure of spontaneous neural activity) alterations in dorsal anterior cingulate as well as posterior cingulate when comparing DBS-OFF and DBS-ON conditions (47). Dorsolateral prefrontal cortex hypoactivity in depression is substantiated by the antidepressant effect of repetitive transcranial magnetic stimulation (rTMS) targeting this area (74–77). Interestingly, the degree of symptom improvement following dorsolateral prefrontal cortex rTMS has been linked to the functional connectivity between this region and SCC (78). Overall, our observation that therapeutic stimulation alters Hb connectivity with these mood-implicated areas tentatively positions Hb as a key player in the brain-wide network of depression (49–52). In particular, Hb may be important for mediating the expression of anhedonia across these circuits (79).

Limitations

This study identified significant post-DBS changes in Hb volume that were not appreciated in a prior volumetric analysis of the same patient cohort (55). This apparent discrepancy may be explained by several factors, including different experimental designs and different methods used to discern longitudinal volume change. The former paper employed a data-driven, hypothesis-free approach (deformation-based morphometry) in which statistical tests were conducted at the voxel level, necessitating stringent multiple comparison correction. By contrast, the current study used the MAGeT segmentation algorithm to specifically estimate bilateral Hb volume in an *a priori* fashion. Finally, while our analyses are novel, they were conducted in relatively small-to-moderately sized patient cohorts ($n = 32$ for the volumetric analysis; $n = 12$ for the fMRI analysis).

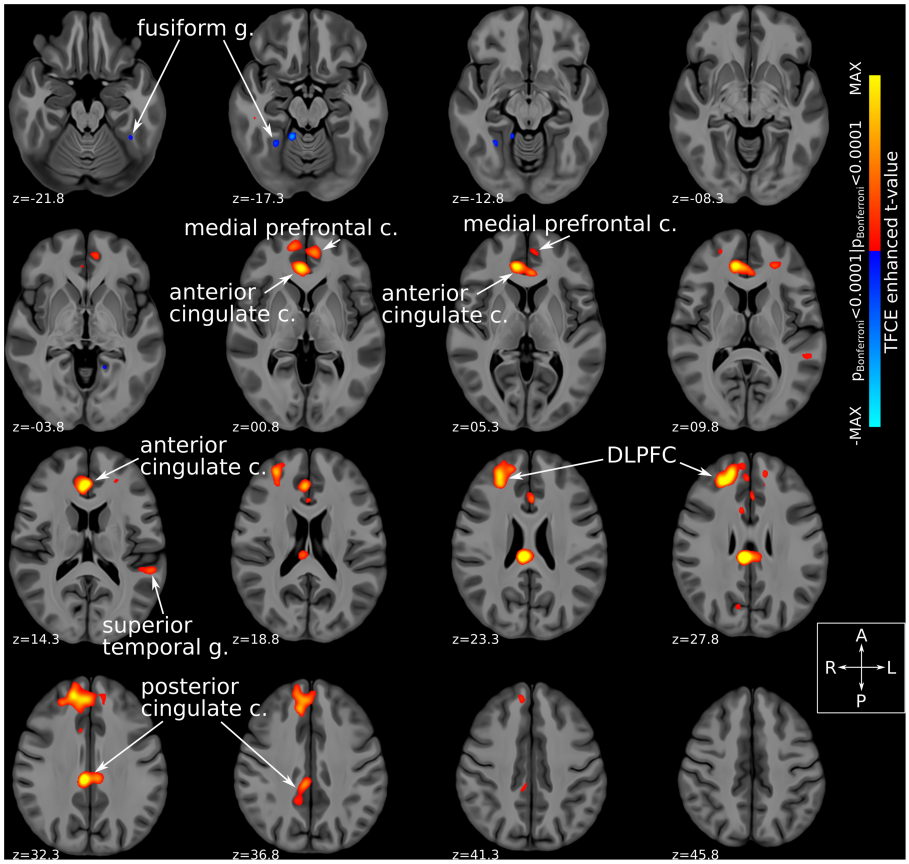

FIGURE 3 | Habenular functional connectivity changes with active SCC-DBS. Change in habenular functional connectivity between DBS-ON and DBS-OFF states. Brain areas that showed a significant change in functional connectivity [TFCE-adjusted $p_{Bonferroni} < 0.0001$ (52)] between these states are superimposed on axial slices of a high resolution, high contrast brain template (53) in MNI standard space (42). Red/yellow colors denote areas that were more functionally connected with the bilateral habenula in the DBS-ON condition, while blue colors denote areas that showed less habenular connectivity in the DBS-ON state. Many of the areas whose habenular connectivity changed with SCC-DBS are known to be part of the "brain-wide network of depression." *c.*, cortex; *DBS*, deep brain stimulation; *DLPFC*, dorsolateral prefrontal cortex; *g.*, gyrus; *MNI*, Montreal Neurological Institute; *SCC*, subcallosal cingulate area; *TFCE*, threshold-free cluster enhancement.

As such, the results outlined here are preliminary and should be confirmed in future work.

To conclude, this study lends support to growing evidence that Hb plays an important role in response to antidepressant therapies (26), suggesting that both structural and functional Hb features may contribute to neurobiological signatures of response to SCC-DBS. In doing so, it also strengthens the case for further exploration of DBS targeting Hb itself for refractory psychiatric disorders.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

All aspects of this study involving human patients were reviewed and approved by the University Health Network Research Ethics Board (REB). Patients participating in the prospective component of this study provided their written informed consent to do so.

AUTHOR CONTRIBUTIONS

GE and JG conceived and designed the study. GE, JG, MB, AP, BW, PG, VB, and SK contributed to data acquisition. GE, JG, AL, and AB contributed to data analysis. GE, JG, AL, and AB contributed to the initial draft of the manuscript and preparation of the figures. AML supervised the study. All authors reviewed and edited the manuscript.

FUNDING

This study was supported by the RR Tasker Chair in Functional Neurosurgery at University Health Network

(AML) and the Canadian Institutes of Health Research (reference # 164235: GE).

ACKNOWLEDGMENTS

This research was enabled in part by support provided by SciNet (www.scinethpc.ca) and Compute Canada (www.computecanada.ca).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.810777/full#supplementary-material

Supplementary Figure 1 | Study flowchart. Three separate analyses were performed: a retrospective baseline volume analysis, a retrospective longitudinal volume analysis, and a prospective fMRI functional connectivity analysis. *DBS*, deep brain stimulation; *FC*, functional connectivity; *SCC*, subcallosal cingulate area.

Supplementary Figure 2 | Habenular segmentation. **(A)** Segmented habenula illustrated on orthogonal slices of the MNI152 brain. This bilateral habenula label was used as a seed for the functional connectivity analysis. **(B)** Exemplar MAGeT habenula segmentation illustrated in orthogonal slices. *MNI*, Montreal Neurological Institute.

Supplementary Table 1 | Imaging acquisition parameters for structural MR imaging.

Supplementary Table 2 | Imaging acquisition parameters for functional MR imaging.

REFERENCES

1. Namboodiri VMK, Rodriguez-Romaguera J, Stuber GD. The habenula. *Curr Biol.* (2016) 26:R873–R7. doi: 10.1016/j.cub.2016.08.051
2. Hikosaka O. The habenula: from stress evasion to value-based decision-making. *Nat Rev Neurosci.* (2010) 11:503–13. doi: 10.1038/nrn2866
3. Hu H, Cui Y, Yang Y. Circuits and functions of the lateral habenula in health and in disease. *Nat Rev Neurosci.* (2020) 21:277–95. doi: 10.1038/s41583-020-0292-4
4. Mizumori SJY, Baker PM. The lateral habenula and adaptive behaviors. *Trends Neurosci.* (2017) 40:481–93. doi: 10.1016/j.tins.2017.06.001
5. Baker PM, Jhou T, Li B, Matsumoto M, Mizumori SJY, Stephenson-Jones M, et al. The lateral habenula circuitry: reward processing and cognitive control. *J Neurosci.* (2016) 36:11482–8. doi: 10.1523/JNEUROSCI.2350-16.2016
6. Benekareddy M, Stachniak TJ, Bruns A, Knoflach F, von Kienlin M, Künnecke B, et al. Identification of a corticohabenular circuit regulating socially directed behavior. *Biol Psychiatry.* (2018) 83:607–17. doi: 10.1016/j.biopsych.2017.10.032
7. Boulos L-J, Darcq E, Kieffer BL. Translating the habenula-from rodents to humans. *Biol Psychiatry.* (2017) 81:296–305. doi: 10.1016/j.biopsych.2016.06.003
8. Torrisi S, Nord CL, Balderston NL, Roiser JP, Grillon C, Ernst M. Resting state connectivity of the human habenula at ultra-high field. *Neuroimage.* (2017) 147:872–9. doi: 10.1016/j.neuroimage.2016.10.034
9. van Kerkhof LWM, Damsteegt R, Trezza V, Voorn P, Vanderschuren LJMJ. Functional integrity of the habenula is necessary for social play behaviour in rats. *Eur J Neurosci.* (2013) 38:3465–75. doi: 10.1111/ejn.12353
10. Lawson RP, Nord CL, Seymour B, Thomas DL, Dayan P, Pilling S, et al. Disrupted habenula function in major depression. *Mol Psychiatry.* (2017) 22:202–8. doi: 10.1038/mp.2016.81
11. Lawson RP, Seymour B, Loh E, Lutti A, Dolan RJ, Dayan P, et al. The habenula encodes negative motivational value associated with primary punishment in humans. *Proc Natl Acad Sci U S A.* (2014) 111:11858–63. doi: 10.1073/pnas.1323586111
12. Germann J, Gouveia FV, Martinez RCR, Zanetti MV, de Souza Duran FL, Chaim-Avancini TM, et al. Fully automated habenula segmentation provides robust and reliable volume estimation across large magnetic resonance imaging datasets, suggesting intriguing developmental trajectories in psychiatric disease. *Biol Psychiatry Cogn Neurosci Neuroimaging.* (2020) 5:923–9. doi: 10.1016/j.bpsc.2020.01.004
13. Fakhoury M. The habenula in psychiatric disorders: more than three decades of translational investigation. *Neurosci Biobehav Rev.* (2017) 83:721–35. doi: 10.1016/j.neubiorev.2017.02.010
14. Browne CA, Hammack R, Lucki I. Dysregulation of the lateral habenula in major depressive disorder. *Front Synaptic Neurosci.* (2018) 10:46. doi: 10.3389/fnsyn.2018.00046
15. Hikosaka O, Sesack SR, Lecourtier L, Shepard PD. Habenula: crossroad between the Basal Ganglia and the Limbic System. *J Neurosci.* (2008) 28:11825–9. doi: 10.1523/JNEUROSCI.3463-08.2008
16. Lecca S, Meye FJ, Mameli M. The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview. *Eur J Neurosci.* (2014) 39:1170–8. doi: 10.1111/ejn.12480
17. Tchenio A, Lecca S, Valentinova K, Mameli M. Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. *Nat Commun.* (2017) 8:1135. doi: 10.1038/s41467-017-01192-1
18. Nuno-Perez A, Tchenio A, Mameli M, Lecca S. Lateral habenula gone awry in depression: bridging cellular adaptations with therapeutics. *Front Neurosci.* (2018) 12:485. doi: 10.3389/fnins.2018.00485
19. Germann J, Gouveia FV, Brentani H, Bedford SA, Tullo S, Chakravarty MM, et al. Involvement of the habenula in the pathophysiology of autism spectrum disorder. *Sci Rep.* (2021). 11:21168. doi: 10.1038/s41598-021-00603-0
20. Proulx CD, Hikosaka O, Malinow R. Reward processing by the lateral habenula in normal and depressive behaviors. *Nat Neurosci.* (2014) 17:1146–52. doi: 10.1038/nn.3779
21. Matsumoto M, Hikosaka O. Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature.* (2007) 447:1111–5. doi: 10.1038/nature05860
22. Ranft K, Dobrowolny H, Krell D, Bielau H, Bogerts B, Bernstein H-G. Evidence for structural abnormalities of the human habenular complex in affective disorders but not in schizophrenia. *Psychol Med.* (2010) 40:557–67. doi: 10.1017/S0033291709990821
23. Savitz JB, Nugent AC, Bogers W, Roiser JP, Bain EE, Neumeister A, et al. Habenula volume in bipolar disorder and major depressive disorder: a high-resolution magnetic resonance imaging study. *Biol Psychiatry.* (2011) 69:336–43. doi: 10.1016/j.biopsych.2010.09.027
24. Gao J, Li Y, Wei Q, Li X, Wang K, Tian Y, et al. Habenula and left angular gyrus circuit contributes to response of electroconvulsive therapy in major depressive disorder. *Brain Imaging Behav.* (2020) 15:2246–53. doi: 10.1007/s11682-020-00418-z
25. Rivas-Grajales AM, Salas R, Robinson ME, Qi K, Murrough JW, Mathew SJ. Habenula connectivity and intravenous ketamine in treatment-resistant depression. *Int J Neuropsychopharmacol.* (2020) 24:383–91. doi: 10.1093/ijnp/pyaa089
26. Gosnell SN, Curtis KN, Velasquez K, Fowler JC, Madan A, Goodman W, et al. Habenular connectivity may predict treatment response in depressed psychiatric inpatients. *J Affect Disord.* (2019) 242:211–9. doi: 10.1016/j.jad.2018.08.026
27. Lozano AM, Lipsman N, Bergman H, Brown P, Chabardes S, Chang JW, et al. Deep brain stimulation: current challenges and future directions. *Nat Rev Neurol.* (2019) 15:148–60. doi: 10.1038/s41582-018-0128-2
28. Lozano AM, Lipsman N. Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron.* (2013) 77:406–24. doi: 10.1016/j.neuron.2013.01.020
29. Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH. Subcallosal cingulate gyrus deep brain stimulation

for treatment-resistant depression. *Biol Psychiatry.* (2008) 64:461–7. doi: 10.1016/j.biopsych.2008.05.034
30. Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, et al. Deep brain stimulation for treatment-resistant depression. *Neuron.* (2005) 45:651–60. doi: 10.1016/j.neuron.2005.02.014
31. Lozano AM, Giacobbe P, Hamani C, Rizvi SJ, Kennedy SH, Kolivakis TT, et al. A multicenter pilot study of subcallosal cingulate area deep brain stimulation for treatment-resistant depression. *J Neurosurg.* (2012) 116:315–22. doi: 10.3171/2011.10.JNS102122
32. Guinjoan SM, Mayberg HS, Costanzo EY, Fahrer RD, Tenca E, Antico J, et al. Asymmetrical contribution of brain structures to treatment-resistant depression as illustrated by effects of right subgenual cingulum stimulation. *J Neuropsychiatry Clin Neurosci.* (2010) 22:265–77. doi: 10.1176/jnp.2010.22.3.265
33. Franzini A, Cordella R, Messina G, Marras CE, Romito LM, Albanese A, et al. Targeting the brain: considerations in 332 consecutive patients treated by deep brain stimulation (DBS) for severe neurological diseases. *Neurological Sciences.* (2012) 33:1285–303. doi: 10.1007/s10072-012-0937-9
34. Holtzheimer PE, Husain MM, Lisanby SH, Taylor SF, Whitworth LA, McClintock S, et al. Subcallosal cingulate deep brain stimulation for treatment-resistant depression: a multisite, randomised, sham-controlled trial. *Lancet Psychiatry.* (2017) 4:839–49. doi: 10.1016/S2215-0366(17)30371-1
35. Lipsman N, Woodside DB, Giacobbe P, Hamani C, Carter JC, Norwood SJ, et al. Subcallosal cingulate deep brain stimulation for treatment-refractory anorexia nervosa: a phase 1 pilot trial. *Lancet.* (2013) 381:1361–70. doi: 10.1016/S0140-6736(12)62188-6
36. Lipsman N, Lam E, Volpini M, Sutandar K, Twose R, Giacobbe P, et al. Deep brain stimulation of the subcallosal cingulate for treatment-refractory anorexia nervosa: 1 year follow-up of an open-label trial. *Lancet Psychiatry.* (2017) 4:285–94. doi: 10.1016/S2215-0366(17)30076-7
37. Merkl A, Aust S, Schneider G-H, Visser-Vandewalle V, Horn A, Kühn AA, et al. Deep brain stimulation of the subcallosal cingulate gyrus in patients with treatment-resistant depression: a double-blinded randomized controlled study and long-term follow-up in eight patients. *J Affect Disord.* (2018) 227:521–9. doi: 10.1016/j.jad.2017.11.024
38. Merkl A, Schneider G-H, Schönecker T, Aust S, Kühl K-P, Kupsch A, et al. Antidepressant effects after short-term and chronic stimulation of the subgenual cingulate gyrus in treatment-resistant depression. *Exp Neurol.* (2013) 249:160–8. doi: 10.1016/j.expneurol.2013.08.017
39. Harmsen IE, Elias GJB, Beyn ME, Boutet A, Pancholi A, Germann J, et al. Clinical trials for deep brain stimulation: current state of affairs. *Brain Stimul.* (2020) 13:378–85. doi: 10.1016/j.brs.2019.11.008
40. Elias GJB, Boutet A, Joel SE, Germann J, Gwun D, Neudorfer C, et al. Probabilistic mapping of deep brain stimulation: insights from 15 years of therapy. *Ann Neurol.* (2021) 89:426–43. doi: 10.1002/ana.25975
41. Kennedy SH, Giacobbe P, Rizvi SJ, Placenza FM, Nishikawa Y, Mayberg HS, et al. Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. *Am J Psychiatry.* (2011) 168:502–10. doi: 10.1176/appi.ajp.2010.10081187
42. Fonov VS, Evans AC, McKinstry RC, Almli CR, Collins DL. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *Neuroimage.* (2009) 47:S102. doi: 10.1016/S1053-8119(09)70884-5
43. Hancu I, Boutet A, Fiveland E, Ranjan M, Prusik J, Dimarzio M, et al. On the (Non-) equivalency of monopolar and bipolar settings for deep brain stimulation fMRI studies of Parkinson's disease patients. *J Magn Reson Imaging.* (2019) 49:1736–49. doi: 10.1002/jmri.26321
44. Boutet A, Hancu I, Saha U, Crawley A, Xu DS, Ranjan M, et al. 3-Tesla MRI of deep brain stimulation patients: safety assessment of coils and pulse sequences. *J Neurosurg.* (2019) 132:586–94. doi: 10.3171/2018.11.JNS181338
45. Boutet A, Rashid T, Hancu I, Elias GJB, Gramer RM, Germann J, et al. Functional MRI safety and artifacts during deep brain stimulation: experience in 102 patients. *Radiology.* (2019) 293:174–83. doi: 10.1148/radiol.2019190546
46. Boutet A, Madhavan R, Elias GJB, Joel SE, Gramer R, Ranjan M, et al. Predicting optimal deep brain stimulation parameters for Parkinson's disease using functional MRI and machine learning. *Nat Commun.* (2021) 12:3043. doi: 10.1038/s41467-021-23311-9
47. Elias GJB, Germann J, Boutet A, Loh A, Bryan L, Asma N, Nicole B, Jessica P, Bhat V, Giacobbe P, et al. 3T MRI of rapid brain activity changes driven by subcallosal cingulate deep brain stimulation. *Brain.* (2021). doi: 10.1093/brain/awab447
48. Chakravarty MM, Steadman P, van Eede MC, Calcott RD, Gu V, Shaw P, et al. Performing label-fusion-based segmentation using multiple automatically generated templates. *Hum Brain Mapp.* (2013) 34:2635–54. doi: 10.1002/hbm.22092
49. Pipitone J, Park MTM, Winterburn J, Lett TA, Lerch JP, Pruessner JC, et al. Multi-atlas segmentation of the whole hippocampus and subfields using multiple automatically generated templates. *Neuroimage.* (2014) 101:494–512. doi: 10.1016/j.neuroimage.2014.04.054
50. Xu K, Liu Y, Zhan Y, Ren J, Jiang T. BRANT: a versatile and extendable resting-state fMRI toolkit. *Front Neuroinform.* (2018) 12:52. doi: 10.3389/fninf.2018.00052
51. Friston KJ, Williams S, Howard R, Frackowiak RSJ, Turner R. Movement-related effects in fMRI time-series. *Magn Resonan Med.* (1996) 35:346–55. doi: 10.1002/mrm.1910350312
52. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage.* (2009) 44:83–98. doi: 10.1016/j.neuroimage.2008.03.061
53. Neudorfer C, Germann J, Elias GJB, Gramer R, Boutet A, Lozano AM. A high-resolution *in vivo* magnetic resonance imaging atlas of the human hypothalamic region. *Sci Data.* (2020) 7:305. doi: 10.1038/s41597-020-00644-6
54. Fox MD, Buckner RL, Liu H, Chakravarty MM, Lozano AM, Pascual-Leone A. Resting-state networks link invasive and noninvasive brain stimulation across diverse psychiatric and neurological diseases. *Proc Natl Acad Sci U S A.* (2014) 111:E4367–E75. doi: 10.1073/pnas.1405003111
55. Elias GJB, Germann J, Boutet A, Pancholi A, Beyn ME, Bhatia K, et al. Structuro-functional surrogates of response to subcallosal cingulate deep brain stimulation for depression. *Brain.* (2021). doi: 10.1093/brain/awab284
56. Padmanabhan JL, Cooke D, Joutsa J, Siddiqi SH, Ferguson M, Darby RR, et al. A human depression circuit derived from focal brain lesions. *Biol Psychiatry.* (2019) 86:749–58. doi: 10.1016/j.biopsych.2019.07.023
57. Dichter GS, Gibbs D, Smoski MJ. A systematic review of relations between resting-state functional-MRI and treatment response in major depressive disorder. *J Affect Disord.* (2015) 172:8–17. doi: 10.1016/j.jad.2014.09.028
58. Wang Z, Cai X, Qiu R, Yao C, Tian Y, Gong C, et al. Case report: lateral habenula deep brain stimulation for treatment-resistant depression. *Front Psychiatry.* (2020) 11:616501. doi: 10.3389/fpsyt.2020.616501
59. Sartorius A, Kiening KL, Kirsch P, von Gall CC, Haberkorn U, Unterberg AW, et al. Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biol Psychiatry.* (2010) 67:e9–e11. doi: 10.1016/j.biopsych.2009.08.027
60. Germann J, Mameli M, Elias GJB, Loh A, Taha A, Gouveia FV, et al. Deep Brain stimulation of the habenula: systematic review of the literature and clinical trial registries. *Front Psychiatry.* (2021) 12:1410. doi: 10.3389/fpsyt.2021.730931
61. Sankar T, Li SX, Obuchi T, Fasano A, Cohn M, Hodaie M, et al. Structural brain changes following subthalamic nucleus deep brain stimulation in Parkinson's disease. *Mov Disord.* (2016) 31:1423–25. doi: 10.1002/mds.26707
62. Sankar T, Chakravarty MM, Bescos A, Lara M, Obuchi T, Laxton AW, et al. Deep brain stimulation influences brain structure in Alzheimer's disease. *Brain Stimul.* (2015) 8:645–54. doi: 10.1016/j.brs.2014.11.020
63. Kim U, Lee T. Topography of descending projections from anterior insular and medial prefrontal regions to the lateral habenula of the epithalamus in the rat. *Eur J Neurosci.* (2012) 35:1253–69. doi: 10.1111/j.1460-9568.2012.08030.x
64. Cheng W, Rolls ET, Qiu J, Xie X, Wei D, Huang C-C, et al. Increased functional connectivity of the posterior cingulate cortex with the lateral orbitofrontal cortex in depression. *Transl Psychiatry.* (2018) 8:90. doi: 10.1038/s41398-018-0139-1
65. Takahashi T, Yücel M, Lorenzetti V, Walterfang M, Kawasaki Y, Whittle S, et al. An MRI study of the superior temporal subregions in patients with current and past major depression. *Prog Neuropsychopharmacol Biol Psychiatry.* (2010) 34:98–103. doi: 10.1016/j.pnpbp.2009.10.005
66. Harada K, Ikuta T, Nakashima M, Watanuki T, Hirotsu M, Matsubara T, et al. Altered connectivity of the anterior cingulate and the posterior superior

temporal gyrus in a longitudinal study of later-life depression. *Front Aging Neurosci.* (2018) 10:31. doi: 10.3389/fnagi.2018.00031
67. Kandilarova S, Stoyanov D, Sirakov N, Maes M, Specht K. Reduced grey matter volume in frontal and temporal areas in depression: contributions from voxel-based morphometry study. *Acta Neuropsychiatr.* (2019) 31:252–7. doi: 10.1017/neu.2019.20
68. Li M, Xu H, Lu S. Neural basis of depression related to a dominant right hemisphere: a resting-state fMRI study. *Behav Neurol.* (2018) 2018:5024520. doi: 10.1155/2018/5024520
69. Treadway MT, Waskom ML, Dillon DG, Holmes AJ, Park MTM, Chakravarty MM, et al. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. *Biol Psychiatry.* (2015) 77:285–94. doi: 10.1016/j.biopsych.2014.06.018
70. Yoshimura S, Okamoto Y, Onoda K, Matsunaga M, Ueda K, Suzuki S-I, et al. Rostral anterior cingulate cortex activity mediates the relationship between the depressive symptoms and the medial prefrontal cortex activity. *J Affect Disord.* (2010) 122:76–85. doi: 10.1016/j.jad.2009.06.017
71. Delaveau P, Jabourian M, Lemogne C, Guionnet S, Bergouignan L, Fossati P. Brain effects of antidepressants in major depression: a meta-analysis of emotional processing studies. *J Affect Disord.* (2011) 130:66–74. doi: 10.1016/j.jad.2010.09.032
72. Pizzagalli D, Pascual-Marqui RD, Nitschke JB, Oakes TR, Larson CL, Abercrombie HC, et al. Anterior cingulate activity as a predictor of degree of treatment response in major depression: evidence from brain electrical tomography analysis. *Am J Psychiatry.* (2001) 158:405–15. doi: 10.1176/appi.ajp.158.3.405
73. Kennedy SH, Evans KR, Krüger S, Mayberg HS, Meyer JH, McCann S, et al. Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *Am J Psychiatry.* (2001) 158:899–905. doi: 10.1176/appi.ajp.158.6.899
74. Kazemi R, Rostami R, Khomami S, Baghdadi G, Rezaei M, Hata M, et al. Bilateral transcranial magnetic stimulation on DLPFC changes resting state networks and cognitive function in patients with bipolar depression. *Front Hum Neurosci.* (2018) 12:356. doi: 10.3389/fnhum.2018.00356
75. Cao X, Deng C, Su X, Guo Y. Response and remission rates following high-frequency vs. low-frequency repetitive transcranial magnetic stimulation (rTMS) over right DLPFC for treating major depressive disorder (MDD): a meta-analysis of randomized, double-blind trials. *Front Psychiatry.* (2018) 9:413. doi: 10.3389/fpsyt.2018.00413
76. Ichikawa N, Lisi G, Yahata N, Okada G, Takamura M, Hashimoto R-I, et al. Primary functional brain connections associated with melancholic major depressive disorder and modulation by antidepressants. *Sci Rep.* (2020) 10:3542. doi: 10.1038/s41598-020-73436-y
77. Elias GJB, Boutet A, Parmar R, Wong EHY, Germann J, Loh A, et al. Neuromodulatory treatments for psychiatric disease: a comprehensive survey of the clinical trial landscape. *Brain Stimul.* (2021) 14:1393–403. doi: 10.1016/j.brs.2021.08.021
78. Fox MD, Buckner RL, White MP, Greicius MD, Pascual-Leone A. Efficacy of transcranial magnetic stimulation targets for depression is related to intrinsic functional connectivity with the subgenual cingulate. *Biol Psychiatry.* (2012) 72:595–603. doi: 10.1016/j.biopsych.2012.04.028
79. Pulcu E, Guinea C, Cowen PJ, Murphy SE, Harmer CJ. A translational perspective on the anti-anhedonic effect of ketamine and its neural underpinnings. *Mol Psychiatry.* (2021) doi: 10.1038/s41380-021-01183-1

Conflict of Interest: AML is the co-founder of Functional Neuromodulation (a DBS-related company), is a consultant for Boston Scientific, Medtronic, and Abbott (companies that produce DBS hardware). PG and SK are consultants for Abbott. GE, AB, and AML have intellectual property in the field of DBS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Psychiatry

MINI REVIEW
published: 17 February 2022
doi: 10.3389/fpsyt.2022.817302

Habenula as a Neural Substrate for Aggressive Behavior

Flavia Venetucci Gouveia[1]* *and George M. Ibrahim*[1,2,3,4,5]

[1] Neuroscience and Mental Health, Hospital for Sick Children Research Institute, Toronto, ON, Canada, [2] Division of Neurosurgery, The Hospital for Sick Children, Toronto, ON, Canada, [3] Division of Neurosurgery, Department of Surgery, University of Toronto, Toronto, ON, Canada, [4] Institute of Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [5] Institute of Medical Science, University of Toronto, Toronto, ON, Canada

Over the past decades, an ever growing body of literature has explored the anatomy, connections, and functions of the habenula (Hb). It has been postulated that the Hb plays a central role in the control of the monoaminergic system, thus influencing a wide range of behavioral responses, and participating in the pathophysiology of a number of psychiatric disorders and neuropsychiatric symptoms, such as aggressive behaviors. Aggressive behaviors are frequently accompanied by restlessness and agitation, and are commonly observed in patients with psychiatric disorders, intellectual disabilities, and neurodegenerative diseases of aging. Recently, the Hb has been explored as a new target for neuromodulation therapies, such as deep brain stimulation, with promising results. Here we review the anatomical organization of the habenula and discuss several distinct mechanisms by which the Hb is involved in the modulation of aggressive behaviors, and propose new investigations for the development of novel treatments targeting the habenula to reduce aggressive behaviors.

Keywords: habenula, aggressive behavior (AB), neuropsychiatric symptoms, preclinical studies, review

OPEN ACCESS

Edited by:
Ali Saffet Gonul,
Ege University, Turkey

Reviewed by:
Yukiori Goto,
Kyoto University, Japan
Suresh Jesuthasan,
Nanyang Technological University, Singapore

***Correspondence:**
Flavia Venetucci Gouveia
fvenetucci@gmail.com

Specialty section:
This article was submitted to Neuroimaging and Stimulation, a section of the journal Frontiers in Psychiatry

Received: *17 November 2021*
Accepted: *25 January 2022*
Published: *17 February 2022*

Citation:
Gouveia FV and Ibrahim GM (2022) Habenula as a Neural Substrate for Aggressive Behavior. Front. Psychiatry 13:817302. doi: 10.3389/fpsyt.2022.817302

INTRODUCTION

The habenula (Hb) is an epithalamic structure that presents rich connections with several cortical and subcortical structures, including the limbic system, and areas responsible for the production and regulation of monoamines (i.e., raphe nuclei for serotonin, ventral tegmental area and substantia nigra for dopamine, and locus coeruleus for noradrenaline) (1–5). These connections place the Hb in a central position for the regulation of motivated behaviors, and thus has been implicated in the pathophysiology of several disorders, such as autism spectrum disorder (ASD) (6), depression (7, 8), bipolar disorder (8–10), and schizophrenia (10, 11), as well as neuropsychiatric symptoms, such as aggressive behaviors (12, 13).

Aggressive behaviors can be verbal and physical insults directed toward oneself (i.e., self-injury behavior), others, or objects (14), and are highly correlated with restlessness and excessive motor agitation (15, 16). Several distinct classifications of human aggressive behavior have been proposed, with the classification in proactive (also known as premeditated aggression) or reactive (also known as impulsive aggression) being widely accepted (17–21). While proactive aggression is believed to involve planned behaviors to achieve a specific goal, reactive aggression is unrelated to a specific goal, being mainly associated with frustration, provocation or stress. Another important difference between these two types of aggressive behavior, is the association with high levels of autonomic arousal and impulsivity in subjects presenting with reactive aggression, that is absence in the proactive aggression (17–21). Aggressive behaviors, mainly reactive aggression, are frequent among

patients with psychiatric conditions, especially in those suffering from intermittent explosive disorder, borderline/antisocial personality disorders, patients with neurodevelopmental conditions, such as ASD (17, 22), and those with neurodegenerative diseases of aging (e.g., Alzheimer's disease) (23–25).

The neurocircuitry underlying aggressive behaviors include prefrontal cortical regions and areas of the mesolimbic system, especially the hypothalamus, amygdala and periaqueductal gray matter (14–17, 26). It is believed that decreased serotonergic transmission in the prefrontal cortex reduces the top-down inhibitory control over the limbic system, resulting in motor activation and hormonal production, preparing the organism for a fight-or-flight situation (17, 21, 26). However, simplistic this mechanism might seem, there are several distinct neural-pathways involved in the association of external and internal stimuli that will result in the expression of an appropriate or inappropriate aggressive behavioral response. As such, the mechanisms by which the Hb is involved in the modulation of aggressive behaviors are numerous and still not fully understood.

In this review, we explore the anatomical organization of the habenula, describe the relevant literature on the involvement of the Hb in the modulation of aggressive behaviors and discuss future perspectives and novel therapies.

Anatomical Organization of the Habenula

The Hb is a bilateral, phylogenetically old, epithalamic structure surrounded by the third ventricle and the thalamus (lateral and dorsal borders), the posterior commissure (ventral and posterior borders), and the stria medullaris of the thalamus (anterior limit, **Figure 1A**) (1, 4, 9). In mammals, the Hb is divided into two sub-regions—the medial habenula (MHb) and the lateral habenula (LHb)—based on their cellular and genetic profiles, neuroanatomical connectivity, and associated functions (**Figure 1B**) (1, 4, 5). Through the fasciculus retroflexus, both MHb and LHb project to distinct brain areas. MHb efferents form the core of the fiber bundle that reaches the interpeduncular nucleus (IP) in a 90° rotation pattern, with dorsal projections reaching the lateral aspect of the IP, medial projections to the ventral aspect of IP and the lateral projections ending on the dorsal aspect of IP (2). Projections from the IP then reaches the periaqueductal gray matter (27), an area critically involved in the neural network of aggressive behavior (17, 21, 26). Discrete projections from the MHb can also be found in the LHb, supra-commissural septum and median raphe nucleus (2, 28). Inputs form the medial, lateral and triangular septal nuclei and septofimbrial nucleus, *via* the medial stria medullaris comprise the main afferent projections to the MHb (2, 5, 29). LHb efferents forms the mantle portion of the fasciculus retroflexus, that reaches the ventral tegmental area, hypothalamus (i.e., lateral, posterior and dorsomedial hypothalamic nuclei, lateral preoptic area), ventromedial thalamic nucleus, substantia innominata, ventrolateral septum, substantia nigra pars compacta, medial and dorsal raphe nuclei, and tegmental reticular formation (2). Discrete additional projections can be found in the pretectal area, superior colliculus, nucleus reticularis tegmenti pontis, parabrachial nuclei, and locus coeruleus (2). Afferent projections from limbic regions are mainly found in the more medial aspect of the LHb while projections from the globus pallidus reach the lateral aspect of the LHb (**Figure 1C**) (2).

Medial Habenula

The MHb can be further divided into five subregions, namely superior (MHbS), inferior (MHbI), central (MHbC), lateral (MHbL), and commissural (MHbCo) parts (**Figure 1B**). The MHbS consists exclusively of densely packed glutamatergic neurons that strongly express interleukin-18, with nuclei on a typical triangular appearance, thin dendrites and tightly packed synaptic vesicles in axon terminals (3, 30). The cell characteristics of neurons in the MHbI are similar to the one in the MHbS, however the nuclei are typically round and the proximal dendrites are thicker. Also, these neurons are not exclusively glutamatergic as they co-transmits acetylcholine from the axonal terminals (3, 30). Likewise, the MHbL is also composed of cholinergic and glutamatergic neurons, however these are smaller, with oval nuclei and nuclear membrane surrounded by nucleoli or chromatin plaques (3, 30). The MHbC can be viewed as a transition area, as it is composed of a combination of cell clusters composed of the diverse cell types observed in the adjacent regions, that are separated from each-other by the terminal fiber bundles of the stria medullaris. While the dorsal region of the MHbC is composed of neurons that co-express substance-P and glutamate, the ventral region is both cholinergic and glutamatergic (3, 30). Finally, the MHbCo displays the largest glutamatergic neurons in the MHb area, with unusual nuclei in a semilunar shape (3, 30).

Lateral Habenula

The LHb is significantly larger than the MHb and is subdivided into medial (LHbM) and lateral (LHbL) parts (**Figure 1B**). Each of these aspects are further subdivided resulting in a total of ten LHb subregions (3). Irrespective of cell morphology and location, neurons in the LHb are predominantly glutamatergic (30), however it has recently been shown that a very discrete population of GABAergic neurons are present in the medial part of the LHb (31, 32). The LHbM is divided in anterior (LHbMA), superior (LHbMS), parvocellular (LHbMPc), central (LHbMC), and marginal (LHbMMg) subregions. Small neurons are found in the LHbMA, LHbMMg and LHbMPc parts, with LHbMA showing round multipolar cells with heavily folded nuclei, LHbMMg having an elongated version of the LHbMA cells, and LHbMPc presenting a spindle-shaped cell with deeply invaginated nucleus. Greatly larger neurons are found in the LHbMS and LHbMC subregions, however LHbMS neurons are characterized by an oval perikarya, and LHbMC cell nuclei are often invaginated and display a brighter and finer karyoplasm (3). The LHbL is divided into parvocellular (LHbLPc), magnocellular (LHbLMc), oval (LHbLO), basal (LHbLB), and marginal (LHbLMg) subregions (3). Neurons in the LHbLPc and LHbLMg parts are predominantly small to medium-sized. Cells in the LHbLMc, and LHbLO are large, and while neurons in the LHbLMc and LHbLB parts present thick and long dendrites, neurons in the LHbLO extend thin ones (3).

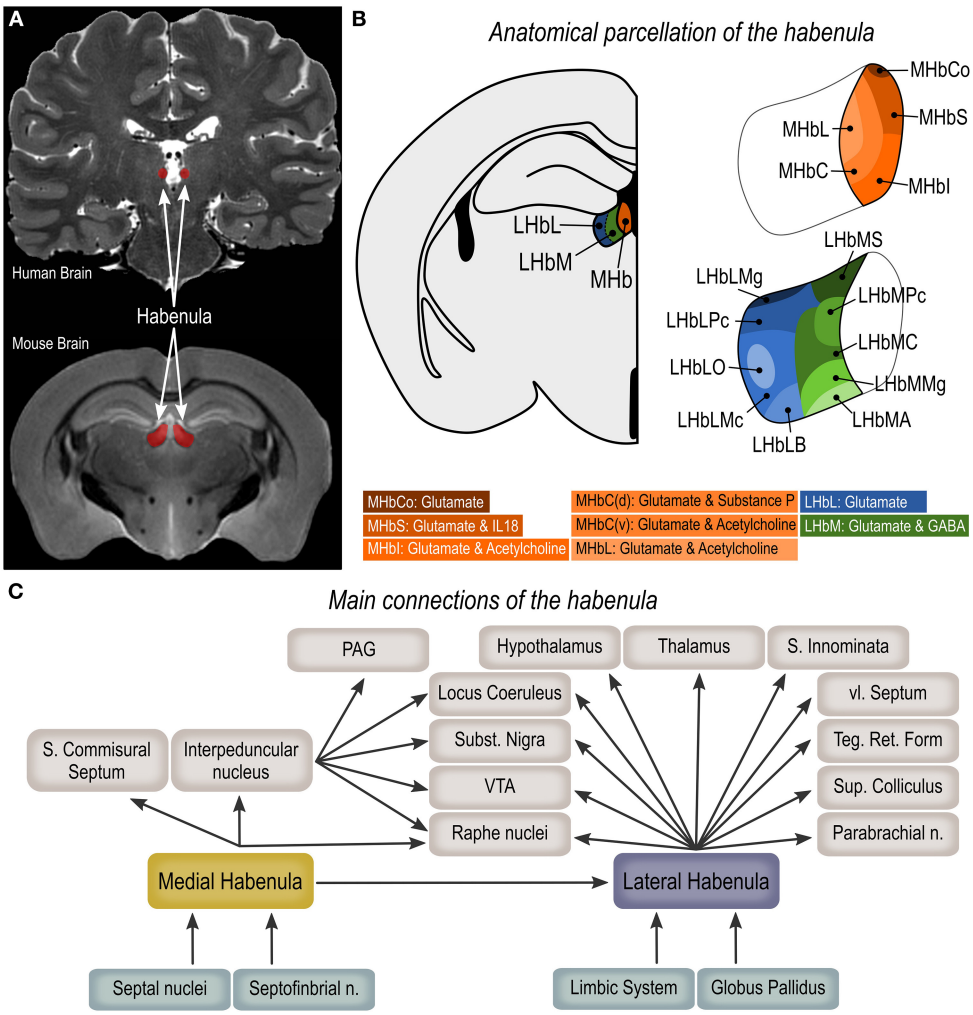

FIGURE 1 | Anatomical organization of the habenula. **(A)** Magnetic resonance imaging (coronal plane) showing the human and mouse habenula (https://openneuro.org/datasets/ds002179/versions/1.1.0). **(B)** Anatomical parcellation of the medial (MHb) and lateral habenula (LHb). **(C)** Main habenula connections. MHbS, medial habenula superior part; MHbI, medial habenula inferior part; MHbC, medial habenula central part; MHbL, medial habenula lateral part; MHbCo, medial habenula commissural part; LHbMA, lateral habenula medial part anterior subregion; LHbMS, lateral habenula medial part superior subregion; LHbMPc, lateral habenula medial part parvocellular subregion; LHbMC, lateral habenula medial part central subregion; LHbMMg, lateral habenula medial part marginal subregion; LHbLPc, lateral habenula lateral part parvocellular subregion; LHbLMc, lateral habenula lateral part magnocellular subregion; LHbLO, lateral habenula lateral part oval subregion; LHbLB, lateral habenula lateral part basal subregion; LHbLMg, lateral habenula lateral part marginal subregion; PAG, periaqueductal gray matter; VTA, ventral tegmental area.

Habenula as a Key Relay for Aggressive Behavior

Several clinical and preclinical studies have investigated the involvement of the Hb in the modulation of aggressive behaviors. Studies using transgenic models have provided further evidence of the involvement of the Hb in the regulation of aggressive behaviors. Using double and triple transgenic zebrafish, Chou et al. (33) demonstrated that the dorsal habenula–interpeduncular nucleus pathway [homologous to the mammalian MHb-IP pathway (34)], is key for the modulation of aggressive behaviors, with connectivity between the medial subregion of dHb and IP being associated with increase aggression (33). GPR3 is an G-protein-coupled-receptor broadly expressed in the central nervous system, with maximal expression in the Hb, that has been implicated in the regulation of cAMP signaling and, consequently, modulation of emotional behavior. Knockout Gpr3$^{-/-}$ mice present null expression of GPR3 in the Hb, high levels of aggressive behavior and accentuated reduction of serotonin, noradrenaline and its metabolites in the hypothalamus and frontal cortex (35). The Disrupted-In-Schizophrenia-1 (DISC1-Q31L) mouse model of depression, bipolar disorder and schizophrenia, presents heightened inter-male aggressive behavior along with increased neuronal density in both the LHb and MHb (36). On the other hand, male

Mecp2^{-} mice (i.e., knockout of the X-linked methyl-CpG-binding protein 2, gene associated to Rett syndrome) present absence of aggressive behavior and accentuated reduction in oxytocinergic innervation in the lateral habenula (37). It is important to highlight, however, that transgenic animals may also present with additional brain alterations in function and connection and, thus, the altered behavior observed in these studies may be the result of the sum of all these changes and not rely solely on alterations observed in the habenula.

In lactating females, aggressive behaviors toward an intruder are mediated by the medial prefrontal cortex-LHb-dorsal raphe nucleus pathway, as demonstrated by increased co-labeling of c-Fos- and Fluorogold-positive neurons in the mPFC and LHb following aggressive encounters (38). Pharmacological manipulation of NMDA and AMPA/kainate receptors *via* microinjection of receptor antagonists in the dorsal raphe nucleus of lactating females is capable of inhibiting this behavior (38). Interestingly, injection of arginine-vasopressin V1a receptor antagonists in the LHb or dorsal raphe nucleus, of both male and female mice, is not sufficient to alter aggressive behaviors, suggesting that the arginine-vasopressin system does not play a crucial role in this neurocircuitry (39). Tear fluid is rich in pheromones capable of eliciting several context-specific behavioral responses in both males and females rodents (40). It has been shown that female mouse tears suppress aggressive behaviors in males and induce a great increase in c-Fos immunoreactivity in the medial aspect of the LHb (41).

A study investigating habenula resting-state functional connectivity in highly reactive aggressive men showed an association between high levels of trait aggression to lower global efficiency of the left habenula and atypical habenula-prefrontal connectivity (42). In a recent work from our group, we showed that the Hb—along with the dorsal raphe nuclei, substantia nigra, ventral tegmental area, and locus coeruleus—is part of the functional connectivity map associated with symptom alleviation in a patient treated with deep brain stimulation of the posterior hypothalamus for reduction of severe and treatment refractory aggressive behavior (43).

Reward Value of Aggressive Behavior

Another line of evidence is based on the well-known involvement of the LHb in reward-related behaviors. The suppression of midbrain dopaminergic neurons, *via* GABAergic indirect connections, is thought to be the main mechanism by which the LHb drives reinforcement learning (1). The LHb is active in response to the negative value of a stimulus, unexpected reward omissions, and cues associated with these stimuli (44, 45), such as in situations of drug withdrawal (46–48). Moreover, the functional integrity of the LHb is necessary to integrate proactive and retroactive information to guide behavioral flexibility when the reward contingencies change (49). Golden et al. (50) investigated the involvement of basal forebrain projections to the LHb in the modulation of aggression reward. Using a conditioned place preference (CPP) or place aversion (CPA) paradigm, the authors have shown that aggressive mice presented CPP for the chamber where aggressive encounters occurred, while non-aggressive mice developed a CPA to the same chamber. Using optogenetic techniques to stimulate or inhibit the basal forebrain projections to the LHb, it was found that the stimulation of these terminals promotes CPP and reduces LHb firing, and the inhibition results in CPA and increases LHb firing (50). These results are in line with previous findings indicating that decreased LHb activity is associated with rewarding components of behavior. Flanigan et al. (32) showed that optogenetic stimulation of orexin terminals located in GABAergic neurons within the LHb promotes inter-male aggressive behavior and CPP for aggression-paired contexts.

The reward value of aggressive behaviors is also mediated *via* the LHb-ventral tegmental area (VTA)-nucleus accumbens (nAcc) network that results in increased dopamine release in the nAcc when animals are expecting a conditioned aggressive encounter (51). Moreover, antagonism of both dopamine receptor types 1 and 2 in the nAcc have been described as reducing the rewarding value of aggressive behaviors (52–54). In line with these findings, two case reports (55, 56) and one case series (57) reporting on patients treated with deep brain stimulation of the nAcc for severe refractory aggressive behaviors, have shown long lasting positive results (58).

The Circadian Cycle and Aggressive Behavior

There is a strong body of evidence on the role of the Hb in regulating the circadian cycle, thus influencing internal physiology, brain activity patterns and day-night behavioral rhythm (59–62). Anatomically, the Hb shares the epithalamus with the pineal gland, a brain structure responsible for the production of the hormone melatonin, that serves among others, as a major regulator of the sleep-wake cycle (5, 63). Similar to the pineal gland, the Hb expresses mRNA for arylalkylamine-N-acetyltransferase, the enzyme responsible for melatonin synthesis, thus being implicated as a supplementary location for melatonin biosynthesis (63). Dysregulations of the circadian cycle are known to negatively influence cognition, emotions and behavior, and is associated with worsening of symptoms in several neuropsychiatric disorders (64, 65). Poor sleep routines (i.e., short sleep duration, inadequate sleep quality) in children and adolescents is associated with increased irritability, conduct problems, anxiety, and hyperactivity (66–68), and in adults, is associated with increased hostility, anger, aggression and suicidal ideation (49–52). However, it is important to highlight that the Hb circadian clock is independent of the suprachiasmatic nucleus (60), and although disruption of the Hb circadian clock does not disrupt the sleep cycle, it is capable of altering the subjects response to stressors, suggesting that a sleep-independent effect on aggression may exist (69).

Among the various neurotransmitters, neuropeptides, and neurohormones involved in the modulation of the circadian cycle, dysfunctions in serotonin transmission are thought to be central in the association between poor sleep and aggressiveness. *Via* GABAergic interneurons, the LHb modulates the activity of serotonergic neurons in the dorsal raphe nucleus (70, 71), that have an increased activity during wakefulness, along with brain-wide increase in serotonin levels (72, 73). The prolonged exposure to high serotonin levels caused by reduced sleep time causes gradual desensitization of serotonin receptors (74), thus

contributing to a reduced serotonergic effective transmission in the prefrontal cortex and consequently reduction in the top-down inhibitory control of emotions described above.

DISCUSSION

Excessive aggressive behaviors are highly prevalent, particularly among patients with psychiatric disorders and presents a major obstacle for patient care, increasing institutionalization rates and reducing patients' quality of life (15, 17). The clinical and preclinical studies described here show evidence of the involvement of the habenula in the neuro-circuitry of aggressive behavior, and suggest that the modulation of neurons in this area could result in symptom alleviation in patients presenting psychiatric disorders associated with aggressive behavior. Furthermore, typical and atypical antipsychotics and antidepressants are commonly used for the treatment of aggressive behaviors *via* antagonizing dopaminergic receptors or selectively inhibiting serotonin reuptake enzymes (17). As described above, the Hb is highly connected with areas responsible for the production and regulation of monoamines (e.g., dopamine and serotonin) and thus, involved in the response to standard treatments (1–5). However, the chronic systemic exposure to these compounds is associated with refractoriness to treatment, and may produce severe side effects that can escalate to the point of being impeditive of treatment (14, 16, 75, 76). Thus, further studies are necessary to better understand the brain mechanisms associated with aggressive behaviors and develop novel treatments that are tailored to safely and effectively improve patient outcomes.

Focused ultrasound is being intensively investigated as a novel non-invasive tool for neuromodulation that could be used to deliver pharmacological agents to localized brain areas, without the need of a systemic distribution (77). By repurposing a commercially available ultrasound contrast, Lea-Banks and colleagues fabricated an ultrasound-sensitive nanodroplet loaded with or without anesthetic drug, that was injected intravenously and then vaporized in a discrete brain target using focused ultrasound (78, 79). The authors showed that the use of the unloaded nanodroplets increased local neuronal activity, while drug-loaded nanodroplets suppressed (78, 79). Considering that the Hb is involved in the regulation of monoamines, such as those modulated by drug therapy, and the current evidence on the possibility of reducing the reward value of aggressive behavior by increasing neuronal firing in the LHb, one could envision further exploring this innovative technique to selectively deliver nanodroplets to the LHb and modulate its activity, to reduce aggressive behavior in patients with psychiatric disorders that do not present adequate response to conventional therapy.

Deep brain stimulation is a neuromodulation therapy that involves the precise placement of electrodes into deep brain structures to modulate neuronal activity *via* the application of an electrical current that can be precisely titrated (80). Although only a few case studies on Hb deep brain stimulation have been published, they report beneficial outcomes in patients suffering from schizophrenia (81), depression (82, 83), obsessive-compulsive disorder (84), and bipolar disorder (85). New clinical trials are currently being performed, demonstrating a growing interest to target this region for the treatment of psychiatric disorders [for a review on deep brain stimulation of the habenula see (86)]. Considering the strong evidence on the involvement of the Hb in the pathophysiology of aggressive behaviors and the possibility of safely targeting this area with deep brain stimulation, it would be interesting to investigate Hb deep brain stimulation in the context of aggressive behaviors.

In this article we provide a detailed review of the anatomical organization of the Hb, by describing cell characteristics and connections of the lateral and medial aspects of the Hb and its subdivisions. We discussed several distinct mechanisms by which the Hb modulates aggressive behavior, detailing studies investigating transgenic models, neuronal modulation and neuroimaging, and the literature about the involvement of the Hb in reward-related behaviors and regulation of circadian cycle. We concluded this review discussing how innovative neuromodulatory techniques could be investigated in the context of Hb and aggressive behaviors to improve patient outcome.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. Hikosaka O. The habenula: from stress evasion to value-based decision-making. *Nat Rev Neurosci*. (2010) 11:503–13. doi: 10.1038/nrn2866
2. Herkenham M, Nauta WJ. Efferent connections of the habenular nuclei in the rat. *J Comp Neurol*. (1979) 187:19–47. doi: 10.1007/978-1-4684-7920-1_17
3. Andres KH, von Düring M, Veh RW. Subnuclear organization of the rat habenular complexes. *J Comp Neurol*. (1999) 407:130–50. doi: 10.1002/(sici)1096-9861(19990428)407:1<130::aid-cne10>3.0.co;2-8
4. Hikosaka O, Sesack SR, Lecourtier L, Shepard PD. Habenula: crossroad between the basal ganglia and the limbic system. *J Neurosci*. (2008) 28:11825–9. doi: 10.1523/JNEUROSCI.3463-08.2008
5. Namboodiri VMK, Rodriguez-Romaguera J, Stuber GD. The habenula. *Curr Biol*. (2016) 26:R873–7. doi: 10.1016/j.cub.2016.08.051
6. Germann J, Gouveia FV, Brentani H, Bedford SA, Tullo S, Chakravarty MM, et al. Involvement of the habenula in the pathophysiology of autism spectrum disorder. *Sci Rep*. (2021) 11:21168. doi: 10.1038/s41598-021-00603-0
7. Sartorius A, Henn FA. Deep brain stimulation of the lateral habenula in treatment resistant major depression. *Med Hypotheses*. (2007) 69:1305–8. doi: 10.1016/j.mehy.2007.03.021
8. Savitz JB, Nugent AC, Bogers W, Roiser JP, Bain EE, Neumeister A, et al. Habenula volume in bipolar disorder and major depressive disorder: a high-resolution magnetic resonance imaging study. *Biol Psychiatry*. (2011) 69:336–43. doi: 10.1016/j.biopsych.2010.09.027
9. Germann J, Gouveia FV, Martinez RCR, Zanetti MV, de Souza Duran FL, Chaim-Avancini TM, et al. Fully automated habenula segmentation provides robust and reliable volume estimation across large magnetic resonance imaging datasets, suggesting intriguing developmental trajectories

in psychiatric disease. *Biol Psychiatry Cogn Neurosci Neuroimaging*. (2020) 5:923–9. doi: 10.1016/j.bpsc.2020.01.004
10. Schafer M, Kim J-W, Joseph J, Xu J, Frangou S, Doucet GE. Imaging habenula volume in schizophrenia and bipolar disorder. *Front Psychiatry*. (2018) 9:456. doi: 10.3389/fpsyt.2018.00456
11. Zhang L, Wang H, Luan S, Yang S, Wang Z, Wang J, et al. Altered volume and functional connectivity of the habenula in schizophrenia. *Front Hum Neurosci*. (2017) 11:636. doi: 10.3389/fnhum.2017.00636
12. Flanigan M, Aleyasin H, Takahashi A, Golden SA, Russo SJ. An emerging role for the lateral habenula in aggressive behavior. *Pharmacol Biochem Behav*. (2017) 162:79–86. doi: 10.1016/j.pbb.2017.05.003
13. Webster JF, Wozny C. Behavior: local lateral habenula interneurons mediate aggression. *Curr Biol*. (2020) 30:R954–6. doi: 10.1016/j.cub.2020.06.065
14. Gouveia FV, Germann J, Devenyi GA, Fonoff ET, Morais RMCB, Brentani H, et al. Bilateral amygdala radio-frequency ablation for refractory aggressive behavior alters local cortical thickness to a pattern found in non-refractory patients. *Front Hum Neurosci*. (2021) 15:653631. doi: 10.3389/fnhum.2021.653631
15. Gouveia FV, Germann J, de Morais R, Fonoff ET, Hamani C, Alho EJ, et al. Longitudinal changes after amygdala surgery for intractable aggressive behavior: clinical, imaging genetics, and deformation-based morphometry study-a case series. *Neurosurgery*. (2021) 88:E158–69. doi: 10.1093/neuros/nyaa378
16. Gouveia FV, Germann J, Devenyi GA, Morais RMCB, Santos APM, Fonoff ET, et al. Refractoriness of aggressive behaviour to pharmacological treatment: cortical thickness analysis in autism spectrum disorder. *BJPsych Open*. (2020) 6:e85. doi: 10.1192/bjo.2020.71
17. Gouveia FV, Hamani C, Fonoff ET, Brentani H, Alho EJL, de Morais RMCB, et al. Amygdala and hypothalamus: historical overview with focus on aggression. *Neurosurgery*. (2019) 85:11–30. doi: 10.1093/neuros/nyy635
18. Batrinos ML. Testosterone and aggressive behavior in man. *Int J Endocrinol Metab*. (2012) 10:563–8. doi: 10.5812/ijem.3661
19. Miczek KA, de Almeida RMM, Kravitz EA, Rissman EF, de Boer SF, Raine A. Neurobiology of escalated aggression and violence. *J Neurosci*. (2007) 27:11803–6. doi: 10.1523/JNEUROSCI.3500-07.2007
20. Rosell DR, Siever LJ. The neurobiology of aggression and violence. *CNS Spectr*. (2015) 20:254–79. doi: 10.1017/S109285291500019X
21. Siever LJ. Neurobiology of aggression and violence. *Am J Psychiatry*. (2008) 165:429–42. doi: 10.1176/appi.ajp.2008.07111774
22. Brentani H, de Paula CS, Bordini D, Rolim D, Sato F, Portolese J, et al. Autism spectrum disorders: an overview on diagnosis and treatment. *Braz J Psychaitry*. (2013) 35:S62–72. doi: 10.1590/1516-4446-2013-S104
23. Lanctôt KL, Herrmann N. Mirtazapine for agitation in dementia: moving forward. *Lancet*. (2021) 398:1462–3. doi: 10.1016/S0140-6736(21)01421-5
24. Ruthirakuhan M, Herrmann N, Andreazza AC, Verhoeff NPLG, Gallagher D, Black SE, et al. Agitation, oxidative stress, and cytokines in alzheimer disease: biomarker analyses from a clinical trial with nabilone for agitation. *J Geriatr Psychiatry Neurol*. (2020) 33:175–84. doi: 10.1177/0891988719874118
25. Ruthirakuhan M, Lanctôt KL, Di Scipio M, Ahmed M, Herrmann N. Biomarkers of agitation and aggression in Alzheimer's disease: a systematic review. *Alzheimers Dement*. (2018) 14:1344–76. doi: 10.1016/j.jalz.2018.04.013
26. Blair RJR. The neurobiology of impulsive aggression. *J Child Adolesc Psychopharmacol*. (2016) 26:4–9. doi: 10.1089/cap.2015.0088
27. Agetsuma M, Aizawa H, Aoki T, Nakayama R, Takahoko M, Goto M, et al. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. *Nat Neurosci*. (2010) 13:1354–6. doi: 10.1038/nn.2654
28. Kim U, Chang S-Y. Dendritic morphology, local circuitry, and intrinsic electrophysiology of neurons in the rat medial and lateral habenular nuclei of the epithalamus. *J Comp Neurol*. (2005) 483:236–50. doi: 10.1002/cne.20410
29. Viswanath H, Carter AQ, Baldwin PR, Molfese DL, Salas R. The medial habenula: still neglected. *Front Hum Neurosci*. (2013) 7:931. doi: 10.3389/fnhum.2013.00931
30. Aizawa H, Kobayashi M, Tanaka S, Fukai T, Okamoto H. Molecular characterization of the subnuclei in rat habenula. *J Comp Neurol*. (2012) 520:4051–66. doi: 10.1002/cne.23167
31. Zhang L, Hernández VS, Swinny JD, Verma AK, Giesecke T, Emery AC, et al. A GABAergic cell type in the lateral habenula links hypothalamic homeostatic and midbrain motivation circuits with sex steroid signaling. *Transl Psychiatry*. (2018) 8:50. doi: 10.1038/s41398-018-0099-5
32. Flanigan ME, Aleyasin H, Li L, Burnett CJ, Chan KL, LeClair KB, et al. Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice. *Nat Neurosci*. (2020) 23:638–50. doi: 10.1038/s41593-020-0617-7
33. Chou M-Y, Amo R, Kinoshita M, Cherng B-W, Shimazaki H, Agetsuma M, et al. Social conflict resolution regulated by two dorsal habenular subregions in zebrafish. *Science*. (2016) 352:87–90. doi: 10.1126/science.aac9508
34. Concha M. An evolutionary perspective on habenular asymmetry in humans. *J Neurol Neuromed*. (2016) 1:44–50. doi: 10.29245/2572.942X/2016/8.1094
35. Valverde O, Célérier E, Baranyi M, Vanderhaeghen P, Maldonado R, Sperlagh B, e al. GPR3 receptor, a novel actor in the emotional-like responses. *PLoS ONE*. (2009) 4:e4704. doi: 10.1371/journal.pone.0004704
36. Serykh A, Khrapova MV, Dubrovina NI, Petrova ES, Mikhnevich N, Starostina MV, et al. The increased density of the habenular neurons, high impulsivity, aggression and resistant fear memory in Disc1-Q31L genetic mouse model of depression. *Behav Brain Res*. (2020) 392:112693. doi: 10.1016/j.bbr.2020.112693
37. Martínez-Rodríguez E, Martín-Sánchez A, Kul E, Bose A, Martínez-Martínez FJ, Stork O, et al. Male-specific features are reduced in Mecp2-null mice: analyses of vasopressinergic innervation, pheromone production and social behaviour. *Brain Struct Funct*. (2020) 225:2219–38. doi: 10.1007/s00429-020-02122-6
38. Muroi Y, Ishii T. Glutamatergic signals in the dorsal raphe nucleus regulate maternal aggression and care in an opposing manner in mice. *Neuroscience*. (2019) 400:33–47. doi: 10.1016/j.neuroscience.2018.12.034
39. Rigney N, Beaumont R, Petrulis A. Sex differences in vasopressin 1a receptor regulation of social communication within the lateral habenula and dorsal raphe of mice. *Horm Behav*. (2020) 121:104715. doi: 10.1016/j.yhbeh.2020.104715
40. Tsunoda M, Miyamichi K, Eguchi R, Sakuma Y, Yoshihara Y, Kikusui T, et al. Identification of an intra- and inter-specific tear protein signal in rodents. *Curr Biol*. (2018) 28:1213–23.e6. doi: 10.1016/j.cub.2018.02.060
41. Cavaliere RM, Silvotti L, Percudani R, Tirindelli R. Female mouse tears contain an anti-aggression pheromone. *Sci Rep*. (2020) 10:2510. doi: 10.1038/s41598-020-59293-9
42. Gan G, Zilverstand A, Parvaz MA, Preston-Campbell RN, d'Oleire Uquillas F, Moeller SJ, et al. Habenula-prefrontal resting-state connectivity in reactive aggressive men—a pilot study. *Neuropharmacology*. (2019) 156:107396. doi: 10.1016/j.neuropharm.2018.10.025
43. Gouveia FV, Germann J, Elias GJ, Hamani C, Fonoff ET, Martinez RCR. Case report: 5 Years follow-up on posterior hypothalamus deep brain stimulation for intractable aggressive behaviour associated with drug-resistant epilepsy. *Brain Stimul*. (2021) 14:1201–4. doi: 10.1016/j.brs.2021.07.062
44. Baker PM, Jhou T, Li B, Matsumoto M, Mizumori SJY, Stephenson-Jones M, et al. The lateral habenula circuitry: reward processing and cognitive control. *J Neurosci*. (2016) 36:11482–8. doi: 10.1523/JNEUROSCI.2350-16.2016
45. Congiu M, Trusel M, Pistis M, Mameli M, Lecca S. Opposite responses to aversive stimuli in lateral habenula neurons. *Eur J Neurosci*. (2019) 50:2921–30. doi: 10.1111/ejn.14400
46. Valentinova K, Tchenio A, Trusel M, Clerke JA, Lalive AL, Tzanoulinou S, et al. Morphine withdrawal recruits lateral habenula cytokine signaling to reduce synaptic excitation and sociability. *Nat Neurosci*. (2019) 22:1053–6. doi: 10.1038/s41593-019-0421-4
47. Tan D, Nuno-Perez A, Mameli M, Meye FJ. Cocaine withdrawal reduces GABA R transmission at entopeduncular nucleus—lateral habenula synapses. *Eur J Neurosci*. (2019) 50:2124–33. doi: 10.1111/ejn.14120
48. Meye FJ, Trusel M, Soiza-Reilly M, Mameli M. Neural circuit adaptations during drug withdrawal—Spotlight on the lateral habenula. *Pharmacol Biochem Behav*. (2017) 162:87–93. doi: 10.1016/j.pbb.2017.08.007
49. Baker PM, Raynor SA, Francis NT, Mizumori SJY. Lateral habenula integration of proactive and retroactive information mediates behavioral flexibility. *Neuroscience*. (2017) 345:89–98. doi: 10.1016/j.neuroscience.2016.02.010
50. Golden SA, Heshmati M, Flanigan M, Christoffel DJ, Guise K, Pfau ML, et al. Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature*. (2016) 534:688–92. doi: 10.1038/nature18601
51. Ferrari PF, van Erp AMM, Tornatzky W, Miczek KA. Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *Eur J Neurosci*. (2003) 17:371–8. doi: 10.1046/j.1460-9568.2003.02447.x

52. Beiderbeck DI, Reber SO, Havasi A, Bredewold R, Veenema AH, Neumann ID. High and abnormal forms of aggression in rats with extremes in trait anxiety–involvement of the dopamine system in the nucleus accumbens. *Psychoneuroendocrinology*. (2012) 37:1969–80. doi: 10.1016/j.psyneuen.2012.04.011
53. Couppis MH, Kennedy CH. The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. *Psychopharmacology*. (2008) 197:449–56. doi: 10.1007/s00213-007-1054-y
54. Becker EA, Marler CA. Postcontest blockade of dopamine receptors inhibits development of the winner effect in the California mouse. (*Peromyscus californicus*). *Behav Neurosci*. (2015) 129:205–13. doi: 10.1037/bne0000043
55. Harat M, Rudaś M, Zieliński P, Birska J, Sokal P. Deep brain stimulation in pathological aggression. *Stereotact Funct Neurosurg*. (2015) 93:310–5. doi: 10.1159/000431373
56. Park HR, Kim IH, Kang H, Lee DS, Kim B-N, Kim DG, et al. Nucleus accumbens deep brain stimulation for a patient with self-injurious behavior and autism spectrum disorder: functional and structural changes of the brain: report of a case and review of literature. *Acta Neurochir*. (2017) 159:137–43. doi: 10.1007/s00701-016-3002-2
57. Harat M, Kiec M, Rudaś M, Birski M, Furtak J. Treating aggression and self-destructive behaviors by stimulating the nucleus accumbens: a case series. *Front Neurol*. (2021) 12:706166. doi: 10.3389/fneur.2021.706166
58. Yan H, Elkaim LM, Venetucci Gouveia F, Huber JF, Germann J, Loh A, et al. Deep brain stimulation for extreme behaviors associated with autism spectrum disorder converges on a common pathway: a systematic review and connectomic analysis. *J Neurosurg*. (2022) 1–10. doi: 10.3171/2021.11.JNS21928. [Epub ahead of print].
59. Baño-Otálora B, Piggins HD. Contributions of the lateral habenula to circadian timekeeping. *Pharmacol Biochem Behav*. (2017) 162:46–54. doi: 10.1016/j.pbb.2017.06.007
60. Salaberry NL, Hamm H, Felder-Schmittbuhl M-P, Mendoza J. A suprachiasmatic-independent circadian clock(s) in the habenula is affected by Per gene mutations and housing light conditions in mice. *Brain Struct Funct*. (2019) 224:19–31. doi: 10.1007/s00429-018-1756-4
61. Shuboni-Mulligan DD, Cavanaugh BL, Tonson A, Shapiro EM, Gall AJ. Functional and anatomical variations in retinorecipient brain areas in and: implications for the circadian and masking systems. *Chronobiol Int*. (2019) 36:1464–81. doi: 10.1080/07420528.2019.1651325
62. Sakhi K, Belle MDC, Gossan N, Delagrange P, Piggins HD. Daily variation in the electrophysiological activity of mouse medial habenula neurones. *J Physiol*. (2014) 592:587–603. doi: 10.1113/jphysiol.2013.263319
63. Yu EZ, Hallenbeck JM, Cai D, McCarron RM. Elevated arylalkylamine-N-acetyltransferase. (AA-NAT) gene expression in medial habenular and suprachiasmatic nuclei of hibernating ground squirrels. *Brain Res Mol Brain Res*. (2002) 102:9–17. doi: 10.1016/S0169-328X(02)00138-9
64. Bronsard G, Bartolomei F. Rhythms, rhythmicity and aggression. *J Physiol Paris*. (2013) 107:327–34. doi: 10.1016/j.jphysparis.2013.03.002
65. Benca R, Duncan MJ, Frank E, McClung C, Nelson RJ, Vicentic A. Biological rhythms, higher brain function, and behavior: gaps, opportunities, and challenges. *Brain Res Rev*. (2009) 62:57–70. doi: 10.1016/j.brainresrev.2009.09.005
66. O'Brien LM. The neurocognitive effects of sleep disruption in children and adolescents. *Child Adolesc Psychiatr Clin N Am*. (2009) 18:813–23. doi: 10.1016/j.chc.2009.04.008
67. Dahl RE. Sleeplessness and aggression in youth. *J Adolesc Health Care*. (2006) 38:641–2. doi: 10.1016/j.jadohealth.2006.03.013
68. Reid GJ, Hong RY, Wade TJ. The relation between common sleep problems and emotional and behavioral problems among 2- and 3-year-olds in the context of known risk factors for psychopathology. *J Sleep Res*. (2009) 18:49–59. doi: 10.1111/j.1365-2869.2008.00692.x
69. Basnakova A, Cheng R-K, Chia JSM, D'Agostino G, Suryadi, Tan GJH, et al. The habenula clock influences response to a stressor. *Neurobiol Stress*. (2021) 15:100403. doi: 10.1016/j.ynstr.2021.100403
70. Wang RY, Aghajanian GK. Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science*. (1977) 197:89–91. doi: 10.1126/science.194312
71. Varga V, Kocsis B, Sharp T. Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. *Eur J Neurosci*. (2003) 17:280–6. doi: 10.1046/j.1460-9568.2003.02465.x
72. Portas CM, Bjorvatn B, Ursin R. Serotonin and the sleep/wake cycle: special emphasis on microdialysis studies. *Prog Neurobiol*. (2000) 60:13–35. doi: 10.1016/S0301-0082(98)00097-5
73. Monti JM. Serotonin control of sleep-wake behavior. *Sleep Med Rev*. (2011) 15:269–81. doi: 10.1016/j.smrv.2010.11.003
74. Roman V, Walstra I, Luiten PGM, Meerlo P. Too little sleep gradually desensitizes the serotonin 1A receptor system. *Sleep*. (2005) 28:1505–10. doi: 10.1093/sleep/28.12.1505
75. Edelsohn GA, Abright AR. Editorial: Safer Use of Antipsychotics in Youth (SUAY): should treatment be guided by symptoms? *J Am Acad Child Adolesc Psychiatry*. (2021) 61:34–36. doi: 10.1016/j.jaac.2021.07.009
76. Vaudreuil C, Farrell A, Wozniak J. Psychopharmacology of treating explosive behavior. *Child Adolesc Psychiatr Clin N Am*. (2021) 30:537–60. doi: 10.1016/j.chc.2021.04.006
77. Lea-Banks H, O'Reilly MA, Hynynen K. Ultrasound-responsive droplets for therapy: a review. *J Control Release*. (2019) 293:144–54. doi: 10.1016/j.jconrel.2018.11.028
78. Lea-Banks H, Meng Y, Wu S-K, Belhadjhamida R, Hamani C, Hynynen K. Ultrasound-sensitive nanodroplets achieve targeted neuromodulation. *J Control Release*. (2021) 332:30–9. doi: 10.1016/j.jconrel.2021.02.010
79. Lea-Banks H, O'Reilly MA, Hamani C, Hynynen K. Localized anesthesia of a specific brain region using ultrasound-responsive barbiturate nanodroplets. *Theranostics*. (2020) 10:2849–58. doi: 10.7150/thno.41566
80. Lozano AM, Lipsman N. Probing and regulating dysfunctional circuits using deep brain stimulation. *Neuron*. (2013) 77:406–24. doi: 10.1016/j.neuron.2013.01.020
81. Wang Y, Zhang C, Zhang Y, Gong H, Li J, Jin H, et al. Habenula deep brain stimulation for intractable schizophrenia: a pilot study. *Neurosurg Focus*. (2020) 49:E9. doi: 10.3171/2020.4.FOCUS20174
82. Sartorius A, Kiening KL, Kirsch P, von Gall CC, Haberkorn U, Unterberg AW, et al. Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biol Psychiatry*. (2010) 67:e9–11. doi: 10.1016/j.biopsych.2009.08.027
83. Wang Z, Cai X, Qiu R, Yao C, Tian Y, Gong C, et al. Case report: lateral habenula deep brain stimulation for treatment-resistant depression. *Front Psychiatry*. (2020) 11:616501. doi: 10.3389/fpsyt.2020.616501
84. Zhang C, Zhang Y, Li D, Deng Z, Nuttin B, Voon V, et al. Habenular stimulation for neurosurgery resistant obsessive-compulsive disorder: a case report. *Front Psychiatry*. (2020) 11:29. doi: 10.3389/fpsyt.2020.00029
85. Zhang C, Kim S-G, Li D, Zhang Y, Li Y, Husch A, et al. Habenula deep brain stimulation for refractory bipolar disorder. *Brain Stimul*. (2019) 12:1298–300. doi: 10.1016/j.brs.2019.05.010
86. Germann J, Mameli M, Elias GJB, Loh A, Taha A, Gouveia FV, et al. Deep brain stimulation of the habenula: systematic review of the literature and clinical trial registries. *Front Psychiatry*. (2021) 12:730931. doi: 10.3389/fpsyt.2021.730931

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Systems Neuroscience

PERSPECTIVE
published: 03 March 2022
doi: 10.3389/fnsys.2022.826475

Lateral Habenula Beyond Avoidance: Roles in Stress, Memory, and Decision-Making With Implications for Psychiatric Disorders

Phillip M. Baker[1], Victor Mathis[2], Lucas Lecourtier[3]*, Sarah C. Simmons[4], Fereshteh S. Nugent[4]*, Sierra Hill[1] and Sheri J. Y. Mizumori[5]**

[1] Department of Psychology, Seattle Pacific University, Seattle, WA, United States, [2] CNRS UPR 3212, Institut des Neurosciences Cellulaires et Intégratives, Center National de la Recherche Scientifique, University of Strasbourg, Strasbourg, France, [3] CNRS, Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), UMR 7364, Université de Strasbourg, Strasbourg, France, [4] Department of Pharmacology and Molecular Therapeutics, School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [5] Department of Psychology, University of Washington, Seattle, WA, United States

In this Perspective review, we highlight some of the less explored aspects of lateral habenula (LHb) function in contextual memory, sleep, and behavioral flexibility. We provide evidence that LHb is well-situated to integrate different internal state and multimodal sensory information from memory-, stress-, motivational-, and reward-related circuits essential for both survival and decision making. We further discuss the impact of early life stress (ELS) on LHb function as an example of stress-induced hyperactivity and dysregulation of neuromodulatory systems within the LHb that promote anhedonia and motivational deficits following ELS. We acknowledge that recent technological advancements in manipulation and recording of neural circuits in simplified and well-controlled behavioral paradigms have been invaluable in our understanding of the critical role of LHb in motivation and emotional regulation as well as the involvement of LHb dysfunction in stress-induced psychopathology. However, we also argue that the use of ethologically-relevant behaviors with consideration of complex aspects of decision-making is warranted for future studies of LHb contributions in a wide range of psychiatric illnesses. We conclude this Perspective with some of the outstanding issues for the field to consider where a multi-systems approach is needed to investigate the complex nature of LHb circuitry interactions with environmental stimuli that predisposes psychiatric disorders.

Keywords: lateral habenula, memory, reward, motivation, sleep, psychiatric illnesses, early life stress

OPEN ACCESS

Edited by:
Federico Bermudez-Rattoni, National Autonomous University of Mexico, Mexico

Reviewed by:
Yukiori Goto, Kyoto University, Japan
Maria-Isabel Miranda, National Autonomous University of Mexico, Mexico

****Correspondence:***
Phillip M. Baker
bakerp5@spu.edu
Lucas Lecourtier
l.lecourtier@unistra.fr
Fereshteh S. Nugent
fereshteh.nugent@usuhs.edu
Sheri J. Y. Mizumori
mizumori@uw.edu

Received: *30 November 2021*
Accepted: *10 February 2022*
Published: *03 March 2022*

Citation:
Baker PM, Mathis V, Lecourtier L, Simmons SC, Nugent FS, Hill S and Mizumori SJY (2022) Lateral Habenula Beyond Avoidance: Roles in Stress, Memory, and Decision-Making With Implications for Psychiatric Disorders. Front. Syst. Neurosci. 16:826475. doi: 10.3389/fnsys.2022.826475

INTRODUCTION

The lateral habenula (LHb) clearly plays a role in learning and memory since LHb disruption produces deficits on tasks that require the processing of contextual information (Baker et al., 2015; Durieux et al., 2020), spatial working memory (Mathis and Lecourtier, 2017; Mathis et al., 2017), and/or stimuli associated with negative valence outcomes (Stamatakis et al., 2016; Knowland and Lim, 2018; Sosa et al., 2021). Across these diverse types of memory and cognitive processing, a fundamental contribution of the LHb may be to constantly monitor one's current internal state

relative to external environmental conditions so that behaviors can be modified as needed (Baker et al., 2015; Mathis and Lecourtier, 2017; Lecca et al., 2020). Such a contribution appears to rely on the integration and signaling of cognitive, motivational/emotional, and behavioral state information (Sutherland, 1982; Chastrette et al., 1991; Nair et al., 2013; Mendoza, 2017; Shepard and Nugent, 2021). For example, LHb responds to positive and negative choice outcomes (Matsumoto and Hikosaka, 2009; Li et al., 2019), the generation of prediction error signals (Hong and Hikosaka, 2013; Tian and Uchida, 2015), changes in motivational and physiological states [e.g., stress, time of day, etc., (Shepard et al., 2018b; Salaberry et al., 2019; Langlois et al., 2021)], and changes in behavioral state (Baker et al., 2015; Nuno-Perez et al., 2018; Lecca et al., 2020).

Functional efferent and afferent connections of the habenula [reviewed in detail in Baker et al. (2015) and Quina et al. (2015)] to areas including the frontal cortical areas (Mathis et al., 2017), the basal ganglia (Wallace et al., 2017), the ventral tegmental area (Stamatakis et al., 2013; Liu et al., 2021). Despite increasing supporting evidence of this broad view of LHb function, a number of significant issues remain to be resolved if we are to sufficiently understand the adaptive relevance of the LHb for everyday memory function. These advances will aid in the development of novel interventions for neuropsychiatric conditions that have been linked to LHb dysfunction such as depression, anxiety, and addiction.

In the following, we focus on key outstanding issues related to two widely held concepts regarding LHb function: 1) The LHb serves as a critical interface for context memory and internal emotional state information, and 2) This integrative role positions the LHb to play a key role in specific psychopathological symptoms due to poor integration of context and emotional information, such as that which occurs when stressed. Evidence to support these general concepts of LHb function is highlighted along with examples of research that exemplify important unresolved issues. It is then suggested that our understanding of the contribution of the LHb to behavior can be substantially enhanced by greater inclusion of more ethologically-relevant tasks. Finally we conclude with suggestions for paths forward.

ROLE OF THE LATERAL HABENULA IN MEMORY PROCESSES: AN INTERFACE BETWEEN CONTEXT AND INTERNAL EMOTIONAL STATE

A growing number of studies have demonstrated, in rodents, that pharmacological or chemogenetic inhibition of LHb induced deficits of several types of memory, including long-term spatial memory in the water maze (Mathis et al., 2015), contextual memory in an object-based recognition task (Goutagny et al., 2013), short-term memory in a delayed non-matching to position task (Mathis and Lecourtier, 2017), fear memory in a trace fear-conditioning paradigm (Durieux et al., 2020) as well as inhibitory avoidance (Tomaiuolo et al., 2014) [see also Song et al. (2017)]. One noteworthy aspect of these examinations is that the engagement of the LHb in learning and memory appears to relate to two aspects of the ongoing situation: its emotional valence and the context in which it occurs.

It does not seem surprising that the LHb is particularly engaged in memory tasks requiring the processing of contextual cues during negative emotional situations, as it has a major role in signaling aversion (Hennigan et al., 2015; Li et al., 2019) and it shows strong activation in response to a large number of stressors (Chastrette et al., 1991; Lecca et al., 2017; Li et al., 2019). In the water maze, LHb dysfunctions not only induced memory deficits, i.e., a greater distance to reach the hidden platform during training and a lower time spent in the target quadrant (i.e., the area where the platform—which has since been withdrawn—was located) during the retention test [see Mathis et al. (2015)], but also led to signs of exacerbated stress, i.e., excessive thigmotaxic behavior (swimming along the edge of the pool) in conjunction with an increased corticosterone (CORT) release [(Mathis et al., 2015, 2018); see also Jacinto et al. (2017)].

These types of results following LHb dysfunction suggest that one of its main roles could be to process different modalities of an ongoing situation, including external environmental cues and internal emotional state, and to participate in the elaboration of appropriate behavioral responses. Hence, the LHb integrates external information as well as physiological, internal, signals. In that regard recent studies showed that the LHb signals stress and punishment in a context-dependent manner, as combination of stressors or contextual illumination reduces LHb stress response (Zhang et al., 2016; Huang et al., 2019). These findings suggest a yet underdetermined influence of external conditions over the LHb functions. Further studies are required to better understand how and in which conditions the LHb can simultaneously deal with external (context, nature of the threat) and internal (CORT levels, circadian rhythm) information. Such a role for the LHb in both both stress- and memory-related information processing raise an important question: are cognitive deficits a primary consequence of LHb dysfunction, secondarily inducing defective stress coping, or is an impossibility to cope with a stressful situation the primary consequence of LHb dysfunction, secondarily inducing learning and memory deficits?

At this point it is hard to answer this question. Indeed, most of the behavioral tests used to assess memory in rodents often include an aversive component to motivate the animals; electrical foot shocks in fear conditioning, cool water to swim in in order to find a hidden platform in the water maze, or food restriction in a variety of tasks using delayed non-matching to position paradigms (although the latter also imply reward-related processes). On the contrary, it might seem simpler to address stress response processes. Hence, as mentioned above, the LHb seems to be a crucial structure engaged in the response to stressors and in signaling aversive situations. The impact of stress over cognitive performances is well described. While low levels of stress can improve performances, a high or

prolonged stress will eventually induce deficits (Arnsten, 2015), especially memory deficits (Kim and Diamond, 2002; Roozendaal et al., 2009). A simple hypothesis would be to consider that, if altered, the engagement of the LHb in stress integration will interfere with memory processes, subsequently leading to performance deficits. This would explain why pharmacological inhibition of the LHb during the acquisition phase of each training day prevented learning in a water maze paradigm (Mathis et al., 2015). Such intervention likely increased the stress load across training days, resulting in a flat learning curve. Indeed, impaired rats showed an increased level of thigmotaxic behavior (Mathis et al., 2015), which can be attributed to defective stress coping, and exacerbated CORT levels (Mathis et al., 2018). This is in accordance with the fact that LHb dysfunction induces anxiety-like behaviors on the elevated plus maze (Mathis et al., 2015). However, it might seem contradictory with the fact that when LHb inhibition occurred at the probe test following a drug-free training phase that should have attenuated potential stress responses (during which one can therefore postulate that rats had been used to the stressful aspect of the situation and had been able to deal with it), it nonetheless created retrieval deficits (Mathis et al., 2015). In addition, during this probe test rats showed a reduced swim speed, suggesting a "calm" exploration of the apparatus. We have also found using a different paradigm, that following habituation to the testing condition and drug-free training, LHb inhibition impaired memory of object locations in an open field when one of three objections is moved from a previous location and replaced with a novel object (Goutagny et al., 2013). All together these results suggest that the LHb role in stress processing is not likely the only reason for the observed memory deficits.

These findings appear to support the idea that cognitive deficits are a primary consequence of LHb dysfunctions, secondarily inducing exacerbated stress. Indeed, the thigmotaxic behavior observed in the water maze following LHb inhibition might reflect the engagement of a default behavioral response as a consequence of a lack of knowledge about the platform location. Such a behavior might be interpreted as a "low-cost" strategy triggered when no memory-based strategy is available. The CORT elevation would then be a consequence required for the physical effort and partially reflecting stress.

Finally, a third case would be that the LHb processes stress- and memory-related information in an independent manner. However, as said earlier, the existing paradigms assessing cognitive processes do not necessarily give the possibility to address stress and memory independently and then together. Indeed, the intrinsic aversive aspect of most of the behavioral tests assessing memory prevents from dissociating these two aspects. One possibility though could be to add a supplementary stressor and assess the effect of this other stressors on memory performances.

Beside the behavioral paradigms, understanding how the LHb receives contextual and stress-related information could help to answer this chicken and egg question. Indeed, the LHb position in the central nervous system is of great interest with regard to stress and cognitive processes. The LHb belongs to the dorsal diencephalic conduction system conveying information from the prefrontal cortex, several septal nuclei, the hypothalamus or the entopeduncular nucleus to midbrain monoaminergic areas such as the raphe, ventral tegmental area and the locus coeruleus (Roman et al., 2020).

Understanding how the LHb receives contextual and stressful information would help to answer this chicken and egg question. Interestingly, upon cognitive testing, a functional connectivity between the LHb and both the mPFC (Mathis et al., 2017) and HPC (Baker et al., 2019; Durieux et al., 2020) has been shown to exist. In addition, the LHb and HPC, although not directly anatomically connected, likely communicate whether it is during exploration of an unfamiliar environment or during rapid eye movement (REM) sleep episodes (Aizawa et al., 2013; Goutagny et al., 2013). The link with sleep is of particular interest as communication between the LHb and HPC could be related to past experiences and therefore be part of the mechanisms underlying HPC-dependent learning and memory processes. A specific role of the LHb in sleep-dependent processes seems also in accordance with the fact that the LHb shows circadian oscillatory activity and is implicated in circadian-related behaviors (Guilding et al., 2010; Baño-Otálora and Piggins, 2017; Mendoza, 2017; Huang et al., 2019; Salaberry et al., 2019). A better understanding of the LHb-related network conveying memory-related information would help untangle whether memory deficits are at the origin or the consequences of the observed exacerbated stress response in the different memory tasks aforementioned (e.g., water maze, fear conditioning).

Further investigations are needed to fully understand how the different types of information (contextual vs. stress-related) are integrated by the LHb. This could be performed using behavioral paradigms that include repeated stressful situations, in order to potentially capture habituation processes and coping strategies. It would be interesting, in such paradigms, to investigate the activity of the LHb in conjunction with those of prefrontal cortical, hippocampal, and amygdalar regions, and explore the level of communication between those structures according to the different aspects of the paradigm, including the acute response to the stressful procedure, and the coping mechanisms upon repetition of it. Examinations could also include important stress-related structures which send input to the LHb, such as the hypothalamus (Lecca et al., 2017; Trusel et al., 2019), the entopeduncular nucleus (Stephenson-Jones et al., 2016; Li et al., 2019), frontal cortical areas (Kim and Lee, 2012; Fillinger et al., 2017), and the VTA (Stamatakis et al., 2013) which likely send information related to the emotional valence of the situation, thus positioning the LHb as a cerebral "hub," linking different macro-systems (Geisler and Trimble, 2008).

It will also be important to better describe the influence of the context over the stress-related aspect of the paradigm. The recent results showing that environmental illumination conditions directly influence the LHb capacity to signal stress through a retino-thalamo-habenular circuit, and participates in the effect of light therapy in depression, is a first step toward this goal (Huang et al., 2019). The recent advances in neuroscience allow

in vivo circuit specific investigation and will likely participate in elucidating these issues.

LATERAL HABENULA REPRESENTS A KEY NODE FOR INCREASED RISK OF PSYCHOPATHOLOGY FOLLOWING EARLY LIFE ADVERSITY

It is well-established that exposure to childhood adversity/early life stress (ELS) is a strong predictor for several later life mental disorders, including substance use disorders (SUDs), anxiety and depression (Heim et al., 2010; Lippard and Nemeroff, 2020; Shepard and Nugent, 2020, 2021). Common forms of childhood adversity include child abuse and neglect, domestic violence, and family economic hardship. The recent COVID-19 pandemic shutdowns across the globe have caused detrimental effects on child mental health with the increased risk for domestic violence, child abuse and neglect, compounded by food and housing insecurity (Gotlib et al., 2020; Humphreys et al., 2020; Lawson et al., 2020; Yard et al., 2021). Poor responsivity of psychiatric patients with a prior history of ELS to psychotherapy and/or pharmacotherapy further necessitates a better understanding of the mechanisms and neural circuits that link ELS with mental illnesses to identify potential novel interventional therapeutic targets.

Prominent ELS rodent and primate models employ early disruptions in mother-infant relationship such as a single 24 h maternal deprivation (MD), repeated daily maternal separation (MS), and limited bedding and nesting (LBN) (Macrì et al., 2007; Nishi et al., 2014; Shepard et al., 2018a; Okhuarobo et al., 2020). Although these ELS models may not reflect all types of early adverse experiences, they are associated with persistent depressive-and anhedonia-like behaviors (Tchenio et al., 2017; Authement et al., 2018; Bolton et al., 2018a; Shepard et al., 2018b; Simmons et al., 2020) and altered drug reward (Bolton et al., 2018b; Okhuarobo et al., 2020; Langlois et al., 2021; Levis et al., 2021) suggesting the translational validity of these models for child neglect. However, it should be noted that not all animals that experience ELS develop stress psychopathology or substance use disorders later in life which is also the case for children exposed to adversity (Kalinichev et al., 2002; Moffett et al., 2006; Ordoñes Sanchez et al., 2021). Thus, in preclinical ELS research, differences between predictable (MS) and unpredictable (single prolonged MD and limited bedding and nesting) stressors as well as the duration of separation and alterations in maternal behavior should be taken into account which may confer resistance or vulnerability and directly impact the outcomes in terms of addictive behaviors, depression and mood phenotypes in these models.

Several neural pathways and neurobiological mechanisms such as the hypothalamic-pituitary-adrenal (HPA) axis and extra-hypothalamic corticotropin-releasing factor (CRF) circuits have been identified by which ELS may increase the risk for mood dysregulation, stress-related disorders and addiction (Nemeroff, 2016). Emerging evidence now suggests that ELS-induced alterations of reward- and stress-related brain regions such as ventral tegmental area (VTA), amygdala, nucleus accumbens, prefrontal cortex and LHb may underlie the increased risk for ELS-induced psychopathology (Authement et al., 2015, 2018; Peña et al., 2017, 2019; Tchenio et al., 2017; Bolton et al., 2018a; Shepard et al., 2020; Simmons et al., 2020; Langlois et al., 2021; Oh et al., 2021; Shepard and Nugent, 2021). Specifically, recent studies provided compelling evidence that the LHb is a critical converging brain region for ELS-induced dysregulation of reward circuits (Tchenio et al., 2017; Authement et al., 2018; Bolton et al., 2018b; Simmons et al., 2020). The LHb links forebrain limbic structures with midbrain monoaminergic centers (Schultz, 2010; Cohen et al., 2012; Proulx et al., 2014) and is involved in reward/aversion-related learning and memory processing associated with avoidance from stressful and aversive situations through suppression of dopamine and serotonin systems. Specifically, anatomically and/or functionally diverse neuronal populations within the LHb modulate motivated behaviors through cell type-specific projections to non-overlapping targets including the VTA, substantia nigra compacta, rostromedial tegmental area (RMTg), or raphe nuclei (Stamatakis et al., 2016; Wallace et al., 2017; Cerniauskas et al., 2019; Hu et al., 2020; Lecca et al., 2020). Not surprisingly, LHb dysfunction contributes to a myriad of cognitive, learning, and affective impairments associated with depression, anxiety, psychosis and drug addiction (Graziane et al., 2018; Nuno-Perez et al., 2018; Proulx et al., 2018).

The common finding among studies using ELS models MD (Authement et al., 2018; Shepard et al., 2018b; Simmons et al., 2020; Langlois et al., 2021) and MS (Tchenio et al., 2017) is that ELS promotes LHb hyperexcitability although the underlying mechanisms vary from downregulation of small conductance (SK2) potassium channels and increased protein kinase (PKA) activity in LHb (Authement et al., 2018) to decreased postsynaptic $GABA_BR$-GIRK signaling arising from entopeduncular nucleus GABAergic inputs to LHb (Tchenio et al., 2017). Additionally, MD in rats persistently increases both tonic and bursting LHb activity from early adolescence to adulthood (Authement et al., 2018; Shepard et al., 2018b; Simmons et al., 2020; Langlois et al., 2021) consistent with the literature that LHb hyperactivity in general (and bursting in particular) contributes to the development of depression-like motivational and social deficits, and anhedonic phenotypes (Yang et al., 2018; Klein et al., 2020). Either chemogenetic inhibition of LHb neurons or deep brain stimulation that reduces LHb activity ameliorates MS-induced depressive-like phenotype in mice (the lack of motivation of mice to avoid an aversive context which is an escapable foot-shock) (Tchenio et al., 2017). Interestingly, juvenile MD rats show an increased active coping behavior in the forced swim test (with an increase in climbing behavior) while late adolescent rats exhibit an increased immobility in the forced swim test, both behavioral phenotypes are reversed by ketamine treatment (Shepard et al., 2018b). More importantly, long-lasting anti-depressant effects of ketamine on MD-induced behavior in young adult rats is

associated with a return to normal levels of LHb neuronal excitability (Shepard et al., 2018b). MD also triggers an anhedonic phenotype in natural sucrose reward while also decreasing morphine intake in morphine self-administration acquisition associated with MD-induced glutamatergic plasticity in LHb neurons (Langlois et al., 2021).

Consistently, it has been shown that synaptic transmission from the LHb to the RMTg, a nucleus that suppresses dopamine neuronal activity and signaling, increases during transitions to immobility in the forced swim test to escape this aversive context. Activation of this LHb to RMTg circuit also decreases motivation of rats to work harder to receive sucrose reward in a progressive ratio schedule of operant appetitive task suggesting a critical role for the LHb in regulation of motivation (Proulx et al., 2018). Therefore, it is possible that MD-induced LHb glutamatergic plasticity and LHb hyperactivity could increase the excitatory drive from the LHb to the RMTg and underlie motivational deficits in MD rats. In the future, it is necessary to employ similar circuit-based studies of MD effects on motivation such as progressive ratio schedule in sucrose self-administration, morphine self-administration, or other motivation based effort tasks (see below). Overall, these findings highlight the role of LHb hyperactivity in ELS-induced induction of anhedonic states and altered opioid seeking where limiting LHb activity using novel fast-acting antidepressants such as ketamine or deep brain stimulation could have therapeutic potential. It remains unclear at this point, however, whether these effects are concurrent with broader cognitive and behavioral effects as has been noted with the aforementioned memory related tasks.

The deleterious effects of ELS on reward circuits also involve alterations of innate stress neuromodulators such as CRF/CRFR1 and dynorphin (Dyn)/kappa opioid receptor (KOR) systems that contribute to the development of stress-induced drug seeking behaviors and negative affective states including anhedonia, social deficits and decreased motivation (as hallmark features of depression) following ELS (Land et al., 2008; Bruchas et al., 2010; Koob, 2010; Pautassi et al., 2012; Karkhanis et al., 2016; Mantsch et al., 2016; Bolton et al., 2018a; Knowland and Lim, 2018; Tejeda and Bonci, 2019). Recent work on the neuromodulatory regulation of LHb excitability and synaptic transmission by CRF/CRFR1 and Dyn/KOR signaling and their dysregulation by MD in male rats (Authement et al., 2018; Simmons et al., 2020) further highlight involvement of critical neuromodulators within LHb circuits that could underlie ELS-induced anhedonia, motivational deficits, drug seeking behaviors, and flexibility related behaviors. Intriguingly, LBN-induced anhedonia is also associated with high c-fos expression (indicative of increased neuronal activity) in the LHb and increased extrahypothalamic CRF neurotransmission from central amygdala (Bolton et al., 2018b), a brain region that also projects to the LHb (Hu et al., 2020). Therefore, additional insight into molecular mechanisms underlying CRF/CRFR1 and Dyn/KOR neuromodulation within LHb and its circuits in ELS models may offer novel therapeutic interventions with specificity for uncoupling these pathologically hyperactive stress signaling pathways following ELS (**Figure 1B**).

POTENTIAL INSIGHTS INTO LATERAL HABENULA FUNCTION UTILIZING MORE COMPLEX, ETHOLOGICALLY RELEVANT BEHAVIORS

Initial reports examining the role of the habenular complex, of which the LHb is a part, placed a wide range of behaviors from sexual functions, to circadian signaling under its control (Sutherland, 1982). As the toolkit to examine brain area contributions to behaviors has advanced, the range of behaviors typically associated with the LHb has narrowed to principally include aversive outcome signaling such as the omission of an expected reward, memory related functions described above, and adaptive behavioral selection such as during probabilistic reversal learning where reward contingencies in a T-maze are reversed once animals learn task contingencies (Nair et al., 2013; Baker et al., 2015; Sosa et al., 2021). Some of this is likely due to the ability to restrict interventions to spare fibers of passage or target specific cell identities. This no doubt ruled out contributions more likely to have come from nearby areas and the like. However, another likely contributor has been a reduction in the range of behavioral conditions examined due to limitations imposed by advanced recording and manipulation techniques.

What may have been lost with an increased focus on simplified behaviors is a greater appreciation for the complex ways a brain area can contribute to dynamic situations. Indeed, the prior two sections demonstrate that despite rapid advances in molecular and circuit understanding of the LHb, the nature of its contribution to integrating stress and memory related behaviors remains unclear. Recent work in the fear literature has demonstrated that ethologically relevant behaviors can reveal additional insight or even challenge long established roles for brain areas in behavior (Gross and Canteras, 2012; Gomez-Marin et al., 2014; Kim and Jung, 2018). Specifically, the inclusion of different scents, visual stimuli, or sounds may help more closely match what an animal experiences in the wild (Kim and Jung, 2018). For example, one such experiment involved placing rats in a continuous closed economy where food had to be accessed by risking shock during one period of the light dark cycle. Results revealed an amygdala dependent modulation of circadian rhythms can be elicited when the fear is timed to circadian cues (Pellman et al., 2015). In addition, realistic predator stimuli such as a plastic owl that surges from behind a hidden curtain while a hungry rat is foraging for food elicits opposite habituation to the fear related cues and willingness to enter a fear context in males and females than what is observed when using footshock and freezing as measures (Zambetti et al., 2019). Specifically, female rats are much less likely to approach the zone in which the owl surges than male rats.

When considering the role of the LHb in complex human psychiatric conditions, it is likely that similar additional insights into complex situations will also be gained by including ethological behavioral paradigms in animal models (Gomez-Marin et al., 2014) such as the closed economy, or simulated predator described above. Prior research utilizing ethologically relevant, complex behaviors have revealed a wealth

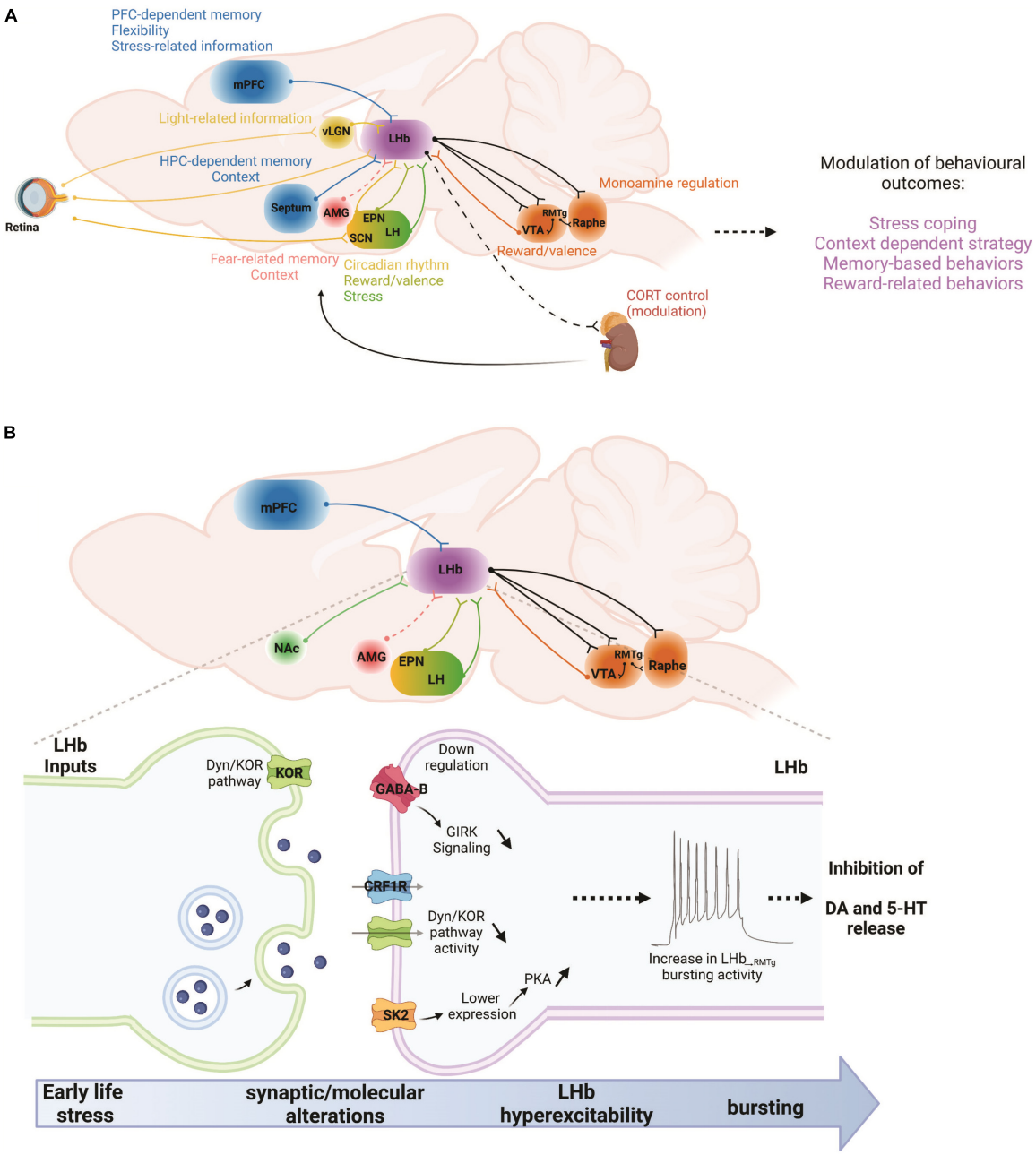

FIGURE 1 | (A) In a given situation, the LHb can be viewed as a recipient of multiple types of information stemming from cortical and subcortical regions. Those include memory- and flexibility-related information from the mPFC, or hippocampal memory-related information from the septum. Emotional information arises from the AMG while stress-related information from the mPFC and the LH. Reward and valence-related information reach the LHb from the EPN and the dopamine system. Finally, light-related information reaches the LHb from the retina, either directly or through the vLGN and SCN. Once the LHb has processed this information, they are communicated to the dopamine and serotonin systems, as well as to the HPA axis in order to adapt behavioral responses to changes in environmental constraints. AMG, amygdala; CORT, corticosterone; EPN, entopeduncular nucleus; HPC, hippocampus; LH, lateral hypothalamus; LHb, lateral habenula; mPFC, medial prefrontal cortex; RMTg, rostromedial tegmental nucleus; SCN, suprachiasmatic nucleus; vLGN, ventral lateral geniculate nucleus; VTA, ventral tegmental area. **(B)** Early life stress, such as maternal deprivation (MD) and repeated daily maternal separation (MS) lead to LHb hyperexcitability. Maternal deprivation in particular also increases LHb neuronal bursting and intrinsic excitability. MS-induced LHb hyperactivity is the consequence of altered input communication from at least the EPN with down-regulation of GABABR-GIRK signaling. On the other hand, MD dysregulates Dyn/KOR and CRF-CRFR1 signaling pathways while increasing PKA activity that promotes the downregulation of small conductance (SK2) potassium channels. Increases in LHb bursting and activity can in turn downregulate dopaminergic and serotonergic transmissions through hyperactivation of the RMTg, and therefore promote stress-related disorders such as anxiety and depression. Future studies will require an exploration of the potential contributions of other LHb inputs to such intra-LHb molecular disturbances, for example from the mPFC, NAc, AMG, and LH. AMG, amygdala; EPN, entopeduncular nucleus; LH, lateral hypothalamus; LHb, lateral habenula; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; RMTg, rostromedial tegmental nucleus; VTA, ventral tegmental area. Dashed lines indicate that there are no known direct connections.

TABLE 1 | Outstanding issues and questions.

1.	What role does the LHb play in memory processing by healthy brains? To answer this question, we need to better understand the precise short- vs. long-term roles of the mPFC and hippocampus in LHb memory processing. Also enhancing the ecologically-relevance and complexity of the behavioral assessments should facilitate our ability to further dissect the role of the LHb in memory.
2.	What types of context memory information are conveyed to the LHb from the mPFC and hippocampus, and how is this information modified during stress or neuropsychiatric states such as depression and anxiety?
3.	Does the neural mechanism of communication between limbic cortex and the LHb change over time, and does this communication become altered in neuropsychiatric conditions?
4.	What are the cellular and network mechanisms by which the LHb integrates context memory (e.g., from mPFC and hippocampus) and motivational information related to emotional state? Future studies focused on the synaptic basis of memory formation and stress dysregulation of synaptic plasticity at these specific synaptic inputs to LHb are also warranted.
5.	It is well documented that sleep is essential for normal memory and emotion regulation. The strong anatomical connection between the suprachiasmatic nucleus and the LHb, and functional ties between the hippocampus and LHb, suggest that at least part of a memory influence of the LHb may be related to its processing during sleep. This is an understudied area of LHb function.
6.	LHb theories postulate that LHb output guides response flexibility and behavioral adaptation. The mechanisms involved in such behavioral guidance, however, are not clear, nor are the details by which stress might modify these output messages.
7.	Often memory disruption is thought to be the consequence of a disordered behavioral state such as that observed after stress. It is possible, however, that a memory disruption could lead to a stressed state. Distinguishing these interpretations is important yet challenging given the dynamic nature of neural systems.
8.	Are there differential impacts of maternal separation (predictable stress) and single maternal deprivation (unpredictable stress) when it comes to resistance or vulnerability to addictive behaviors, depression and other mood phenotypes?
9.	What are the critical neuromodulations within LHb that could underly anhedonia, motivational deficits, and drug seeking behaviors?

of information into LHb contributions to decision-making. For example, Thornton and Evans (1982) observed that when rats were faced with an inescapable swimming scenario in the Morris water maze followed by a means of escape *via* rope climbing, habenula lesioned animals showed less flexible behavior (e.g., switching from trying to climb out *via* the edge of the pool to swimming to the middle to climb the rope) and a reduced likelihood of achieving escape. Combining such varied behaviors alongside the highly targeted molecular and physiological techniques now at the neuroscientist's disposal may elaborate previously unknown, or recently forgotten roles for the LHb across a range of behaviors. For example, recent advances in behavioral tracking has led to the ability to precisely track positional information at a frame by frame granularity using automated behavioral coding (Nath et al., 2019; Nilsson et al., 2020). Understanding the neurophysiological changes associated with shifts in complex behaviors (such as during social interaction) and cue recognition could be critical to understanding how the LHb combines input from forebrain and memory related areas with stress related signals to influence downstream modulatory systems (Proulx et al., 2014).

To some extent, a revisiting of ethological behaviors in the LHb literature is already underway. Recent examples including realistic social aggression paradigms where mice are chronically socially defeated by a larger more aggressive strain (Flanigan et al., 2020), experiences of maternal deprivation during rearing described above (Shepard et al., 2018b), and social behavior in zebrafish examining decisions to fight or flee (Okamoto et al., 2021), are particularly relevant behaviors in the context of psychiatric conditions. In addition, when neural recordings have been obtained in freely behaving animals in dynamic environments, a much more complex picture of its role in ongoing behavior has emerged beyond signaling aversive stimuli (Baker et al., 2015; Lecca et al., 2020). Specifically, neural signals correlate strongly with velocity of animals as they seek rewards in open fields or in a T-maze (Sharp et al., 2006; Baker et al., 2015; Lecca et al., 2020). It is no doubt that behaviors such as conditioned place preference/avoidance, highly controlled delivery of aversive or appetitive stimuli, or sucrose preference have informed important theories of LHb function. Examining these theories within the broader view of the LHb in behavior summarized by Sutherland (1982), among others, will likely help clarify in what ways the conclusions from simplified paradigms contribute to more complex decision-making situations. For example, comparing results from effort based operant tasks such as progressive ratio (Zapata et al., 2017), with a more ethological behavior such as rats exerting effort to climb barriers (Sevigny et al., 2021) could help reveal the extent to which effort or fatigue is related to anatomical and physiological changes in LHb. This will further clarify potential LHb contributions to a wide range of psychiatric conditions.

SUMMARY/CONCLUSION

Over the past 20 or so years, there has been significant evolution in our understanding of the functional importance of the LHb. This has led to the generally-accepted views that the LHb plays a key role in associating context-dependent memory with one's emotional state, and that dysfunction of this memory-emotion interface has neuropsychiatric consequences. As investigations continue to detail the dynamical nature of synaptic and circuit interactions of LHb function, it will be important to do so from a multi-level approach so that we will increasingly understand LHb function from its molecules to the circuits in which it is embedded. Having a more detailed comprehension of the LHb role in computing multimodal information regarding the emotional valence of a situation, prior stress experiences, and its contextual properties will likely help understanding some of the symptoms observed in pathologies associated with LHb dysfunctions such as depression (Li et al., 2013; Lecca et al., 2016; Nuno-Perez et al., 2018), addiction (Lecca et al., 2014;

Velasquez et al., 2014; Mathis and Kenny, 2019) frontotemporal dementia (Bocchetta et al., 2016), and possibly schizophrenia (Zhang et al., 2017; Schafer et al., 2018).

The emergence of psychopathological symptoms is particularly striking when examined under stress induced contexts. A growing appreciation of the role of early life stress in LHb processing of emotional context is an example of the importance of understanding the complex interactions between memory and goal-directed behavior (Tchenio et al., 2017; Shepard et al., 2018b; Shepard and Nugent, 2021). Also, the yet unexplored LHb function in sleep appears relevant due to the relation between sleep and stress exposure (Goldstein and Walker, 2014; Vandekerckhove and Wang, 2018), and because sleep disturbances are key features of pathologies involving LHb dysfunction such as depression (Kudlow et al., 2013) and schizophrenia (Carruthers et al., 2021). Such studies examining the interaction between sleep and stress will likely bring new insights about cognitive deficits (memory loss, attention deficits, anhedonia) observed in depressive patients (Hammar and Ardal, 2009; Disner et al., 2011; Culpepper et al., 2017) and other populations with noted sleep disturbances.

Before we can develop efficient interventions to treat dysfunctions in memory, stress, sleep, and emotion processing, a number of questions remain to be addressed to further our understanding of the behavioral and neural mechanisms that underlie the LHb's role in these contexts (**Table 1**). Overall, advances in our understanding of the functional significance of the LHb requires taking a multi-systems approach that includes the nature of the interactions between the LHb and its numerous afferent and efferent partners (**Figure 1**), as well as how the LHb plays central roles in many types of behaviors and types of memory. While behaviors including sleep, emotional processing, and decision-making often require the inclusion of more complex, or ethologically relevant behavioral assays, the insights gained from these studies will likely have important implications for understanding how observed cellular and circuit changes contribute to complex human psychopathologies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work and critically reviewed content and approved the final version of manuscript for submission.

FUNDING

This work was supported by the National Institute of Neurological Disorders and Stroke (NIH/NINDS) Grant #R21NS120628 to FN, the National Institute of Mental Health (NIH/NIMH) Grant #MH119391 to SM, ERA-NET NEURON Grant (NeuroMarket) to LL, and an FRSG Grant (115138) to PB. The funding agencies did not contribute to writing this article or deciding to submit it.

REFERENCES

Aizawa, H., Yanagihara, S., Kobayashi, M., Niisato, K., Takekawa, T., Harukuni, R., et al. (2013). The synchronous activity of lateral habenular neurons is essential for regulating hippocampal theta oscillation. *J. Neurosci.* 33, 8909–8921. doi: 10.1523/JNEUROSCI.4369-12.2013

Arnsten, A. F. (2015). Stress weakens prefrontal networks: molecular insults to higher cognition. *Nat. Neurosci.* 18, 1376–1385. doi: 10.1038/nn.4087

Authement, M. E., Kodangattil, J. N., Gouty, S., Rusnak, M., Symes, A. J., Cox, B. M., et al. (2015). Histone deacetylase inhibition rescues maternal deprivation-induced gabaergic metaplasticity through restoration of AKAP signaling. *Neuron* 86, 1240–1252. doi: 10.1016/j.neuron.2015.05.024

Authement, M. E., Langlois, L. D., Shepard, R. D., Browne, C. A., Lucki, I., Kassis, H., et al. (2018). A role for corticotropin-releasing factor signaling in the lateral habenula and its modulation by early-life stress. *Sci. Signal.* 11:eaan6480. doi: 10.1126/scisignal.aan6480

Baker, P. M., Oh, S. E., Kidder, K. S., and Mizumori, S. J. (2015). Ongoing behavioral state information signaled in the lateral habenula guides choice flexibility in freely moving rats. *Front. Behav. Neurosci.* 9:295. doi: 10.3389/fnbeh.2015.00295

Baker, P. M., Rao, Y., Rivera, Z. M. G., Garcia, E. M., and Mizumori, S. J. Y. (2019). Selective functional interaction between the lateral habenula and hippocampus during different tests of response flexibility. *Front. Mol. Neurosci.* 12:245. doi: 10.3389/fnmol.2019.00245

Baño-Otálora, B., and Piggins, H. D. (2017). Contributions of the lateral habenula to circadian timekeeping. *Pharmacol. Biochem. Behav.* 162, 46–54. doi: 10.1016/j.pbb.2017.06.007

Bocchetta, M., Gordon, E., Marshall, C. R., Slattery, C. F., Cardoso, M. J., Cash, D. M., et al. (2016). The habenula: an under-recognised area of importance in frontotemporal dementia? *J. Neurol. Neurosurg. Psychiatry* 87, 910–912. doi: 10.1136/jnnp-2015-312067

Bolton, J. L., Molet, J., Regev, L., Chen, Y., Rismanchi, N., Haddad, E., et al. (2018a). Anhedonia following early-life adversity involves aberrant interaction of reward and anxiety circuits and is reversed by partial silencing of amygdala corticotropin-releasing hormone gene. *Biol. Psychiatry* 83, 137–147. doi: 10.1016/j.biopsych.2017.08.023

Bolton, J. L., Ruiz, C. M., Rismanchi, N., Sanchez, G. A., Castillo, E., Huang, J., et al. (2018b). Early-life adversity facilitates acquisition of cocaine self-administration and induces persistent anhedonia. *Neurobiol. Stress* 8, 57–67. doi: 10.1016/j.ynstr.2018.01.002

Bruchas, M. R., Land, B. B., and Chavkin, C. (2010). The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. *Brain Res.* 1314, 44–55. doi: 10.1016/j.brainres.2009.08.062

Carruthers, S. P., Brunetti, G., and Rossell, S. L. (2021). Sleep disturbances and cognitive impairment in schizophrenia spectrum disorders: a systematic review and narrative synthesis. *Sleep Med.* 84, 8–19. doi: 10.1016/j.sleep.2021.05.011

Cerniauskas, I., Winterer, J., de Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., et al. (2019). Chronic stress induces activity, synaptic, and transcriptional remodeling of the lateral habenula associated with deficits in motivated behaviors. *Neuron* 104, 899–915.e8. doi: 10.1016/j.neuron.2019.09.005

Chastrette, N., Pfaff, D. W., and Gibbs, R. B. (1991). Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Res.* 563, 339–344. doi: 10.1016/0006-8993(91)91559-j

Cohen, J. Y., Haesler, S., Vong, L., Lowell, B. B., and Uchida, N. (2012). Neuron-type-specific signals for reward and punishment in the ventral tegmental area. *Nature* 482, 85–88. doi: 10.1038/nature10754

Culpepper, L., Lam, R. W., and McIntyre, R. S. (2017). Cognitive impairment in patients with depression: awareness, assessment, and management. *J. Clin. Psychiatry* 78, 1383–1394. doi: 10.4088/JCP.tk16043ah5c

Disner, S. G., Beevers, C. G., Haigh, E. A., and Beck, A. T. (2011). Neural mechanisms of the cognitive model of depression. *Nat. Rev. Neurosci.* 12, 467–477. doi: 10.1038/nrn3027

Durieux, L., Mathis, V., Herbeaux, K., Muller, M.-A., Barbelivien, A., Mathis, C., et al. (2020). Involvement of the lateral habenula in fear memory. *Brain Struct. Funct.* 225, 2029–2044. doi: 10.1007/s00429-020-02107-5

Fillinger, C., Yalcin, I., Barrot, M., and Veinante, P. (2017). Efferents of anterior cingulate areas 24a and 24b and midcingulate areas 24a' and 24b' in the mouse. *Brain Struct. Funct.* 223, 1747–1778. doi: 10.1007/s00429-017-1585-x

Flanigan, M. E., Aleyasin, H., Li, L., Burnett, C. J., Chan, K. L., LeClair, K. B., et al. (2020). Orexin signaling in GABAergic lateral habenula neurons modulates aggressive behavior in male mice. *Nat. Neurosci.* 23, 638–650. doi: 10.1038/s41593-020-0617-7

Geisler, S., and Trimble, M. (2008). The lateral habenula: no longer neglected. *CNS Spectr.* 13, 484–489.

Goldstein, A. N., and Walker, M. P. (2014). The Role of Sleep in Emotional Brain Function. *Ann. Rev. Clin. Psychol.* 10, 679–708. doi: 10.1146/annurev-clinpsy-032813-153716

Gomez-Marin, A., Paton, J. J., Kampff, A. R., Costa, R. M., and Mainen, Z. F. (2014). Big behavioral data: psychology, ethology and the foundations of neuroscience. *Nat. Neurosci.* 17, 1455–1462. doi: 10.1038/nn.3812

Gotlib, I. H., Borchers, L. R., Chahal, R., Gifuni, A. J., Teresi, G. I., and Ho, T. C. (2020). Early life stress predicts depressive symptoms in adolescents during the COVID-19 pandemic: the mediating role of perceived stress. *Front. Psychol.* 11:603748. doi: 10.3389/fpsyg.2020.603748

Goutagny, R., Loureiro, M., Jackson, J., Chaumont, J., Williams, S., Isope, P., et al. (2013). Interactions between the lateral habenula and the hippocampus: implication for spatial memory processes. *Neuropsychopharmacology* 38, 2418–2426. doi: 10.1038/npp.2013.142

Graziane, N. M., Neumann, P. A., and Dong, Y. (2018). A focus on reward prediction and the lateral habenula: functional alterations and the behavioral outcomes induced by drugs of abuse. *Front. Synaptic Neurosci.* 10:12. doi: 10.3389/fnsyn.2018.00012

Gross, C. T., and Canteras, N. S. (2012). The many paths to fear. *Nat. Rev. Neurosci.* 13, 651–658. doi: 10.1038/nrn3301

Guilding, C., Hughes, A. T., and Piggins, H. D. (2010). Circadian oscillators in the epithalamus. *Neuroscience* 169, 1630–1639. doi: 10.1016/j.neuroscience.2010.06.015

Hammar, A., and Ardal, G. (2009). Cognitive functioning in major depression–a summary. *Front. Hum. Neurosci.* 3:26. doi: 10.3389/neuro.09.026.2009

Heim, C., Shugart, M., Craighead, W. E., and Nemeroff, C. B. (2010). Neurobiological and psychiatric consequences of child abuse and neglect. *Dev. Psychobiol.* 52, 671–690. doi: 10.1002/dev.20494

Hennigan, K., D'Ardenne, K., and McClure, S. M. (2015). Distinct midbrain and habenula pathways are involved in processing aversive events in humans. *J. Neurosci.* 35, 198–208. doi: 10.1523/JNEUROSCI.0927-14.2015

Hong, S., and Hikosaka, O. (2013). Diverse sources of reward value signals in the basal ganglia nuclei transmitted to the lateral habenula in the monkey. *Front. Hum. Neurosci.* 7:778. doi: 10.3389/fnhum.2013.00778

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Huang, L., Xi, Y., Peng, Y., Yang, Y., Huang, X., Fu, Y., et al. (2019). A visual circuit related to habenula underlies the antidepressive effects of light therapy. *Neuron* 102, 128–142.e8. doi: 10.1016/j.neuron.2019.01.037

Humphreys, K. L., Myint, M. T., and Zeanah, C. H. (2020). Increased risk for family violence during the COVID-19 pandemic. *Pediatrics* 146:e20200982. doi: 10.1542/peds.2020-0982

Jacinto, L. R., Mata, R., Novais, A., Marques, F., and Sousa, N. (2017). The habenula as a critical node in chronic stress-related anxiety. *Exp. Neurol.* 289, 46–54. doi: 10.1016/j.expneurol.2016.12.003

Kalinichev, M., Easterling, K. W., and Holtzman, S. G. (2002). Early neonatal experience of Long-Evans rats results in long-lasting changes in reactivity to a novel environment and morphine-induced sensitization and tolerance. *Neuropsychopharmacology* 27, 518–533. doi: 10.1016/S0893-133X(02)00326-3

Karkhanis, A. N., Rose, J. H., Weiner, J. L., and Jones, S. R. (2016). Early-life social isolation stress increases kappa opioid receptor responsiveness and downregulates the dopamine system. *Neuropsychopharmacology* 41, 2263–2274. doi: 10.1038/npp.2016.21

Kim, J. J., and Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nat. Rev. Neurosci.* 3, 453–462.

Kim, J. J., and Jung, M. W. (2018). Fear paradigms: the times they are a-changin'. *Curr. Opin. Behav. Sci.* 24, 38–43. doi: 10.1016/j.cobeha.2018.02.007

Kim, U., and Lee, T. (2012). Topography of descending projections from anterior insular and medial prefrontal regions to the lateral habenula of the epithalamus in the rat. *Eur. J. Neurosci.* 35, 1253–1269. doi: 10.1111/j.1460-9568.2012.08030.x

Klein, M. E., Chandra, J., Sheriff, S., and Malinow, R. (2020). Opioid system is necessary but not sufficient for antidepressive actions of ketamine in rodents. *Proc. Natl. Acad. Sci. U. S. A.* 17, 2656–2662. doi: 10.1073/pnas.1916570117

Knowland, D., and Lim, B. K. (2018). Circuit-based frameworks of depressive behaviors: the role of reward circuitry and beyond. *Pharmacol. Biochem. Behav.* 174, 42–52. doi: 10.1016/j.pbb.2017.12.010

Koob, G. F. (2010). The role of CRF and CRF-related peptides in the dark side of addiction. *Brain Res.* 1314, 3–14. doi: 10.1016/j.brainres.2009.11.008

Kudlow, P. A., Cha, D. S., Lam, R. W., and McIntyre, R. S. (2013). Sleep architecture variation: a mediator of metabolic disturbance in individuals with major depressive disorder. *Sleep Med.* 14, 943–949. doi: 10.1016/j.sleep.2013.04.017

Land, B. B., Bruchas, M. R., Lemos, J. C., Xu, M., Melief, E. J., and Chavkin, C. (2008). The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. *J. Neurosci.* 28, 407–414. doi: 10.1523/JNEUROSCI.4458-07.2008

Langlois, L. D., Berman, R. Y., Shepard, R. D., Simmons, S. C., Tsuda, M. C., Gouty, S., et al. (2021). Potentiation of glutamatergic synaptic transmission onto lateral habenula neurons following early life stress and intravenous morphine self-administration in rats. *Addict. Biol.* 27:e13064. doi: 10.1111/adb.13064

Lawson, M., Piel, M. H., and Simon, M. (2020). Child Maltreatment during the COVID-19 pandemic: consequences of parental job loss on psychological and physical abuse towards children. *Child Abuse Neglect* 110:104709. doi: 10.1016/j.chiabu.2020.104709

Lecca, S., Meye, F. J., and Mameli, M. (2014). The lateral habenula in addiction and depression: an anatomical, synaptic and behavioral overview. *Eur. J. Neurosci.* 39, 1170–1178. doi: 10.1111/ejn.12480

Lecca, S., Meye, F. J., Trusel, M., Tchenio, A., Harris, J., Schwarz, M. K., et al. (2017). Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior. *Elife* 6:e30697. doi: 10.7554/eLife.30697

Lecca, S., Namboodiri, V. M. K., Restivo, L., Gervasi, N., Pillolla, G., Stuber, G. D., et al. (2020). Heterogeneous habenular neuronal ensembles during selection of defensive behaviors. *Cell Rep.* 31:107752. doi: 10.1101/2019.12.22.886457

Lecca, S., Pelosi, A., Tchenio, A., Moutkine, I., Lujan, R., Hervé, D., et al. (2016). Rescue of GABAB and GIRK function in the lateral habenula by protein phosphatase 2A inhibition ameliorates depression-like phenotypes in mice. *Nat. Med.* 22, 254–261. doi: 10.1038/nm.4037

Levis, S. C., Bentzley, B. S., Molet, J., Bolton, J. L., Perrone, C. R., Baram, T. Z., et al. (2021). On the early life origins of vulnerability to opioid addiction. *Mol. Psychiatry* 26, 4409–4416. doi: 10.1038/s41380-019-0628-5

Li, H., Pullmann, D., and Jhou, T. C. (2019). Valence-encoding in the lateral habenula arises from the entopeduncular region. *Elife* 8:e41223. doi: 10.7554/eLife.41223

Li, K., Zhou, T., Liao, L., Yang, Z., Wong, C., Henn, F., et al. (2013). βCaMKII in lateral habenula mediates core symptoms of depression. *Science* 341, 1016–1020. doi: 10.1126/science.1240729

Lippard, E. T. C., and Nemeroff, C. B. (2020). The devastating clinical consequences of child abuse and neglect: increased disease vulnerability and poor treatment response in mood disorders. *Am. J. Psychiatry* 177, 20–36. doi: 10.1176/appi.ajp.2019.19010020

Liu, H., Rastogi, A., Narain, P., Xu, Q., Sabanovic, M., Alhammadi, A. D., et al. (2021). Blunted diurnal firing in lateral habenula projections to dorsal raphe nucleus and delayed photoentrainment in stress-susceptible mice. *PLoS Biol.* 19:e3000709. doi: 10.1371/journal.pbio.3000709

Macrì, S., Spinelli, S., Adriani, W., Dee Higley, J., and Laviola, G. (2007). Early adversity and alcohol availability persistently modify serotonin and hypothalamic-pituitary-adrenal-axis metabolism and related behavior: what

experimental research on rodents and primates can tell us. *Neurosci. Biobehav. Rev.* 31, 172–180. doi: 10.1016/j.neubiorev.2006.06.026

Mantsch, J. R., Baker, D. A., Funk, D., Lê, A. D., and Shaham, Y. (2016). Stress-induced reinstatement of drug seeking: 20 years of progress. *Neuropsychopharmacology* 41, 335–356. doi: 10.1038/npp.2015.142

Mathis, V., and Kenny, P. J. (2019). From controlled to compulsive drug-taking: the role of the habenula in addiction. *Neurosci. Biobehav. Rev.* 106, 102–111. doi: 10.1016/j.neubiorev.2018.06.018

Mathis, V., and Lecourtier, L. (2017). Role of the lateral habenula in memory through online processing of information. *Pharmacol. Biochem. Behav.* 162, 69–78. doi: 10.1016/j.pbb.2017.07.004

Mathis, V., Barbelivien, A., Majchrzak, M., Mathis, C., Cassel, J.-C., and Lecourtier, L. (2017). The lateral habenula as a relay of cortical information to process working memory. *Cereb. Cortex* 27, 5485–5495. doi: 10.1093/cercor/bhw316

Mathis, V., Cosquer, B., Avallone, M., Cassel, J.-C., and Lecourtier, L. (2015). Excitatory transmission to the lateral habenula is critical for encoding and retrieval of spatial memory. *Neuropsychopharmacology* 40, 2843–2851. doi: 10.1038/npp.2015.140

Mathis, V., Cosquer, B., Barbelivien, A., Herbeaux, K., Bothorel, B., Sage-Ciocca, D., et al. (2018). The lateral habenula interacts with the hypothalamo-pituitary adrenal axis response upon stressful cognitive demand in rats. *Behav. Brain Res.* 341, 63–70. doi: 10.1016/j.bbr.2017.12.016

Matsumoto, M., and Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nat. Neurosci.* 12, 77–84. doi: 10.1038/nn.2233

Mendoza, J. (2017). Circadian neurons in the lateral habenula: clocking motivated behaviors. *Pharmacol. Biochem. Behav.* 162, 55–61. doi: 10.1016/j.pbb.2017.06.013

Moffett, M. C., Harley, J., Francis, D., Sanghani, S. P., Davis, W. I., and Kuhar, M. J. (2006). Maternal separation and handling affects cocaine self-administration in both the treated pups as adults and the dams. *J. Pharmacol. Exp. Ther.* 317, 1210–1218. doi: 10.1124/jpet.106.101139

Nair, S. G., Strand, N. S., and Neumaier, J. F. (2013). DREADDing the lateral habenula: a review of methodological approaches for studying lateral habenula function. *Brain Res.* 1511, 93–101. doi: 10.1016/j.brainres.2012.10.011

Nath, T., Mathis, A., Chen, A. C., Patel, A., Bethge, M., and Mathis, M. W. (2019). Using DeepLabCut for 3D markerless pose estimation across species and behaviors. *Nat. Protoc.* 14, 2152–2176. doi: 10.1038/s41596-019-0176-0

Nemeroff, C. B. (2016). Paradise lost: the neurobiological and clinical consequences of child abuse and neglect. *Neuron* 89, 892–909. doi: 10.1016/j.neuron.2016.01.019

Nilsson, S. R. O., Goodwin, N. L., Choong, J. J., Hwang, S., Wright, H. R., Norville, Z. C., et al. (2020). Simple Behavioral Analysis (SimBA) – an open source toolkit for computer classification of complex social behaviors in experimental animals. *bioRxiv* [Preprint]. doi: 10.1101/2020.04.19.049452

Nishi, M., Horii-Hayashi, N., and Sasagawa, T. (2014). Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. *Front. Neurosci.* 8:166. doi: 10.3389/fnins.2014.00166

Nuno-Perez, A., Tchenio, A., Mameli, M., and Lecca, S. (2018). Lateral habenula gone awry in depression: bridging cellular adaptations with therapeutics. *Front. Neurosci.* 12:485. doi: 10.3389/fnins.2018.00485

Oh, W. C., Rodríguez, G., Asede, D., Jung, K., Hwang, I.-W., Ogelman, R., et al. (2021). Dysregulation of the mesoprefrontal dopamine circuit mediates an early-life stress-induced synaptic imbalance in the prefrontal cortex. *Cell Rep.* 35:109074. doi: 10.1016/j.celrep.2021.109074

Okamoto, H., Cherng, B.-W., Nakajo, H., Chou, M.-Y., and Kinoshita, M. (2021). Habenula as the experience-dependent controlling switchboard of behavior and attention in social conflict and learning. *Curr. Opin. Neurobiol.* 68, 36–43. doi: 10.1016/j.conb.2020.12.005

Okhuarobo, A., Bolton, J. L., Igbe, I., Zorrilla, E. P., Baram, T. Z., and Contet, C. (2020). A novel mouse model for vulnerability to alcohol dependence induced by early-life adversity. *Neurobiol. Stress* 13:100269. doi: 10.1016/j.ynstr.2020.100269

Ordoñes Sanchez, E., Bavley, C. C., Deutschmann, A. U., Carpenter, R., Peterson, D. R., Karbalaei, R., et al. (2021). Early life adversity promotes resilience to opioid addiction-related phenotypes in male rats and sex-specific transcriptional changes. *Proc. Natl. Acad. Sci. U. S. A.* 118:e2020173118. doi: 10.1073/pnas.2020173118

Pautassi, R. M., Nizhnikov, M. E., Fabio, M. C., and Spear, N. E. (2012). Early maternal separation affects ethanol-induced conditioning in a nor-BNI insensitive manner, but does not alter ethanol-induced locomotor activity. *Pharmacol. Biochem. Behav.* 100, 630–638. doi: 10.1016/j.pbb.2011.11.005

Pellman, B. A., Kim, E., Reilly, M., Kashima, J., Motch, O., de la Iglesia, H. O., et al. (2015). Time-specific fear acts as a non-photic entraining stimulus of circadian rhythms in rats. *Sci. Rep.* 5:14916. doi: 10.1038/srep14916

Peña, C. J., Kronman, H. G., Walker, D. M., Cates, H. M., Bagot, R. C., Purushothaman, I., et al. (2017). Early life stress confers lifelong stress susceptibility in mice *via* ventral tegmental area OTX2. *Science* 356, 1185–1188. doi: 10.1126/science.aan4491

Peña, C. J., Smith, M., Ramakrishnan, A., Cates, H. M., Bagot, R. C., Kronman, H. G., et al. (2019). Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. *Nat. Commun.* 10:5098. doi: 10.1038/s41467-019-13085-6

Proulx, C. D., Aronson, S., Milivojevic, D., Molina, C., Loi, A., Monk, B., et al. (2018). A neural pathway controlling motivation to exert effort. *Proc. Natl. Acad. Sci. U. S. A.* 115, 5792–5797. doi: 10.1073/pnas.1801837115

Proulx, C. D., Hikosaka, O., and Malinow, R. (2014). Reward processing by the lateral habenula in normal and depressive behaviors. *Nat. Neurosci.* 17, 1146–1152. doi: 10.1038/nn.3779

Quina, L. A., Tempest, L., Ng, L., Harris, J. A., Ferguson, S., Jhou, T. C., et al. (2015) Efferent pathways of the mouse lateral habenula. *J. Comp. Neurol.* 523, 32–60. doi: 10.1002/cne.23662

Roman, E., Weininger, J., Lim, B., Roman, M., Barry, D., Tierney, P., et al. (2020). Untangling the dorsal diencephalic conduction system: a review of structure and function of the stria medullaris, habenula and fasciculus retroflexus. *Brain Struct. Funct.* 225, 1437–1458. doi: 10.1007/s00429-020-02069-8

Roozendaal, B., McEwen, B. S., and Chattarji, S. (2009). Stress, memory and the amygdala. *Nat. Rev. Neurosci.* 10, 423–433. doi: 10.1038/nrn2651

Salaberry, N. L., Hamm, H., Felder-Schmittbuhl, M.-P., and Mendoza, J. (2019). A suprachiasmatic-independent circadian clock(s) in the habenula is affected by Per gene mutations and housing light conditions in mice. *Brain Struct. Funct.* 224, 19–31. doi: 10.1007/s00429-018-1756-4

Schafer, M., Kim, J.-W., Joseph, J., Xu, J., Frangou, S., and Doucet, G. E. (2018). Imaging habenula volume in schizophrenia and bipolar disorder. *Front. Psychiatry* 9:456. doi: 10.3389/fpsyt.2018.00456

Schultz, W. (2010). Dopamine signals for reward value and risk: basic and recent data. *Behav. Brain Funct.* 6:24. doi: 10.1186/1744-9081-6-24

Sevigny, J. P., Bryant, E. N., Encarnacion, É, Smith, D. F., Acosta, R., and Baker, P. M. (2021). Lateral habenula inactivation alters willingness to exert physical effort using a maze task in rats. *Front. Behav. Neurosci.* 15:652793. doi: 10.3389/fnbeh.2021.652793

Sharp, P. E., Turner-Williams, S., and Tuttle, S. (2006). Movement-related correlates of single cell activity in the interpeduncular nucleus and habenula of the rat during a pellet-chasing task. *Behav. Brain Res.* 166, 55–70. doi: 10.1016/j.bbr.2005.07.004

Shepard, R. D., and Nugent, F. S. (2020). Early life stress- and drug-induced histone modifications within the ventral tegmental area. *Front. Cell Dev. Biol.* 8:588476. doi: 10.3389/fcell.2020.588476

Shepard, R. D., and Nugent, F. S. (2021). Targeting endocannabinoid signaling in the lateral habenula as an intervention to prevent mental illnesses following early life stress: a perspective. *Front. Synaptic Neurosci.* 13:689518.

Shepard, R. D., Langlois, L. D., Authement, M. E., and Nugent, F. S. (2020). Histone deacetylase inhibition reduces ventral tegmental area dopamine neuronal hyperexcitability involving AKAP150 signaling following maternal deprivation in juvenile male rats. *J. Neurosci. Res.* 98, 1457–1467. doi: 10.1002/jnr.24613

Shepard, R. D., Langlois, L. D., Browne, C. A., Berenji, A., Lucki, I., and Nugent, F. S. (2018b). Ketamine reverses lateral habenula neuronal dysfunction and behavioral immobility in the forced swim test following maternal deprivation in late adolescent rats. *Front. Synaptic Neurosci.* 10:39. doi: 10.3389/fnsyn.2018.00039

Shepard, R. D., Gouty, S., Kassis, H., Berenji, A., Zhu, W., Cox, B. M., et al. (2018a). Targeting histone deacetylation for recovery of maternal deprivation-induced changes in BDNF and AKAP150 expression in the VTA. *Exp. Neurol.* 309, 160–168. doi: 10.1016/j.expneurol.2018.08.002

Simmons, S. C., Shepard, R. D., Gouty, S., Langlois, L. D., Flerlage, W. J., Cox, B. M., et al. (2020). Early life stress dysregulates kappa opioid receptor signaling within the lateral habenula. *Neurobiol. Stress* 13:100267. doi: 10.1016/j.ynstr.2020.100267

Song, M., Jo, Y. S., Lee, Y.-K., and Choi, J.-S. (2017). Lesions of the lateral habenula facilitate active avoidance learning and threat extinction. *Behav. Brain Res.* 318, 12–17. doi: 10.1016/j.bbr.2016.10.013

Sosa, R., Mata-Luévanos, J., and Buenrostro-Jáuregui, M. (2021). The role of the lateral habenula in inhibitory learning from reward omission. *eNeuro* 8:ENEURO.0016-21.2021. doi: 10.1523/ENEURO.0016-21.2021

Stamatakis, A. M., Jennings, J. H., Ung, R. L., Blair, G. A., Weinberg, R. J., Neve, R. L., et al. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* 80, 1039–1053. doi: 10.1016/j.neuron.2013.08.023

Stamatakis, A. M., Van Swieten, M., Basiri, M. L., Blair, G. A., Kantak, P., and Stuber, G. D. (2016). Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward. *J. Neurosci.* 36, 302–311. doi: 10.1523/JNEUROSCI.1202-15.2016

Stephenson-Jones, M., Yu, K., Ahrens, S., Tucciarone, J. M., van Huijstee, A. N., Mejia, L. A., et al. (2016). A basal ganglia circuit for evaluating action outcomes. *Nature* 539, 289–293. doi: 10.1038/nature19845

Sutherland, R. J. (1982). The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neurosci. Biobehav. Rev.* 6, 1–13. doi: 10.1016/0149-7634(82)90003-3

Tchenio, A., Lecca, S., Valentinova, K., and Mameli, M. (2017). Limiting habenular hyperactivity ameliorates maternal separation-driven depressive-like symptoms. *Nat. Commun.* 8:1135. doi: 10.1038/s41467-017-01192-1

Tejeda, H. A., and Bonci, A. (2019). Dynorphin/kappa-opioid receptor control of dopamine dynamics: implications for negative affective states and psychiatric disorders. *Brain Res.* 1713, 91–101. doi: 10.1016/j.brainres.2018.09.023

Thornton, E. W., and Evans, J. C. (1982). The role of habenular nuclei in the selection of behavioral strategies. *Physiol. Psychol.* 10, 361–367.

Tian, J., and Uchida, N. (2015). Habenula lesions reveal that multiple mechanisms underlie dopamine prediction errors. *Neuron* 87, 1304–1316. doi: 10.1016/j.neuron.2015.08.028

Tomaiuolo, M., Gonzalez, C., Medina, J. H., and Piriz, J. (2014). Lateral habenula determines long-term storage of aversive memories. *Front. Behav. Neurosci.* 8:170. doi: 10.3389/fnbeh.2014.00170

Trusel, M., Nuno-Perez, A., Lecca, S., Harada, H., Lalive, A. L., Congiu, M., et al. (2019). Punishment-predictive cues guide avoidance through potentiation of hypothalamus-to-habenula synapses. *Neuron* 102, 120–127.e4. doi: 10.1016/j.neuron.2019.01.025

Vandekerckhove, M., and Wang, Y.-L. (2018). Emotion, emotion regulation and sleep: an intimate relationship. *AIMS Neurosci.* 5, 1–17. doi: 10.3934/Neuroscience.2018.1.1

Velasquez, K. M., Molfese, D. L., and Salas, R. (2014). The role of the habenula in drug addiction. *Front. Hum. Neurosci.* 8:174. doi: 10.3389/fnhum.2014.00174

Wallace, M. L., Saunders, A., Huang, K. W., Philson, A. C., Goldman, M., Macosko, E. Z., et al. (2017). Genetically distinct parallel pathways in the entopeduncular nucleus for limbic and sensorimotor output of the basal ganglia. *Neuron* 94, 138–152.e5. doi: 10.1016/j.neuron.2017.03.017

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., et al. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature* 554, 317–322. doi: 10.1038/nature25509

Yard, E., Radhakrishnan, L., Ballesteros, M. F., Sheppard, M., Gates, A., Stein, Z., et al. (2021). Emergency department visits for suspected suicide attempts among persons aged 12-25 Years before and during the COVID-19 pandemic - United States, January 2019-May 2021. *MMWR Morb. Mortal. Wkly. Rep.* 70, 888–894. doi: 10.15585/mmwr.mm7024e1

Zambetti, P. R., Schuessler, B. P., and Kim, J. J. (2019). Sex differences in foraging rats to naturalistic aerial predator stimuli. *iScience* 16, 442–452. doi: 10.1016/j.isci.2019.06.011

Zapata, A., Hwang, E.-K., and Lupica, C. R. (2017). Lateral habenula involvement in impulsive cocaine seeking. *Neuropsychopharmacology* 42, 1103–1112. doi: 10.1038/npp.2016.286

Zhang, L., Hernández, V. S., Vázquez-Juárez, E., Chay, F. K., and Barrio, R. A. (2016). Thirst is associated with suppression of habenula output and active stress coping: is there a role for a non-canonical vasopressin-glutamate pathway? *Front. Neural Circ.* 10:13. doi: 10.3389/fncir.2016.00013

Zhang, L., Wang, H., Luan, S., Yang, S., Wang, Z., Wang, J., et al. (2017). Altered volume and functional connectivity of the habenula in schizophrenia. *Front. Hum. Neurosci.* 11:636. doi: 10.3389/fnhum.2017.00636

Author Disclaimer: The opinions and assertions contained herein are the private opinions of the authors and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences or the Department of Defense or the Government of the United States.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Behavioral Neuroscience

REVIEW
published: 14 March 2022
doi: 10.3389/fnbeh.2022.812952

The Role of the Lateral Habenula in Suicide: A Call for Further Exploration

Rocky B. Marks[1†], Janelle Y. Wee[1†], Samantha V. Jacobson[1†], Kimi Hashimoto[1†], Katherine L. O'Connell[1†], Sam Adler Golden[2†], Phillip Michael Baker[3] and Keyne Catherine Law[1*†]*

[1]Department of Clinical Psychology, Seattle Pacific University, Seattle, WA, United States, [2]Department of Biological Structure, University of Washington, Seattle, WA, United States, [3]Department of Psychology, Seattle Pacific University, Seattle, WA, United States

OPEN ACCESS

Edited by:
Anna Katharina Braun,
Otto-von-Guericke Universität
Magdeburg, Germany

Reviewed by:
Aki Takahashi,
University of Tsukuba, Japan
Agata Faron-Górecka,
Maj Institute of Pharmacology (PAN),
Poland

***Correspondence:**
Rocky B. Marks
marksj2@spu.edu
Keyne Catherine Law
lawk3@spu.edu

†ORCID:
Rocky B. Marks
orcid.org/0000-0002-0388-8194
Janelle Y. Wee
orcid.org/0000-0003-4277-8171
Samantha V. Jacobson
orcid.org/0000-0002-6238-7680
Kimi Hashimoto
orcid.org/0000-0002-9971-9298
Katherine L. O'Connell
orcid.org/0000-0003-4869-0584
Sam Adler Golden
orcid.org/0000-0002-2104-2272
Phillip Michael Baker
orcid.org/0000-0002-3023-2983
Keyne Catherine Law
orcid.org/0000-0002-6966-9410

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: 10 November 2021
Accepted: 15 February 2022
Published: 14 March 2022

Citation:
Marks RB, Wee JY, Jacobson SV, Hashimoto K, O'Connell KL, Golden SA, Baker PM and Law KC (2022) The Role of the Lateral Habenula in Suicide: A Call for Further Exploration. Front. Behav. Neurosci. 16:812952. doi: 10.3389/fnbeh.2022.812952

Despite decades of significant effort in research, policy, and prevention, suicide rates have continued to rise to the current peak of 14.6 per 100,000 deaths. This has resulted in a concerted effort to identify biomarkers associated with suicidal behavior in the brain, to provide predictions that are better than the chance of discerning who will die by suicide. We propose that the lateral habenula (LHb), and its dysfunction during a suicidal crisis, is a critical component of the transition from suicidal ideations to self-harm. Moreover, the LHb—a key functional node in brain reward circuitry—has not been ascribed a contributory role in suicidal behavior. We argue that the LHb anchors a "suicide circuit" and call for suicide researchers to directly examine the role of the LHb, and its long-term modulation, in response to the negative affect in suicidal behavior. Discerning the neural mechanisms of this contribution will require the collaboration of neuroscientists and psychologists. Consequently, we highlight and discuss research on LHb as it relates to suicidal ideation, suicidal behavior, or death by suicide. In so doing we hope to address the bench-to-bedside translational issues currently involved in suicide research and suggest a developmental framework that focuses on specific structures motivated by theoretical anchors as a way to incorporate neurobiological findings within the context of clinical theory.

Keywords: suicide, habenula, lateral habenula, habenula, neurobiomarkers, ideation, capability

INTRODUCTION

Out of every 100,000 deaths that occur in the United States, 14.6 result from self-inflicted injuries (CDC WISQARS, 2020). Suicide rates continue to rise yearly, despite significant research, policy, and prevention efforts across commercial, academic, and government sectors. Five decades of suicide research have not improved the ability to predict who will die by suicide (Franklin et al., 2017), or what interventions effectively prevent it (CDC WISQARS, 2020). Currently, leading theories of suicide risk are based on psychosocial models encompassing the complex interplay between environmental, affective, behavioral, and neurobiological factors.

While acknowledging the role of neurobiology, these theories often reduce the exact functions, structures, and circuitry to an abstract, conceptual influence rather than a tractable avenue for intervention. For example, suicide is thought to be moderately heritable, with the family history of suicide emerging as a salient risk factor for suicidal behavior, especially among families with high

rates of psychopathology (Coon et al., 2020). However, the specific genes, the brain structures under their genetic influence, or the neurotransmitters affected by the inherited risk of suicide are much less clear. Conversely, biological research on suicide has largely focused on examining independent mechanisms [e.g., 5-hydroxytryptamine receptors (5-HT), Brain-Derived Neurotropic Factor (BDNF), Hypothalamic-Pituitary-Adrenal (HPA) axis reactivity] that carry the risk of failing to incorporate the dynamic and complex biopsychosocial systems involving suicidality and an individual's environment. Noting this recurrent pattern of academic conceptual isolationism, we call for increased interdisciplinary investigation into neurobiological correlates of suicidality guided by psychosocial suicide research theory. We provide a candidate for this approach, the lateral habenula, a neural structure that we believe will justify this argument through the lens of suicidology. The pairing of neurobiological mechanisms with suicide theory, long overdue, has the potential to dramatically improve clinical practice.

PSYCHOSOCIAL SUICIDE THEORIES AND RESEARCH—A MODERN DAY PRIMER

Many psychosocial theories propose explanations for why people die by suicide and identify factors that may contribute to the transition of suicidal ideation into suicidal behavior. The Stress–Diathesis Model of suicide (van Heeringen, 2012) proposes that stressors such as psychological disorders, crises, poverty, hopelessness, and adverse childhood experiences interact with epigenetic and neurobiological alterations to increase suicide risk. However, not all individuals who experience stressors think about suicide or engage in suicidal behavior, suggesting that neurobiological and genomic vulnerabilities contribute to the development of suicide risk, and thus a stress–diathesis interaction occurs. In support of this theory, structural and functional brain states can serve a role in distinguishing who, from samples of persons who experienced similar painful life events, will exhibit thoughts of suicide (Mann and Rizk, 2020). Further, the Stress–Diathesis Model prominently uses the concept of the "suicidal mode", which defines suicide-specific motivations, cognitions, and affect in the context of the stressors which elicit them. With each activation of the suicidal mode, suicidal behavior becomes progressively more accessible in memory and the suicidal mode is more responsive to minor stimuli. With repeated stimulation, the suicidal mode becomes activated more easily and with a lower threshold for activation (Leon et al., 1990; Oquendo et al., 2004). It stands to reason that the facilitation of the suicidal mode is accompanied by changes in neural plasticity in key brain regions associated with processing these stimuli but, as of the time of this writing, these studies have yet to be done.

Additional theories explain the progress of suicidal ideation to lethal self-injury. The Interpersonal Theory of Suicide (IPTS; Joiner, 2005) states that an individual: (i) must feel as if they are a burden on others; and (ii) feel as if they are disconnected from their social environment, to begin thinking of killing themselves. Additionally, the IPTS specifies that to act on suicidal ideation, an individual must acquire the capability for suicide, which involves an increased pain tolerance and fearlessness of death that allows them to overcome the basic drive towards self-preservation inherent in biological life. In support of this theory, past studies have found that an acquired capability for suicide, the ability to tolerate pain, and fearlessness about death differentiates those who think about suicide from those who make a suicide attempt (May and Klonsky, 2016; Paashaus et al., 2019). It is hypothesized that acquired capability was developed from repeated exposure to painful and provocative experiences (e.g., non-suicidal self-injury; Bender et al., 2012; Bauer et al., 2020). Building on the IPTS, the Three-Step Theory (3ST; Klonsky and May, 2015), incorporates that pain and hopelessness, together, initiate passive suicidal ideation ("I do not want to exist") that is escalated into active suicidal ideation ("I want to kill myself") when there is a lack of connection with others. The 3ST also extends acquired capability for suicide to include practical capability (having knowledge/access to the means for suicide) and dispositional capability (innate neurobiological/genetic factors that enable an individual to enact lethal suicidal behavior). Here again, it is possible that brain regions associated with pain and fear modulation contributes to changes in suicide capability. Confirmation of this would help solidify these theories and provide potential biomarkers that can contribute to the progression from suicidal ideation to suicidal behavior.

Integrating psychosocial theories, the Integrated Motivational Volitional Theory (IMVT; O'Connor and Kirtley, 2018), conceptualizes the progression of suicide into three phases. First is the pre-motivational phase, which includes a diathesis–stress approach that promotes the development of suicidal ideation. Next, the motivational phase leads to the expression of suicidal ideation that emerges as a response to feelings of entrapment, hopelessness, and defeat. In the final volitional phase, acquired capability, exposure to others' suicidal behaviors, detailed suicide plans, and cognitive rehearsal of death and suicide facilitates the transition from suicidal ideation into suicidal behavior.

Other theories have integrated the idea of suicidal mode that explain the fluidity of factors that trigger and contribute to the severity and duration of an individual's suicidal state. When internal or external precipitants trigger the suicidal mode, it acts in a synchronistical fashion to produce a suicidal episode characterized by cognitive themes, physiological arousal, and death-related behaviors. Extending the idea of suicide risk as dynamic, as opposed to stable, is the Fluid Vulnerability Theory (FVT; Rudd, 2006). This theory conceptualizes suicide risk as a combination of baseline risk factors (e.g., past suicide attempts, depressive symptoms, etc.) and acute risk factors (e.g., sudden losses, major life changes, etc.). Based on the conceptualization of dynamic suicide risk, the National Institute of Mental Health has established a priority initiative to examine time-varying suicide risk factors (Kleiman et al., 2017; Hallensleben et al., 2018; Bryan et al., 2019; NIMH, 2020).

Psychosocial research on suicide contains a diverse range of viewpoints, ranging from those emphasizing the physical, such as pain tolerance (Rabasco and Andover, 2020), to the implied, such as cultural and economic diversity and social belongingness (Chu et al., 2010; Li Z. et al., 2011). Whether

genetic and neurostructural influences are defined as diatheses, dispositional capability, or biological vulnerability to suicidal behavior, theories of suicide implicitly emphasize the role of genetics and biology in determining risk. These theories, however, do not define structures or biomarkers that influence suicidality, and in doing so settle for defining the biology of suicide merely through conceptual abstractionism. Furthermore, absent neurobiological candidates, the foundational ideas of psychosocial suicide risk factors are unable to discriminate between those who will develop suicidal ideation, those who will die by suicide, and those who will never think of lethal self-injury at all (Franklin et al., 2017; Millner et al., 2020). The study of suicide must evolve and use integrative systems approaches that more closely approximate the complex picture of suicidality by incorporating ecological, psychosocial, and neurobiological factors leading to the emergence of suicidal behavior.

Neurobiological Trends in Suicidology

There are several promising neurobiological targets that contribute to suicidal behavior, revealed in large part by the increasing popularity of identifying suicide-related biomarkers, that hold the potential to identify the most prominent systems or biomarkers implicated in the neuropathology of suicide (Sudol and Mann, 2017; Calati et al., 2020). One of the earliest targets was the serotonergic system, leading to the identification of suicide-associated genetic variations, receptor activity, and downregulation of the neurochemical itself (Stockmeier et al., 1998; Mann, 1999, 2003; Arango et al., 2001; Oquendo et al., 2003, 2006). However, serotonergic suicide-related data has not been consistent across suicide research studies. For example, low midbrain serotonin transporter bindings have been found among suicide attempters (Miller et al., 2013) while greater serotonin receptor binding potential was found in the brainstem of suicide attempters in other studies (Sullivan et al., 2015). Given the inconsistencies in the serotonergic systems' importance in the neuropathology of suicide, research has extended to examining other neural structures and systems' role in suicide.

While biological research has progressed our understanding of suicide, conversely neurobiological research can run into difficulties taking mechanistically complex processes and translating them into cognitions or behaviors that suicidal persons may struggle with. In addition, neurobiological research can run the risk of describing specific mechanisms of suicidality that simply need more holistic psychosocial factors to create research with high explanatory power. We argue that, by integrating findings from psychosocial studies of suicide, we can better map our research onto the complex systems of suicidality. To that end, we would call for further examination into the LHb, a structure we believe may be key in developing dynamic suicide research.

THE LATERAL HABENULA, SUICIDAL IDEATION, SUICIDAL BEHAVIOR, AND CAPABILITY FOR SUICIDE

The LHb is often ignored in neurostructural research due to the technical challenge of resolving its structure because of its small size and deep position within the brain. However, advances in both the power of, and analytical approaches to, resolving fMRI signaling now provide satisfactory resolution for LHb registration and segmentation (Gan et al., 2019; Hashikawa et al., 2020).

Anatomically, the habenula is an epithalamic structure often subdivided into the medial habenula and the LHb linking forebrain and midbrain regions, connecting a host of neural structures that include the ventral pallidum, the ventromedial prefrontal cortex (vmPFC), the lateral hypothalamic area (LHA), the globus pallidus, the dorsal raphe nuclei (DRN) and the ventral tegmental area (VTA; Aizawa and Zhu, 2019; Qiao et al., 2020). The LHb is largely innervated from the entopeduncular nucleus and the lateral hypothalamic area, serving a function as a co-releaser of glutamate and GABA. Indeed, within the LHb, glutamatergic neurons project to the ventral tegmental area and the GABAergic rostromedial tegmental nucleus (Shabel et al., 2012). As a relay interface between basal ganglia and limbic system, the LHb is involved in motivational and emotional control of behavior, playing a critical role in both the stress response and behavioral responses induced by expected reward. Further, the LHb operates as a hub between a host of other neural structures that transforms motivational representation into appropriate behavioral outputs by regulating monoaminergic neurotransmission (Stamatakis et al., 2013; Li J. et al., 2017; Ambrosi et al., 2019). Our rationale for implicating the LHb in suicidality is founded on a broad base of past research which has associated LHb dysfunction with many different concepts closely linked with suicidality.

The LHb in the Desire and Drive for Suicide

Within the world of suicide research, an emerging therapeutic focus has reconceptualized suicidality on a behavioral continuum. That is to say of the many suicide risk factors established over decades of research, some appear to directly drive suicide-specific thoughts, feelings, and behaviors whereas other risk factors can be thought of as indirect, in that they are circumstances, states, or conditions leading the patient to consider their life not worth living (Tucker et al., 2015; Jobes et al., 2018). The distinction is subtle, yet important, playing into the larger conversation of whether a risk factor largely will influence a person through soliciting thoughts of suicide, or whether a factor may motivate suicidal behavior. In linking the function of the LHb to suicidality, we believe it is helpful to contextualize past research on this continuum.

The LHb and Desire for Suicide

LHb thought to be a downstream structure in the pathway through which sensory information, projected from other neural structures (such as the medial septum), generates sensory-evoked aversion through a net effect of negatively valenced emotions in animal models (Zhang et al., 2018). This pathway, activated through glutamatergic and GABAergic neurons, may be underlying the process through which the LHb increases the processing of negative stimuli and decreases the processing of positive stimuli (Belzung et al., 2015). This contributes to a neurobiological sensitivity to predict punishment even from

neutral stimuli and this process may underlie the pervasive negative emotional state that characterizes depression. Over time, hyperactivity in this pathway's functional connectivity may contribute to chronic dysregulation of the habenula, leading to long-term alterations of dopamine, serotonin, and norepinephrine transmission that primes a neurological disposition towards avoidance and facilitates social withdrawal related to suicidal ideation (Knowland and Lim, 2018; Ambrosi et al., 2019). In addition, LHb activity has been linked to insomnia, deficits in circadian rhythm regulation, and somatization which are all high arousal life stressors linked with suicidal ideation (Fakhoury, 2017; Mendoza, 2017; Gold and Kadriu, 2019; Qiao et al., 2020).

Further, due to its function as a communication center between limbic structures in the forebrain and aminergic centers in the midbrain, and the structure's key role in monoamine transmission and cognition, the LHb is closely linked to both the emergence and maintenance of treatment-resistant depressive symptoms, a condition found to co-occur heavily with hopelessness (Papakostas et al., 2005; Lecourtier and Kelly, 2007; Browne et al., 2018). Hopelessness is thought to be a key factor in differentiating suicidal ideators from those who do not experience suicidal thoughts (Wolfe et al., 2019). Indeed, the LHb has been implicated in anhedonia (Li et al., 2013), learned helplessness (Li B. et al., 2011), and rumination (Belzung et al., 2015) which have all been linked to hopeless depression and suicidality in past literature (Abramson et al., 2000). These deficits can be linked to the social disconnectedness underlying the process through which a nonsuicidal person can begin to develop suicidal ideation.

The LHb and Drive for Suicide

Hyperactivity in the LHb has been found to be associated with depressive symptomology, anti-reward signaling (systems that activate to limit or diminish rewarding behavior), and impairments in reward-seeking behaviors (Matsumoto and Hikosaka, 2007, 2009; Hong et al., 2011; Proulx et al., 2014).

A further specification of the effect of monoamine theories of suicide relates to the endocannabinoid (eCB) system in the LHb. The eCB system consists of receptors, receptor agonists, and classified as lipid mediators and receptors are typically situated in the presynaptic terminal of the neuron, meaning they are synthesized in an activity-dependent manner compared to neurotransmitters (Shepard and Nugent, 2021). Dysregulation of the eCB system has been implicated in neuropsychiatric conditions, including depression and other stress-related disorders (Castillo et al., 2012; Berger et al., 2018). Endocannabinoids are widely prevalent throughout the brain, suggesting that eCBs are fundamental in modulating synaptic functioning. The eCB system interacts with other neuromodulatory systems and may thus represent an extension of the monoamine theory of depression, as it regulates the release of glutamate, GABA, and other monoamines implicated in depression (Castillo et al., 2012). Notably in the LHb, eCB signaling plays a role in controlling synaptic plasticity, neuronal activity, and subsequent associated behavior, therefore alterations in eCB regulation of LHb neurons contribute to LHb dysfunction that is associated with motivational and social deficits seen in depression and stress-related disorders (Shepard and Nugent, 2021). In the Fluid Vulnerability Theory of suicide, eCB levels in the LHb could then play a crucial role in understanding biological predispositions to suicide risk, which then could be compounded by chronic stress (Authement et al., 2018). The LHb is a critical region that controls the expression of aversive behavior that is activated by aversive stimuli or lack of expected reward.

There is growing evidence that implicates the habenula in stress-related behavioral alterations and identifies endocannabinoids' role in these processes (Berger et al., 2018; Vickstrom et al., 2021). Stress engages eCB signaling in the LHb, evident by rat exposure to social defeat stress demonstrating increased levels of endocannabinoids and agonist binding affinity in the LHb compared to non-stressed rats (Berger et al., 2018). Similarly chronic stress, linked with emotional dysregulation, affects synaptic transmission and transcriptional plasticity in the LHb. Thus, we propose that emotional dysregulation induced by acute suicide risk factors may result in a sensitization of the LHb, leading to increased LHb activity that is observed during chronic stress (Cerniauskas et al., 2019). Stress may be affecting the development of suicidal ideation through activation of the LHb and subsequent activation of Brown Adipose Tissue (BAT) thermogenesis, in a process called "emotional hyperthermia." This involves hyperactivity of the LHb and a corresponding subtle increase in body temperature that has been shown to correlate with increases in perseverative and ruminative behaviors frequently associated with suicidal ideation (Law and Tucker, 2018; Brizuela et al., 2019). Maladaptive responses to chronic stress play an important role in the emergence of mood disorders, especially those with biological vulnerability or predisposition. Chronic stress creates a marked increase in LHb activity and increased LHb activity promotes depression-related behavior (Chen and Kenny, 2018; Cerniauskas et al., 2019). As a strong link exists between chronic stress, depression-related thinking, and suicidal ideation (Park et al., 2010), we propose that these biological mechanisms may be a driving force behind processes wherein an individual both begins to desire dying by suicide and further still behind subsequent processes through which an individual is motivated to engage in suicidal behavior.

The LHb and Capability for Suicide

Another link to LHb function and suicidality could be through the interpretation of capability for suicide, a term for deficits in pain and fear processing allowing one to overcome the drive towards survival inherent in all biological life. Activity in the LHb is thought to underscore the interpretation of both physiological and psychological pain as aversive stimuli (Wirtshafter et al., 1994; Li Y. et al., 2017), potentially implicating this structure in the induction of feelings of entrapment necessary for one to act on their suicidal thoughts. Indeed, a growing body of literature has indicated that hyperactivity in the LHb may result in both a "reward deficit syndrome," characterized clinically by anhedonia, and reduced motivation, along with an "enhanced anti-reward syndrome" noticeable as dysphoria, which may be driving suicidal behaviors through entrapment

(Qiao et al., 2020). Further, chronic dysregulation of the habenula may lead to long-term alterations of dopamine, serotonin, and norepinephrine transmission that may facilitate social withdrawal maladaptive coping related to entrapment and thus, suicide-related behaviors (Knowland and Lim, 2018; Ambrosi et al., 2019).

Consistent with cytokine theories of depression (Jeon and Kim, 2016; Shariq et al., 2018) activation or neuroplasticity in the LHb may be associated with neuroinflammation found to be related to greater intensity of suicidal ideation (Brundin et al., 2015) and greater risk of a completed suicide attempt (Batty et al., 2016). Rodent models of depression (Zhao et al., 2018; Guan et al., 2020) have found similar increases in proinflammatory factors and proteins in the LHb, including tumor necrosis factor-a (TNF-a), C-reactive protein (CRP), and pro-inflammatory cytokines such as interleukin-1b (IL-1b), IL-6, and IL-10. This increase in neuroinflammation within the LHb appears to be associated with greater depressive symptoms and reductions in fear processing (Zhao et al., 2018), particularly notable for suicidality given the LHb's involvement in emotion regulation and processing of aversive stimuli (Ambrosi et al., 2019).

The LHb may be involved in modulating fear and pain which are theorized to be core components of suicide capability (Joiner, 2005). The LHb contains a number of pain-activated neurons (PANs), which increase their firing rates when activated by noxious stimulation. As these neurons are activated, the pain threshold decreases, meaning that pain tolerance is reduced and stimuli are more likely to be perceived as painful (Li et al., 2016). Individuals with high pain tolerance, and therefore higher levels of capability for suicide, may experience sensitization in the firing rates of these LHb PANs. Further, this argument suggests that hyperactivity in the LHb may subsequently lead to desensitization to critical cognitive and affective signals such as gratification, danger assessment, and pain sensitivity as shown in past research (Li Y. et al., 2017; Yang et al., 2018). Indeed, a pathway has emerged through with pain signals are received from the spinal cord, leading to PAN activation in the habenula. Over time, chronic stimulation leads to an increase in the excitability of the LHb neurons which suppresses reward responses projected to the VTA (dopaminergic) and DRN (serotonergic), implicating the LHb in both the pain/depression comorbidity (Zhang et al., 2020). Along this pathway, synaptic changes in the LHb resulting from desensitization of these structures would indeed result in the increases in pain tolerance and decreases in pain sensitivity found to consistently co-occur with the painful and provocative events comprising an individual's acquired capability for suicide (Franklin et al., 2011; Koenig et al., 2016).

Additionally, LHb activity has been implicated in the processing of fear, meaning there is a conceptual link between activation in LHb glutamatergic neurons and increases in fearlessness, particularly in the fearlessness of death (Ribeiro et al., 2014). Results from rodent model research have found that inactivation in the LHb influenced contextual memory deficits and enhanced the fear response to a conditioned stimulus (Durieux et al., 2020). Thus, we believe it is likely that hyperactivity in the LHb could lead to deficits in cueing the fear response in response to suicide-related stimuli. That is, LHb activation may be differentiating between individuals who are high in capability for suicide and those who are low by influencing the likelihood that an individual will perceive suicide-related stimuli as aversive. However past research has found a less robust connection between the habituation to self-injurious or suicidal behavior and resultant increases in capability for suicide (Ribeiro et al., 2020), and thus future research should consider examining whether this hyperreactivity is either static for each person or dynamic and responsive to an individual's environment.

CONCLUSIONS: WHERE DO WE GO FROM HERE

Consistent with the associations of the LHb with the anti-reward circuit, depressive symptoms, anhedonia, reductions in appetitive behaviors such as socialization, and deficits in stress-related fear responses, we believe the LHb is likely a critical structure for investigations into the neuropathophysiological correlates of suicidality. Based on existing research, there is strong evidence for the habenula's involvement in suicide and suicide-related behaviors. Specifically, it is plausible that LHb dysfunction may play a critical role in facilitating the transition from suicidal ideation to suicidal behavior.

In the vein of suicide theory, we believe hyperactivity in the LHb can likely be linked to one's tendency to perceive events as painful and provocative, key experiences in the IPTS and 3ST theories of suicide. Further, neuroplasticity in the LHb likely plays a crucial role in influencing one's baseline levels of suicide risk, such that when psychosocial stressors activate acute vulnerabilities for suicide risk (per the IMVT), an individual's ability to recover and remit from suicidal crises may depend, in part, on neurobiological factors in the LHb (e.g., endocannabinoid receptor density, cytokine levels, and monoaminergic signaling). Once activated, the LHb may sustain negative emotional states through key structures in the basal ganglia and limbic system, promoting an anti-reward strategy that leads to vulnerabilities in suicidal thoughts and behaviors. Chronic activation of the LHb may then result in shifts towards hyperactivity in neuronal cell clusters responsive to aversive stimuli, functioning as a byproduct of the brain's reaction to dysregulated emotional states linked to psychosocial or environmental cues. This process would then result in a one-way "suicide circuit," by which the LHb becomes sensitized to aversive stimuli, making the suicidal mode easier and easier to access with each activation. See **Figure 1** for a hypothesis of this proposed pathway. As such, the role of the LHb, and its long-term modulation in response to negative stimuli in suicidal behavior should be examined. Given the technical and methodological challenges of studying the habenula and the limitations of making conclusions from animal models to human models, our proposed model is developed based on research's current understanding of the habenula's role in various cognitive, affective, and

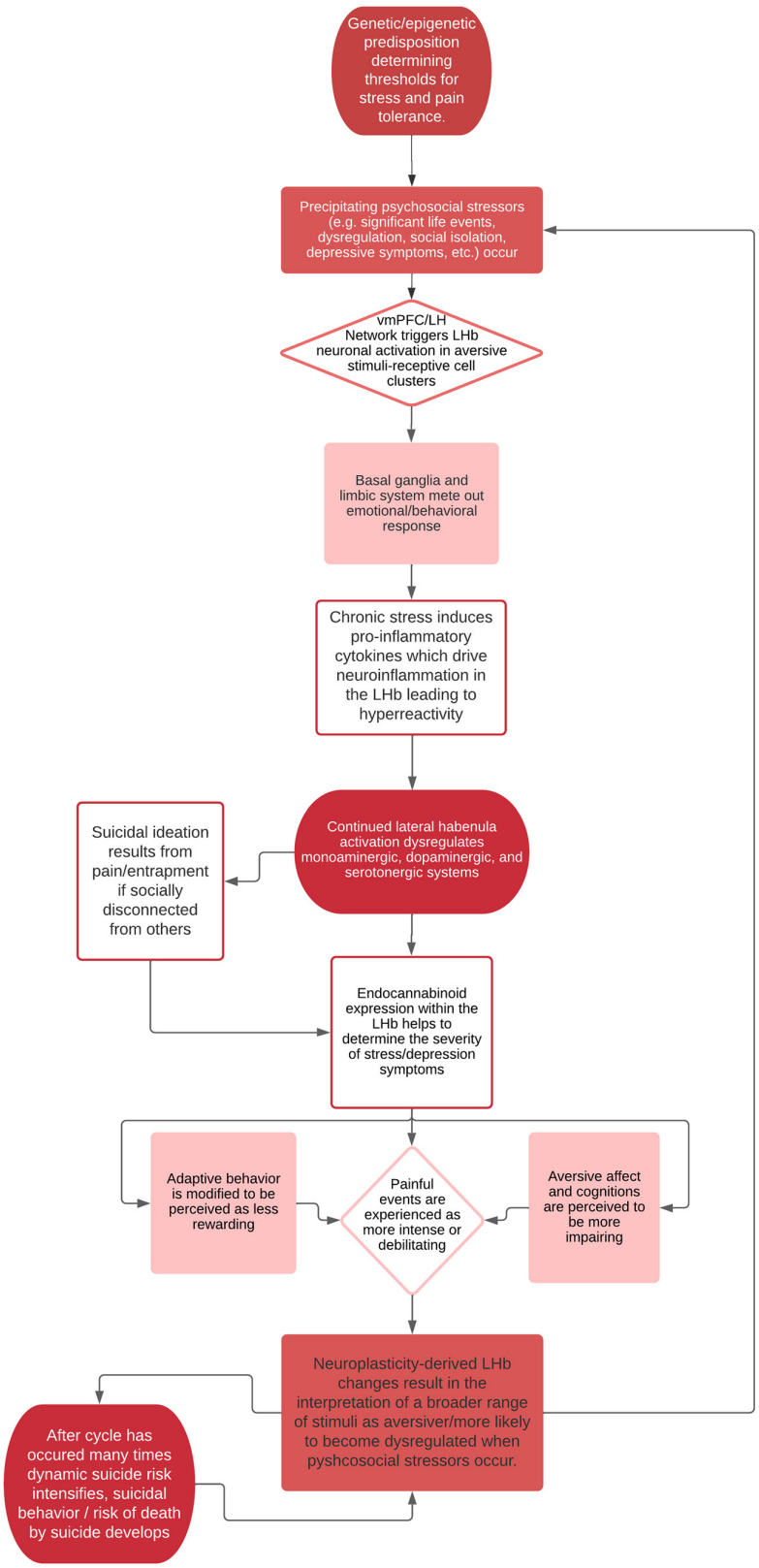

FIGURE 1 | Theoretical process of habenular "suicide pathway".

behavioral processes. Thus, our model may be updated as our understanding of the habenula and related circuitry improves.

We believe that a synergistic partnership between interdisciplinary collaborators can yield potentially life-saving research that can be used to optimize suicide risk assessment and prevention practices. Specifically, it would be helpful to use existing suicide theories generated by psychological research to inform neuroscience research using human models and generate hypotheses that can then be tested in animal models. The findings from neuroscience research can then be used to refine suicide theories and applied to optimize suicide risk assessment and prevention practices. As such, we hope that suicide research moving forward will become more interdisciplinary and see more collaboration between neuroscientists and psychologists.

AUTHOR CONTRIBUTIONS

RM, JW, and KL contributed to the conception and design of this review. RM, JW, SJ, KH, and KO'C gathered and synthesized review articles and wrote sections of the manuscript. RM organized the database and wrote the first draft of the manuscript, which SG, PB, and KL provided edits and areas of focus. All authors contributed to the article and approved the submitted version.

REFERENCES

Abramson, L. Y., Alloy, L. B., Hogan, M. E., Whitehouse, W. G., Gibb, B. E., Hankin, B. L., et al. (2000). "The hopelessness theory of suicidality," in *Suicide Science: Expanding The Boundaries*, eds T. E. Joiner, M. D. , and Rudd (Kluwer Academic/Plenum Publishers), 17–32. doi: 10.1007/0-306-47233-3_3

Aizawa, H., and Zhu, M. (2019). Toward an understanding of the habenula's various roles in human depression. *Psychiatry Clin. Neurosci.* 73, 607–612. doi: 10.1111/pcn.12892

Ambrosi, E., Arciniegas, D. B., Curtis, K. N., Patriquin, M. A., Spalletta, G., Sani, G., et al. (2019). Resting-state functional connectivity of the habenula in mood disorder patients with and without suicide-related behaviors. *J. Neuropsychiatry Clin. Neurosci.* 31, 49–56. doi: 10.1176/appi.neuropsych.17120351

Arango, V., Underwood, M. D., Boldrini, M., Tamir, H., Kassir, S. A., Hsiung, S., et al. (2001). Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology* 25, 892–903. doi: 10.1016/S0893-133X(01)00310-4

Authement, M. E., Langlois, L. D., Shepard, R. D., Browne, C. A., Lucki, I., Kassis, H., et al. (2018). A role for corticotrophin releasing factor signaling in the lateral habenula and its modulation by early life stress. *Sci. Signal.* 11:eaan6480. doi: 10.1126/scisignal.aan6480

Batty, G. D., McIntosh, A. M., Russ, T. C., Deary, I. J., and Gale, C. R. (2016). Psychological distress, neuroticism and cause-specific mortality: early prospective evidence from UK Biobank. *J. Epidemiol. Commun. Health* 70, 1136–1139. doi: 10.1136/jech-2016-207267

Bauer, B. W., Gai, A. R., Joiner, T. E., and Capron, D. W. (2020). The frequency and subjective impact of painful and provocative events on the acquired capability for suicide. *Arch. Suicide Res.* 25, 715–729. doi: 10.1080/13811118.2020.1756017

Belzung, C., Willner, P., and Philippot, P. (2015). Depression: from psychopathology to pathophysiology. *Curr. Opin. Neurobiol.* 30, 24–30. doi: 10.1016/j.conb.2014.08.013

Bender, T. W., Anestis, M. D., Anestis, J. C., Gordon, K. H., and Joiner, T. E. (2012). Affective and behavioral paths toward the acquired capacity for suicide. *J. Soc. Clin. Psychol.* 31, 81–100. doi: 10.1521/jscp.2012.31.1.81

Berger, A. L., Henricks, A. M., Lugo, J. M., Wright, H. R., Warrick, C. R., Sticht, M. A., et al. (2018). The lateral habenula directs coping styles under conditions of stress *via* recruitment of the endocannabinoid system. *Biol. Psychiatry* 84, 611–623. doi: 10.1016/j.biopsych.2018.04.018

Brizuela, M., Antipov, A., Blessing, W. W., and Ootsuka, Y. (2019). Activating dopamine D2 receptors reduces brown adipose tissue thermogenesis induced by psychological stress and by activation of the lateral habenula. *Sci. Rep.* 9:19512. doi: 10.1038/s41598-019-56125-3

Browne, C. A., Hammack, R., and Lucki, I. (2018). Dysregulation of the lateral habenula in major depressive disorder. *Front. Synaptic Neurosci.* 10:46. doi: 10.3389/fnsyn.2018.00046

Brundin, L., Erhardt, S., Bryleva, E. Y., Achtyes, E. D., and Postolache, T. T. (2015). The role of inflammation in suicidal behaviour. *Acta Psychiatr. Scand.* 132, 192–203. doi: 10.1111/acps.12458

Bryan, C. J., Rozek, D. C., Butner, J., and Rudd, M. D. (2019). Patterns of change in suicide ideation signal the recurrence of suicide attempts among high-risk psychiatric outpatients. *Behav. Res. Ther.* 120:103392. doi: 10.1016/j.brat.2019.04.001

Calati, R., Nemeroff, C. B., Lopez-Castroman, J., Cohen, L. J., and Galynker, I. (2020). Candidate biomarkers of suicide crisis syndrome: what to test next? A concept paper. *Int. J. Neuropsychopharmacol.* 23, 192–205. doi: 10.1093/ijnp/pyz063

Castillo, P. E., Younts, T. J., Chavez, A. E., and Hashimotodani, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron* 76, 70–81. doi: 10.1016/j.neuron.2012.09.020

CDC WISQARS. (2020). *Ten Leading Causes of Death by Age Group*. Available online at: https://webappa.cdc.gov/sasweb/ncipc/leadcause.html.

Cerniauskas, I., Winterer, J., de Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., et al. (2019). Chronic stress induces activity, synaptic and transcriptional remodeling of the lateral habenula associated with deficits in motivated behaviors. *Neuron* 104, 899–915.e8. doi: 10.1016/j.neuron.2019.09.005

Chen, Z., and Kenny, P. (2018). Endocannabinoid signaling in the habenula regulates adaptive responses to stress. *Biol. Psychiatry* 84, 553–554. doi: 10.1016/j.biopsych.2018.08.001

Chu, J. P., Goldblum, P., Floyd, R., and Bongar, B. (2010). The cultural theory and model of suicide. *Appl. Prev. Psychol.* 14, 25–40. doi: 10.1016/j.appsy.2011.11.001

Coon, H., Darlington, T. M., DiBlasi, E., Callor, W. B., Ferris, E., Fraser, A., et al. (2020). Genome-wide significant regions in 43 Utah high-risk families implicate multiple genes involved in risk for completed suicide. *Mol. Psychiatry* 25, 3077–3090. doi: 10.1038/s41380-018-0282-3

Durieux, L., Mathis, V., Herbeaux, K., Muller, M., Barbelivien, A., Mathis, C., et al. (2020). Involvement of the lateral habenula in fear memory. *Brain Struct. Funct.* 225, 2029–2044. doi: 10.1007/s00429-020-02107-5

Fakhoury, M. (2017). The habenula in psychiatric disorders: more than three decades of translational investigation. *Neurosci. Biobehav. Rev.* 83, 721–735. doi: 10.1016/j.neubiorev.2017.02.010

Franklin, J. C., Hessel, E. T., and Prinstein, M. J. (2011). Clarifying the role of pain tolerance in suicidal capability. *Psychiatry Res.* 189, 362–367. doi: 10.1016/j.psychres.2011.08.001

Franklin, J. C., Ribeiro, J. D., Fox, K. R., Bentley, K. H., Kleiman, E. M., Huang, X., et al. (2017). Risk factors for suicidal thoughts and behaviors: a meta-analysis of 50 years of research. *Psychol. Bull.* 143, 187–232. doi: 10.1037/bul0000084

Gan, G., Zilverstand, A., Parvaz, M. A., Preston-Campbell, R. N., d'Oleire Uquillas, F., Moeller, S. J., et al. (2019). Habenula-prefrontal resting-state connectivity in reactive aggressive men – A pilot study. *Neuropharmacology* 156:107396. doi: 10.1016/j.neuropharm.2018.10.025

Gold, P. W., and Kadriu, B. (2019). A major role for the lateral habenula in depressive illness: physiologic and molecular mechanisms. *Front. Psychiatry* 10:320. doi: 10.3389/fpsyt.2019.00320

Guan, R., Wang, J., Li, D., Li, Z., Liu, H., Ding, M., et al. (2020). Hydrogen sulfide inhibits cigarette smoke-induced inflammation and injury in alveolar epithelial cells by suppressing PHD2/HIF-1α/MAPK signaling pathway. *Int. Immunopharmacol.* 81:105979. doi: 10.1016/j.intimp.2019.105979

Hallensleben, N., Spangenberg, L., Forkmann, T., Rath, D., Hegerl, U., Kersting, A., et al. (2018). Investigating the dynamics of suicidal ideation. *Crisis* 39, 65–69. doi: 10.1027/0227-5910/a000464

Hashikawa, Y., Hashikawa, K., Rossi, M. A., Basiri, M. L., Liu, Y., Johnston, N. L., et al. (2020). Transcriptional and spatial resolution of cell types in the mammalian habenula. *Neuron* 106, 743–758.e5. doi: 10.1016/j.neuron.2020.03.011

Hong, S., Jhou, T. C., Smith, M., Saleem, K. S., and Hikosaka, O. (2011). Negative reward signals from the lateral habenula to dopamine neurons are mediated by rostromedial tegmental nucleus in primates. *J. Neurosci.* 31, 11457–11471. doi: 10.1523/JNEUROSCI.1384-11.2011

Jeon, S. W., and Kim, Y. K. (2016). Neuroinflammation and cytokine abnormality in major depression: Cause or consequence in that illness? *World J. Psychiatry* 6, 283–293. doi: 10.5498/wjp.v6.i3.283

Jobes, D. A., Gregorian, M. J., and Colborn, V. A. (2018). A stepped care approach to clinical suicide prevention. *Psychol. Serv.* 15, 243–250. doi: 10.1037/ser0000229

Joiner, T. E. (2005). *Why People Die by Suicide.* Cambridge, MA: Harvard University Press.

Kleiman, E. M., Turner, B. J., Fedor, S., Beale, E. E., Huffman, J. C., and Nock, M. K. (2017). Examination of real-time fluctuations in suicidal ideation and its risk factors: results from two ecological momentary assessment studies. *J. Abnorm. Psychol.* 126, 726–738. doi: 10.1037/abn0000273

Klonsky, E. D., and May, A. M. (2015). The three-step theory (3ST): a new theory of suicide rooted in the "ideation-to-action" framework. *Int. J. Cogn. Ther.* 8, 114–129. doi: 10.1521/ijct.2015.8.2.114

Knowland, D., and Lim, B. K. (2018). Circuit-based frameworks of depressive behaviors: the role of reward circuitry and beyond. *Pharmacol. Biochem. Behav.* 174, 42–52. doi: 10.1016/j.pbb.2017.12.010

Koenig, J., Thayer, J. F., and Kaess, M. (2016). A meta-analysis on pain sensitivity in self-injury. *Psychol. Med.* 46, 1597–1612. doi: 10.1017/S0033291716000301

Law, K. C., and Tucker, R. P. (2018). Repetitive negative thinking and suicide: a burgeoning literature with need for further exploration. *Curr. Opin. Psychol.* 22, 68–72. doi: 10.1016/j.copsyc.2017.08.027

Lecourtier, L., and Kelly, P. H. (2007). A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. *Neurosci. Biobehav. Rev.* 31, 658–672. doi: 10.1016/j.neubiorev.2007.01.004

Leon, A. C., Friedman, R. A., Sweeney, J. A., Brown, R. P., and Mann, J. J. (1990). Statistical issues in the identification of risk factors for suicidal behavior: the application of survival analysis. *Psychiatry Res.* 31, 99–108. doi: 10.1016/0165-1781(90)90112-i

Li, J., Kang, S., Fu, R., Wu, L., Wu, W., Liu, H., et al. (2017). Inhibition of AMPA receptor and CaMKII activity in the lateral habenula reduces depressive-like behavior and alcohol intake in rats. *Neuropharmacology* 126, 108–120. doi: 10.1016/j.neuropharm.2017.08.035

Li, J., Li, Y., Zhang, B., Shen, X., and Zhao, H. (2016). Why depression and pain often coexist and mutually reinforce: role of the lateral habenula. *Exp. Neurol.* 284, 106–113. doi: 10.1016/j.expneurol.2016.08.010

Li, Z., Page, A., Martin, G., and Taylor, R. (2011). Attributable risk of psychiatric and socio-economic factors for suicide from individual-level, population-based studies: a systematic review. *Soc. Sci. Med.* 72, 608–616. doi: 10.1016/j.socscimed.2010.11.008

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C. D., Schulz, D., et al. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* 470, 535–539. doi: 10.1038/nature09742

Li, Y., Wang, Y., Xuan, C., Li, Y., Piao, L., Li, J., et al. (2017). Role of the lateral habenula in pain-associated depression. *Front. Behav. Neurosci.* 11:31. doi: 10.3389/fnbeh.2017.00031

Li, K., Zhou, T., Liao, L., Yang, Z., Wong, C., Henn, F., et al. (2013). BCaMKII in lateral habenula mediates core symptoms of depression. *Science* 341, 1016–1020. doi: 10.1126/science.1240729

Mann, J. J. (1999). Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology* 21, 99S–105S. doi: 10.1016/S0893-133X(99)00040-8

Mann, J. J. (2003). Neurobiology of suicidal behaviour. *Nat. Rev. Neurosci.* 4, 819–828. doi: 10.1038/nrn1220

Mann, J. J., and Rizk, M. M. (2020). A brain-centric model of suicidal behavior. *Am. J. Psychiatry* 177, 902–916. doi: 10.1176/appi.ajp.2020.20081224

Matsumoto, M., and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447, 1111–1115. doi: 10.1038/nature05860

Matsumoto, M., and Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nat. Neurosci.* 12, 77–84. doi: 10.1038/nn.2233

May, A. M., and Klonsky, E. D. (2016). What distinguishes suicide attempters from suicide ideators? A meta-analysis of potential factors. *Clin. Psychol. Sci. Pract.* 23, 5–20. doi: 10.1037/h0101735

Mendoza, J. (2017). Circadian neurons in the lateral habenula: clocking motivated behaviors. *Pharmacol. Biochem. Behav.* 162, 55–61. doi: 10.1016/j.pbb.2017.06.013

Miller, J. M., Hesselgrave, N., Ogden, R. T., Sullivan, G. M., Oquendo, M. A., Mann, J. J., et al. (2013). Positron emission tomography quantification of serotonin transporter in suicide attempters with major depressive disorder. *Biol. Psychiatry* 74, 287–295. doi: 10.1016/j.biopsych.2013.01.024

Millner, A. J., Robinaugh, D. J., and Nock, M. K. (2020). Advancing the understanding of suicide: the need for formal theory and rigorous descriptive research. *Trends Cogn. Sci.* 24, 704–716. doi: 10.1016/j.tics.2020.06.007

NIMH (2020). *Dysregulation and Proximal Risk for Suicide (R21 Clinical Trial Optional).* Available online at: https://grants.nih.gov/grants/guide/rfa-files/RFA-MH-20-326.html. Accessed October 2, 2021.

O'Connor, R. C., and Kirtley, O. J. (2018). The integrated motivational-volitional model of suicidal behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* 373:20170268. doi: 10.1098/rstb.2017.0268

Oquendo, M. A., Placidi, G. P. A., Malone, K. M., Campbell, C., Keilp, J., Brodsky, B., et al. (2003). Positron emission tomography of regional brain metabolic responses to a serotonergic challenge and lethality of suicide attempts in major depression. *Arch. Gen. Psychiatry* 60, 14–22. doi: 10.1001/archpsyc.60.1.14

Oquendo, M. A., Galfalvy, H., Russo, S., Ellis, S. P., Grunebaum, M. F., Burke, A., et al. (2004). Prospective study of clinical predictors of suicidal acts after a major depressive episode in patients with major depressive disorder or bipolar disorder. *Am. J. Psychiatry* 161, 1433–1441. doi: 10.1176/appi.ajp.161.8.1433

Oquendo, M. A., Russo, S. A., Underwood, M. D., Kassir, S. A., Ellis, S. P., Mann, J. J., et al. (2006). Higher postmortem prefrontal 5-HT2A receptor binding correlates with lifetime aggression in suicide. *Biol. Psychiatry* 59, 235–243. doi: 10.1016/j.biopsych.2005.06.037

Park, S.-M., Cho, S., and Moon, S.-S. (2010). Factors associated with suicidal ideation: role of emotional and instrumental support. *J. Psychosom. Res.* 69, 389–397. doi: 10.1016/j.jpsychores.2010.03.002

Paashaus, L., Forkmann, T., Glaesmer, H., Juckel, G., Rath, D., Schönfelder, A., et al. (2019). Do suicide attempters and suicide ideators differ in capability for suicide? *Psychiatry Res.* 275, 304–309. doi: 10.1016/j.psychres.2019.03.038

Papakostas, G. I., Petersen, T., Pava, J., Masson, E., Worthington, J. J., Alpert, J. E., et al. (2005). Hopelessness and suicidal ideation in outpatients with treatment-resistant depression: prevalence and impact on treatment outcome. *Focus* 3, 140–145. doi: 10.1176/foc.3.1.140

Proulx, C. D., Hikosaka, O., and Malinow, R. (2014). Reward processing by the lateral habenula in normal and depressive behaviors. *Nat. Neurosci.* 17, 1146–1152. doi: 10.1038/nn.3779

Qiao, D., Zhang, A., Sun, N., Yang, C., Li, J., Zhao, T., et al. (2020). Altered static and dynamic functional connectivity of habenula associated with suicidal ideation in first-episode, drug-naïve patients with major depressive disorder. *Front. Psychiatry* 11:608197. doi: 10.3389/fpsyt.2020.608197

Rabasco, A., and Andover, M. S. (2020). The interaction of dissociation, pain tolerance and suicidal ideation in predicting suicide attempts. *Psychiatry Res.* 284:112661. doi: 10.1016/j.psychres.2019.112661

Ribeiro, J. D., Harris, L. M., Linthicum, K. P., and Bryen, C. P. (2020). Do suicidal behaviors increase the capability for suicide? A longitudinal pretest-posttest study of more than 1,000 high-risk individuals. *Clin. Psychol. Sci.* 8, 890–904. doi: 10.1177/2167702620921511

Ribeiro, J. D., Witte, T. K., Van Orden, K. A., Selby, E. A., Gordon, K. H., Bender, T. W., et al. (2014). Fearlessness about death: the psychometric

properties and construct validity of the revision to the acquired capability for suicide scale. *Psychol. Assess.* 26, 115–126. doi: 10.1037/a0034858

Rudd, M. D. (2006). "Fluid vulnerability theory: a cognitive approach to understanding the process of acute and chronic suicide risk," in *Cognition and Suicide: Theory, Research and Therapy*, ed T. E. Ellis (Washington, DC: American Psychological Association), 355–368. doi: 10.1037/11377-016

Shabel, S. J., Proulx, C. D., Trias, A., Murphy, R. T., and Malinow, R. (2012). Input to the lateral habenula from the basal ganglia is excitatory, aversive and suppressed by serotonin. *Neuron* 74, 475–481. doi: 10.1016/j.neuron.2012.02.037

Shariq, A. S., Brietzke, E., Rosenblat, J. D., Barendra, V., Pan, Z., and McIntyre, R. S. (2018). Targeting cytokines in reduction of depressive symptoms: a comprehensive review. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 83, 86–91. doi: 10.1016/j.pnpbp.2018.01.003

Shepard, R. D., and Nugent, F. S. (2021). Targeting endocannabinoid signaling in the lateral habenula as an intervention to prevent mental illnesses following early life stress: a perspective. *Front. Synaptic Neurosci.* 13:689518. doi: 10.3389/fnsyn.2021.689518

Stamatakis, A. M., Jennings, J. H., Ung, R. L., Blair, G. A., Weinberg, R. J., Neve, R. L., et al. (2013). A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* 80, 1039–1053. doi: 10.1016/j.neuron.2013.08.023

Stockmeier, C. A., Shapiro, L. A., Dilley, G. E., Kolli, T. N., Friedman, L., and Rajkowska, G. (1998). Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression—postmortem evidence for decreased serotonin activity. *J. Neurosci.* 18, 7394–7401. doi: 10.1523/JNEUROSCI.18-18-07394.1998

Sudol, K., and Mann, J. J. (2017). Biomarkers of suicide attempt behavior: towards a biological model of risk. *Curr. Psychiatry Rep.* 19:31. doi: 10.1007/s11920-017-0781-y

Sullivan, G. M., Oquendo, M. A., Milak, M., Miller, J. M., Burke, A., Ogden, R. T., et al. (2015). Positron emission tomography quantification of serotonin(1A) receptor binding in suicide attempters with major depressive disorder. *JAMA Psychiatry* 72, 169–178. doi: 10.1001/jamapsychiatry.2014.2406

Tucker, R. P., Crowley, K. J., Davidson, C. L., and Gutierrez, P. M. (2015). Risk factors, warning signs and drivers of suicide: what are they, how do they differ and why does it matter? *Suicide Life Threat. Behav.* 45, 679–689. doi: 10.1111/sltb.12161

van Heeringen, K. (2012). "Stress-diathesis model of suicidal behavior," in *The Neurobiological Basis of Suicide*, ed Y. Dwivedi (Boca Raton, FL: CRC Press/Taylor & Francis), 113–123.

Vickstrom, C. R., Liu, X., Liu, S., Hu, M.-M., Mu, L., Hu, Y., et al. (2021). Role of endocannabinoid signaling in a septohabenular pathway in the regulation of anxiety- and depressive-like behavior. *Mol. Psychiatry* 26, 3178–3191. doi: 10.1038/s41380-020-00905-1

Wirtshafter, D., Asin, K. E., and Pitzer, M. R. (1994). Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. *Brain Res.* 633, 21–26. doi: 10.1016/0006-8993(94)91517-2

Wolfe, K. L., Nakonezny, P. A., Owen, V. J., Rial, K. V., Moorehead, A. P., Kennard, B. D., et al. (2019). Hopelessness as a predictor of suicide ideation in depressed male and female adolescent youth. *Suicide Life Threat. Behav.* 49, 253–263. doi: 10.1111/sltb.12428

Yang, Y., Wang, H., Hu, J., and Hu, H. (2018). Lateral habenula in the pathophysiology of depression. *Curr. Opin. Neurobiol.* 48, 90–96. doi: 10.1016/j.conb.2017.10.024

Zhang, G.-F., Guo, J., and Yang, J. J. (2020). The lateral Habenula: role in chronic pain and depression. *Transl. Perioper. Pain Med.* 7, 271–278. doi: 10.31480/2330-4871/126

Zhang, G.-W., Shen, L., Zhong, W., Xiong, Y., Zhang, L. I., and Tao, H. W. (2018). Transforming sensory cues into aversive emotion *via* septal-habenular pathway. *Neuron* 99, 1016–1028.e5. doi: 10.1016/j.neuron.2018.07.023

Zhao, Y.-W., Pan, Y.-Q., Tang, M.-M., and Lin, W.-J. (2018). Blocking p38 signaling reduces the activation of pro-inflammatory cytokines and the phosphorylation of p38 in the habenula and reverses depressive-like behaviors induced by neuroinflammation. *Front. Pharmacol.* 9:511. doi: 10.3389/fphar.2018.00511

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Behavioral Neuroscience

BRIEF RESEARCH REPORT
published: 14 March 2022
doi: 10.3389/fnbeh.2022.815461

Lateral Habenula Responses During Eye Contact in a Reward Conditioning Task

Hyunchan Lee and Okihide Hikosaka*

Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health (NIH), Bethesda, MD, United States

For many animals, social interaction may have intrinsic reward value over and above its utility as a means to the desired end. Eye contact is the starting point of interactions in many social animals, including primates, and abnormal patterns of eye contact are present in many mental disorders. Whereas abundant previous studies have shown that negative emotions such as fear strongly affect eye contact behavior, modulation of eye contact by reward has received scant attention. Here we recorded eye movement patterns and neural activity in lateral habenula while monkeys viewed faces in the context of Pavlovian and instrumental conditioning tasks. Faces associated with larger rewards spontaneously elicited longer periods of eye contact from the monkeys, even though this behavior was not required or advantaged in the task. Concurrently, lateral habenula neurons were suppressed by faces signaling high value and excited by faces signaling low value. These results suggest that the reward signaling of lateral habenula may contribute to social behavior and disorders, presumably through its connections with the basal ganglia.

Keywords: lateral habenula, eye contact, reward, primate, electrophysiology

OPEN ACCESS

Edited by:
Jurgen Germann, University Health Network (UHN), Canada

Reviewed by:
Sabrina Ravel, Centre National de la Recherche Scientifique (CNRS), France
Veronika Zlatkina, McGill University, Canada

****Correspondence:***
Hyunchan Lee
dr.hyunchan@gmail.com

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *15 November 2021*
Accepted: *09 February 2022*
Published: *14 March 2022*

Citation:
Lee H and Hikosaka O (2022) Lateral Habenula Responses During Eye Contact in a Reward Conditioning Task. Front. Behav. Neurosci. 16:815461. doi: 10.3389/fnbeh.2022.815461

INTRODUCTION

Eye contact is a key element of social interactions between conspecifics and even across species. This is especially true in primates (Mosher et al., 2011). Abnormal patterns of eye contact are a common behavioral symptom in autism spectrum disorder (Szatmari et al., 2016). Social behavior is an intrinsic source of natural reward and can release reward-associated neuromodulators (e.g., dopamine and serotonin) (Krach et al., 2010; Trezza et al., 2010; Dölen et al., 2013). However, there have been few studies on how eye contact behavior relates to reward and reinforcement learning.

In the primates, interactions are driven by long-term relationships and are thus necessarily shaped by past experience with outcomes of prior interactions. Although some studies have suggested that aversive feelings such as fear and avoidance lead to gaze aversion (Schneier et al., 2011), this effect is not found in all cases (Wieser et al., 2009). Given the complexity of affiliative and antagonistic behaviors in primate societies, we hypothesized that neuronal networks comprising the reward system might regulate eye contact together.

What are brain structures likely to contribute to social aspects of gaze behavior? Previous work in the lab established the role of several basal ganglia structures for learning the emotional value of non-social objects (Hikosaka et al., 2006, 2019). Among them, the lateral habenula (LHb) is highly sensitive to the emotional significance and interacts with brainstem areas and basal ganglia which can control the release of reward-associated neuromodulators (e.g., dopamine and serotonin)

(Matsumoto and Hikosaka, 2007; Hikosaka, 2010; Hu et al., 2020) that could be important in establishing relationships and engaging in social behavior. We thus hypothesized that LHb plays a role in establishing eye contact based on prior emotional experience.

RESULTS

To test this hypothesis, we recorded 33 LHb neurons in two monkeys (15 in monkey CH and 18 in monkey KI) performing an active/passive task in which different face images signaled four different emotional contexts (**Figures 1A,B**). The entire population of recorded cells in the LHb showed consistent responses to the value of each reward stimuli in the task procedure. We thus expect the significant population of cells in the LHb would play a role in modulating the gaze duration/eye contact. In this task, emotional context varied from trial to trial, governed by the possibility of large or small juice rewards ("Rich" or "Poor" contexts) and also by the occurrence or absence of an aversive airpuff stimulus ("Dangerous" or "Safe" contexts). The airpuff was only delivered in the passive task.

The face images were assigned four distinct emotional contexts based on task mode (active or passive) and amount × probability of the outcome. All face stimuli maintained consistent emotional contexts in both active and passive tasks (**Figure 1B**). (1) Rich-Safe (Rwd +, Pun−): monkeys experienced big rewards in both tasks. (2) Rich-Dangerous (Rwd +, Pun +): monkeys experienced big rewards in the active task but punishment in the passive task. (3) Poor-Safe (Rwd−, Pun−): monkeys experienced small rewards in both tasks. (4) Poor-Dangerous (Rwd−, Pun +): monkeys experienced small rewards in the active task and punishment in the passive task. At the time of face stimulus onset, the expected reward amount was higher in the Rich-context (Rich-Safe and Rich-Dangerous) compared with Poor-contexts (Poor-Safe and Poor-Dangerous) (**Figure 1D** and **Supplementary Table 1**).

Each trial started with the appearance of a face image, the identity of which informed the monkey whether the context of the current trial was Rich-Safe, Rich-Dangerous, Poor-Safe, or Poor-Dangerous (**Figure 1A**). After a free viewing of the face for 1 s, an active or passive cue appeared at the center of the screen and respective tasks diverged. In the active task, the monkeys were required to fixate their gaze (700 ms) on the active cue. After the fixation, one of the "good" or "bad" objects appeared. The monkeys were then required to make a saccade to "good" fractal objects to obtain a juice reward and avoid gazing at "bad" objects to preserve the possibility of reward later in the trial. After a "bad" object, there was necessarily a "good" object. When the "bad" objects appeared, after the avoidance for 1 s, the active cue re-appeared and "good" objects appeared. The monkeys then could make a saccade to the "good" object to obtain a juice reward. The good and bad objects were different between 4 faces or scenes. Each face or scene environment contained five different fractals (5 fractals/environment × 16 environments/set = total 80 fractals/set) for "good" and "bad" objects in the active task and "100," "50," and "0%" objects in the passive task. Monkeys could learn these objects within five blocks (Active task, 192 trials/block + Passive task, 192 trials/block = total 384 trials/block). After five blocks of the learning, the gaze pattern and neuronal responses of monkeys were constantly discriminative to the reward value of stimuli (Rich vs. Poor environments, Good vs. Bad, 100 vs. 0% objects in Rich contexts). The passive task was a Pavlovian conditioning procedure entailing three conditioned stimuli (CS) comprising different outcome probabilities (100, 50, or 0%). In the passive task, the monkeys were not required to make a saccade to any object and could freely observe this. Outcomes in the passive task occurred irrespective of the monkey's behavior; thus, the resulting pattern of eye movements can be considered a form of natural viewing.

Reward History Modulates LHb Activity and Eye Contact During Face Viewing

At the start of the trial, LHb neurons were significantly inhibited by Rich faces and excited by Poor faces (**Figure 1C**). Furthermore, in Rich trials, the monkeys' gaze consistently dwelled longer on the face images than on Poor trials (irrespective of Danger vs. Safe) (**Figure 2A**). LHb neurons showed an initial inhibitory response to every face lasting from 50 to 150 ms. On Rich trials, monkeys showed an increase to look at the face at all from 100 to 150 ms after the trial started (**Figure 2B**). After 150 ms both LHb activity and gaze behavior discriminated between Rich and Poor faces. Specifically, on Rich trials as compared to Poor, the monkeys' gaze dwelled longer inside rectangular regions around the eye region, evidently for the sake of discerning what conditions were on the menu for the current trial. Eye contact behavior did not differ between Dangerous vs. Safe trials (**Figure 2C**). Around 450 ms, the monkeys' gaze shifted from the eyes to the center of the screen where the active or passive cue would appear (**Figure 2D**). The probability of gaze within the central window was greater on Rich trials than on Poor trials in a window of 600-1,000 ms. This indicates that the monkey was more motivated by Rich context faces to see the upcoming cue and learn whether an active or a passive trial would follow.

Reward History Modulates LHb Activity and Saccades to Fractal Objects

To assess the impact of task context on reward modulation of LHb neurons over and above the impact of social stimuli, we compared neural responses to saccade targets in the active task and CS fractals in the passive task. In the active task, LHb was suppressed by good objects that monkeys were required to fixate on (> 500 ms), and excited by bad objects that monkeys avoided (**Figures 3A–H**). In the passive task, Safe contexts, LHb was excited by the 0% CS (signaling a disappointing reward omission) and were inhibited to graded degrees by the 50 and 100% CS (**Figures 3I,J**). The probability of saccades to CS increased as a function of reward probability (**Figures 3M,N**). In the Dangerous contexts, LHb was similarly excited (**Figures 3K,L**) and gaze to CS suppressed by all CS objects regardless of punishment probabilities (**Figures 3O,P**). Although this finding suggests that reward expectation exerts a more decisive influence on behavior

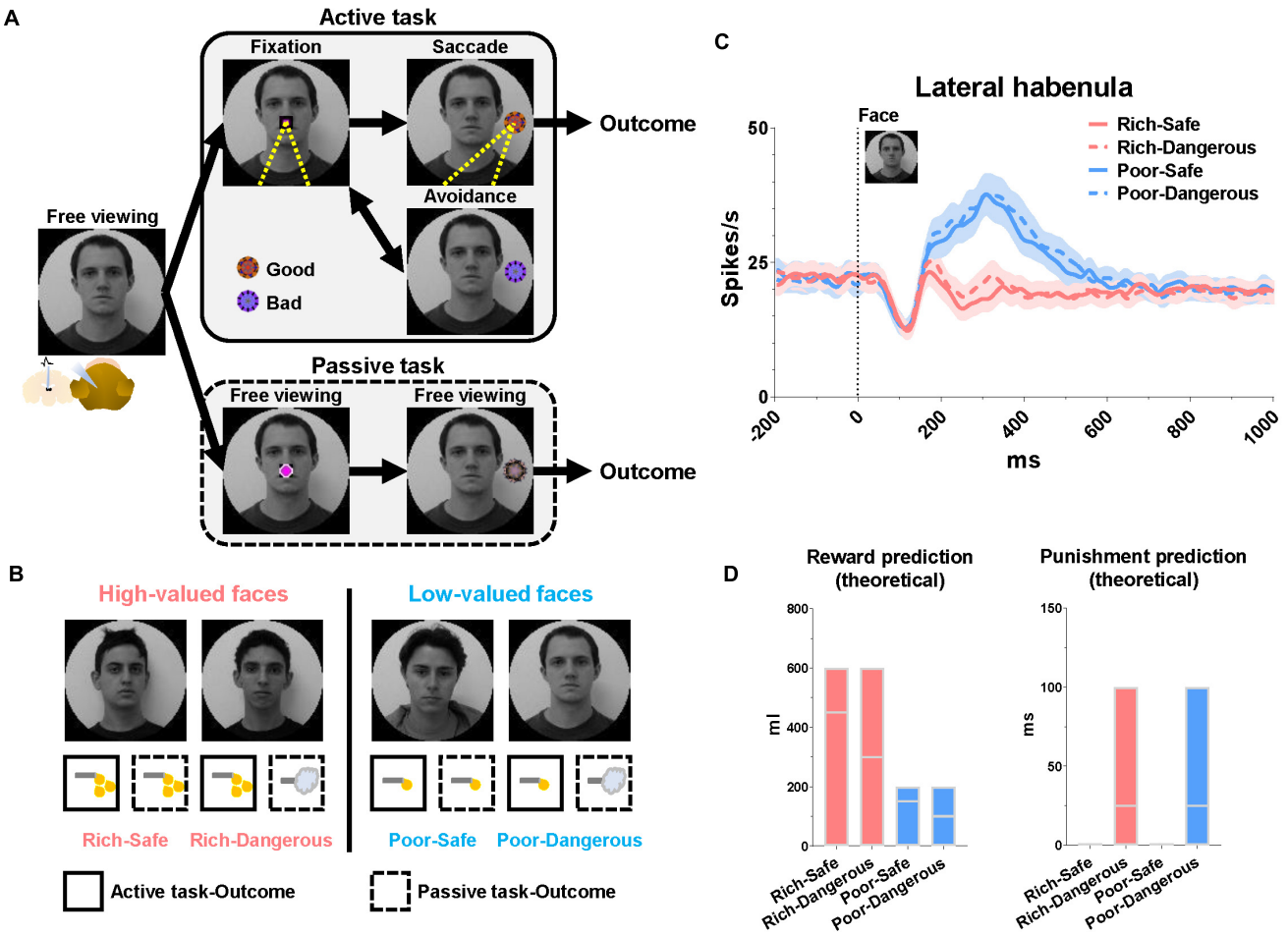

FIGURE 1 | Lateral habenula responses to high- and low-valued faces. **(A)** Face-based procedure with two distinct behavioral tasks (active and passive). **(B)** Examples of face images signaling different conditions. Rich and Poor faces were associated with high-valued and low-valued rewards, respectively. Dangerous faces (but not Safe faces) were associated with punishment in the passive mode. **(C)** Population LHb responses to the faces during the free viewing period before the start of each task. Data were from both monkeys and all recorded LHb neurons averaged across entire sessions. **(D)** Theoretical reward and punishment predictions at face onset. Floating bars indicate the minimum to the maximum value of outcomes in each trial at the face onset. The lines in the bars indicate the mean value of the reward (Rich-Safe, 450 μl; Rich-Dangerous, 300 μl; Poor-Safe, 150 μl; Poor-Dangerous, 100 μl) and punishment (Rich-Safe, 0 ms; Rich-Dangerous, 25 ms; Poor-Safe, 0 ms; Poor-Dangerous, 25 ms) in each trial at the face onset. The mean values were determined by the average of all combinations of outcomes in the active and passive conditions (**Supplementary Tables 1,2**).

and LHb neurons than punishment expectation, these identical responses of LHb activities and gaze to punishment objects could also have a "warning" role whatever the probability of airpuff occurrence.

DISCUSSION

Reward History and Eye Contact: A Perspective of Social Skill/Habitual Behavior

The present study found that the activities of LHb neurons are modulated by the reward history of environmental stimuli (**Figure 1C**). Moreover, the environments that were associated with greater rewards elicited prolonged gaze periods (**Figure 2**). This relationship between reward experience and gaze duration was consistently observed in both social (faces, **Figure 2**) and non-social stimuli (landscape scenes, **Supplementary Figures 1A–C**). In the social environment, monkeys' eye contact and face-to-face gaze were strongly modulated by the history of prior reward experiences. Additionally, the pattern of LHb activity and gaze behavior was consistently changed by reward experiences of objects in both natural free viewing (**Figures 3M–P**, no action required) and instrumental viewing (3E–H, action required).

In real life, many animals have inter-species social gaze/interaction as well as the behaviors within conspecifics. Significantly, the interactions with human are solid and crucial to captive or domestic animals (Tuber et al., 1996;

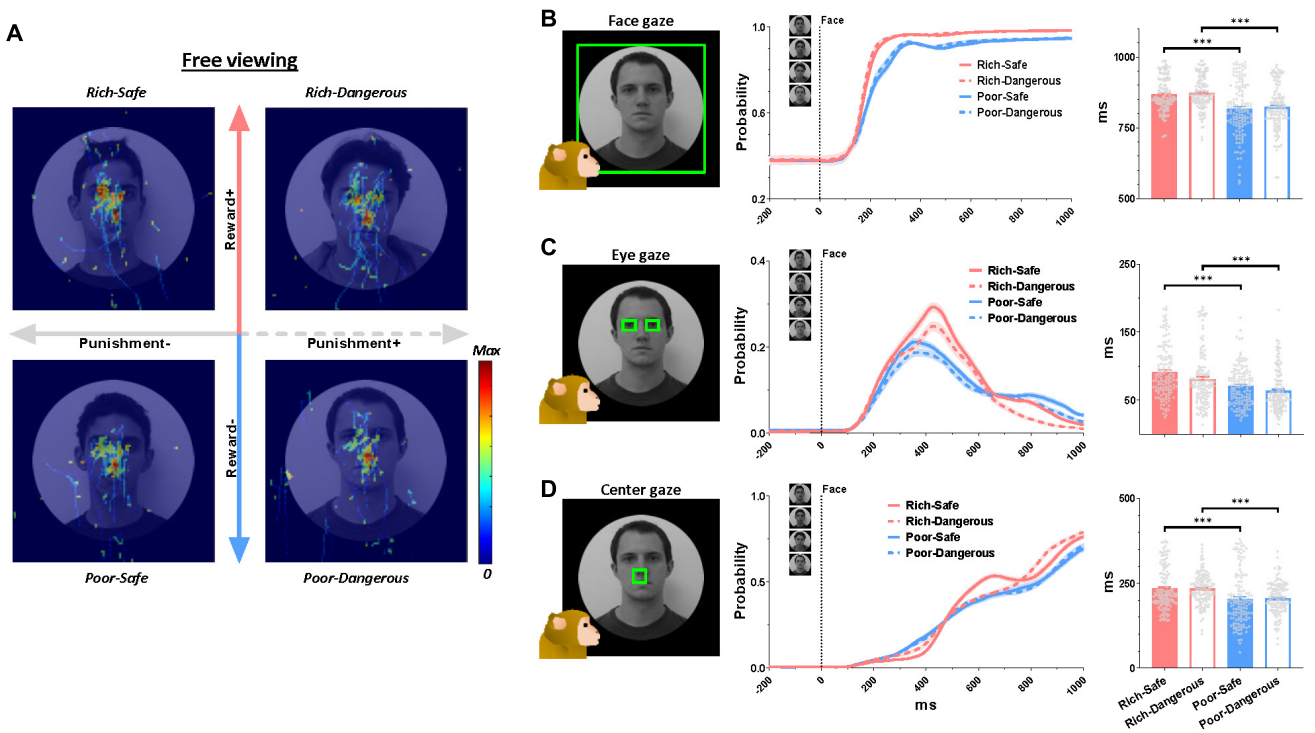

FIGURE 2 | Eye gaze to high- and low-valued faces. **(A)** Normalized eye scan patterns during the free viewing period. Hot colors indicate higher gaze probability. **(B–D)** Left, green boxes indicate regions of interest. Middle, probability of gaze within the said region over all trials ever recorded in the active and passive conditions during the entirety of 138 behavioral sessions. Right, quantification of gaze on the face regions. The average duration of gaze inside the region of interest inside the following time windows: gaze on the face, 0-1,000 ms (top row), gaze at eyes, 0-1,000 ms (middle row), gaze at screen center, 600-1,000 ms (bottom row). Data were from both monkeys and all behaviors averaged across entire sessions.

Brosnan and De Waal, 2003; Range et al., 2009; Dettmer et al., 2016). Here, a social bond and emotional history between the animals and humans could play critical roles in the social behaviors of animals beyond their species-specific responses (Stoeger et al., 2012; Katayama et al., 2019). We thus tested LHb activities and monkey's social gaze using human faces. We then propose that the face and eyes of animals could function as a goal-oriented rewarding object in social contexts and that LHb neurons play an important role in learning this behavior through habitual practice and cultural acclimation.

Consistent with this notion, one study reported that face patches are not observed in newborn primates (Livingstone et al., 2017), and experience with faces is necessary to develop normal face viewing behavior and dedicated face processing modules in the brain (Arcaro et al., 2017). Moreover, in humans, the interpretation of eye contact varies widely across cultures (Uono and Hietanen, 2015). This raises the possibility that the culturally expected pattern of eye contact behavior is a social skill acquired over the course of normal social development. A previous study has reported that macaque monkeys have a strong hierarchical social structure that dominants monopolize 87% of food in the social tolerance test (Burkart and van Schaik, 2013). This finding implies that appropriate social recognition and behaviors based on their social relationship and culture could be critical sources to their living on the social structures.

How then does the brain modulate eye gaze based on emotional histories? We recently suggested that parallel circuits in basal ganglia play an important role in automatic skills and habitual eye movements (Hikosaka et al., 2019). These studies showed that primates spontaneously make saccades to objects associated with reward over the long term. To facilitate neuronal plasticity of basal ganglia neurons and establish the automatic behavior, LHb may play an important role in this form of learning by relaying reward prediction errors signals to dopamine neurons. In turn, these dopamine neurons facilitate synaptic plasticity in basal ganglia neurons, thus paving the way for automatic behavior.

Hypothetical Network Implementing Lateral Habenula Modulation of Gaze Holding

How might neural activity in LHb lead to sustained eye contact on rewarding objects? A crucial mechanism for stopping eye movements in the brain is modulated by omnidirectional pause neurons (OPN) in the raphe interpositus nucleus (Optican and Pretegiani, 2017). The OPN tonically fire during fixation and abruptly cease their firings before and during a saccade. Then OPN can directly control saccadic eye movements by inhibiting and disinhibiting excitatory/inhibitory burst neurons (EBN/IBN)

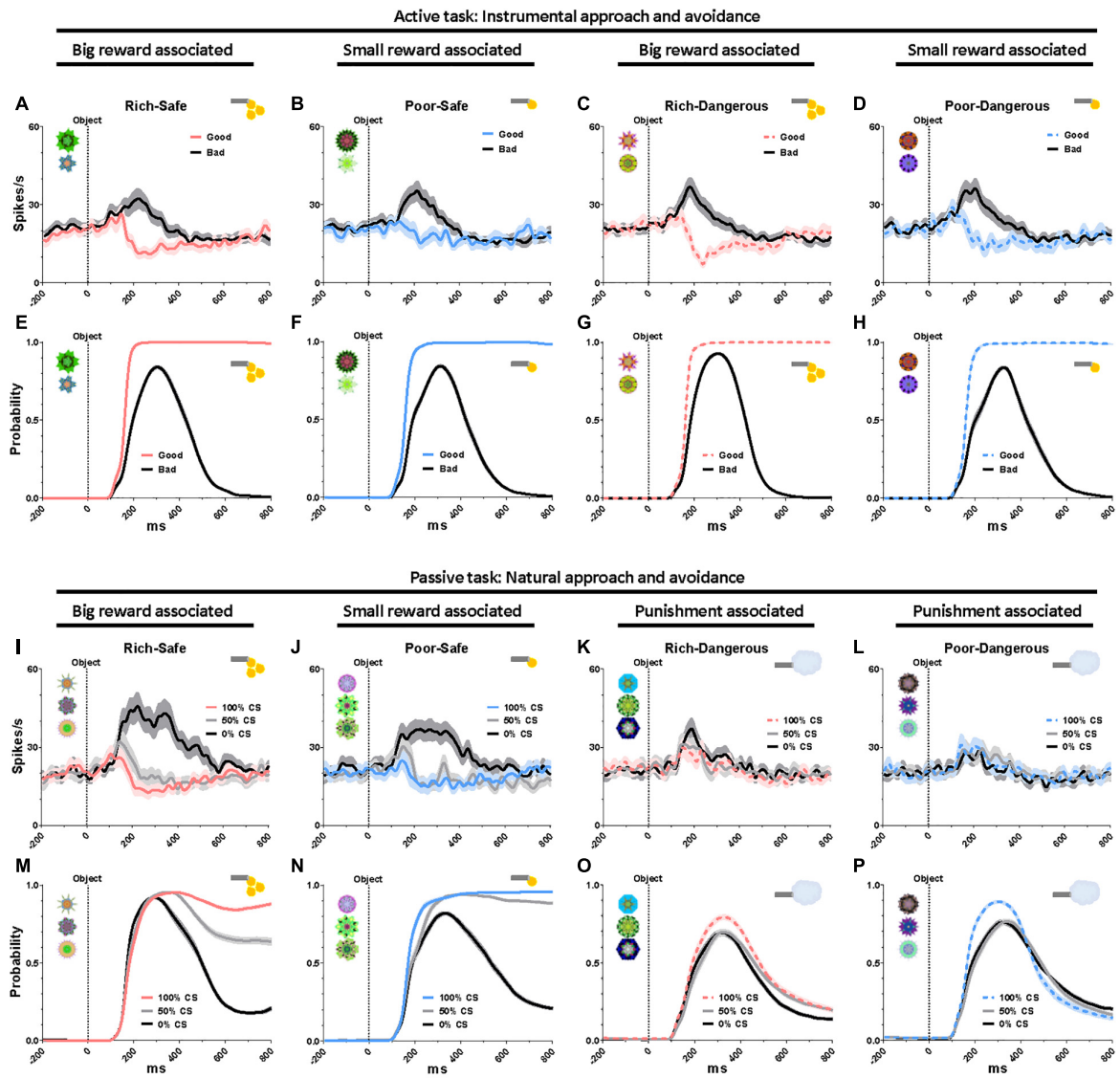

FIGURE 3 | Eye gaze to high- and low-valued objects. **(A–D)** LHb responses to good and bad objects during the active task. **(E–H)** Probability of gaze on good and bad objects during the active task. **(I–L)** LHb responses to the conditioned stimuli (CS) objects during the passive task. **(M–P)** Probability of gaze on the CS objects during the passive task. Data were from both monkeys and all recorded LHb neurons and behaviors averaged across entire sessions.

(Hikosaka and Kawakami, 1977; Igusa et al., 1980; Nakao et al., 1980; Yoshida et al., 1982), premotor neurons of the oculomotor nerve (abducens nerve) (Hikosaka et al., 1977).

In a previous retrograde tracing study in primates, cells projecting to OPN were found in brainstem areas including reticular formation, periaqueductal gray, superior colliculus (SC), and the habenulopeduncular tract (Langer and Kaneko, 1990). SC is a well-studied brain area that projects to OPN (Yoshida et al., 2001; Takahashi et al., 2005) and the premotor burst neurons that control eye movements (Sugiuchi et al., 2005; Izawa et al., 2007; Takahashi et al., 2014). Moreover, the basal ganglia-SC pathway is critical to modulating automatic skills and habitual eye movements established by reward history (Kim et al., 2015; Amita and Hikosaka, 2019; Kunimatsu et al., 2021). LHb neurons access the basal ganglia-SC loop at the level of striatum and dopamine neurons and are thus well-positioned to modulate emotional factors driving learning in oculomotor behavior (**Figure 4**) (Matsumoto and Hikosaka, 2007; Hong and Hikosaka, 2013). Whereas the outputs of LHb have been extensively studied in midbrain dopamine neurons, LHb projections to other brain stem nuclei are still not clearly appreciated.

Nonetheless, LHb has massive projections to the periaqueductal gray (Li et al., 1993), reticular formation, and parabrachial nucleus (Herkenham and Nauta, 1979). These brainstem areas play an essential role in the motivation of behavior (Kim et al., 2020) as well as direct motor control of facial movement and vocalization (Magoun et al., 1937) that execute quintessentially social gestures. We suggest that this

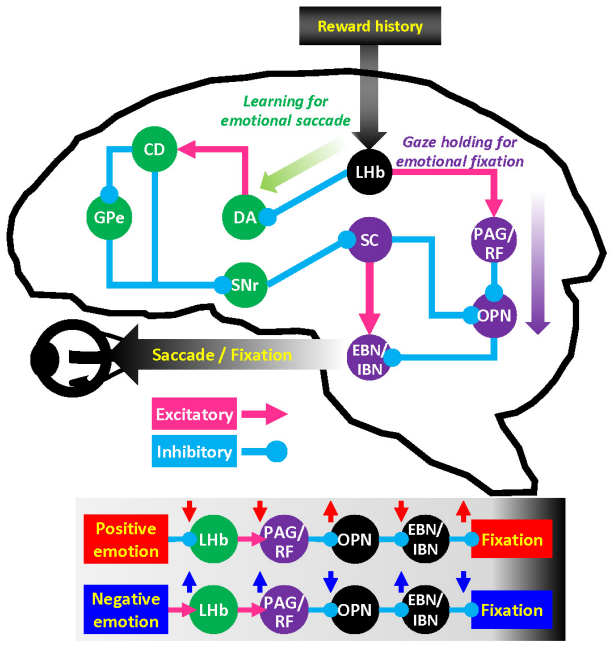

FIGURE 4 | Hypothetical network for the influence of emotion on gaze. LHb neurons regulate dopamine (DA) neurons in substantia nigra pars compacta and ventral tegmental area through rostromedial tegmental nucleus. Dopamine neurons in turn send signals to caudate head (CD) nucleus that projects to superior colliculus (SC) via the external segment of the globus pallidus (GPe) and substantia nigra pars reticulata (SNr). This basal ganglia–SC circuit can modulate learning of goal-directed saccadic eye movements. LHb also has substantial projections to the brainstem structures such as periaqueductal gray (PAG) and reticular formation (RF). These brainstem areas can regulate premotor burst neuron (EBN/IBN) and omnipause neuron (OPN) that control the oculomotor nerve.

LHb-brain stem pathway may play a pivotal role in gaze holding through the OPN mechanisms and facilitate the execution of social behaviors in general.

Why Is Eye Contact Important?

Eye contact is a crucial affiliative social behavior that has been reported from the start of infancy (Ferrari et al., 2009). Primates, in particular, possess facial features (e.g., prominent irises and eyebrows, articulate mouth, and facial muscles) optimized to attract gaze and signal intention and feelings (Zhang et al., 2021). More broadly, many animals depend on collective behaviors for survival that must be coordinated and executed at the level of a pack, flock, swarm, or school (Norris and Schilt, 1988; Stander, 1992). Eye contact might support the organization of gregarious behavior in many species.

In addition to eyes and faces, hands are a frequent target of socially guided eye movements (Ninomiya et al., 2020). A previous study showed that, in monkeys raised without exposure to faces, eye movements dwelled longer on human hands than on faces (Arcaro et al., 2017). For captive primates, the human hand might be an object of critical interest for predicting rewards, for instance during enrichment activities or at feeding time.

Like this, there are many social and non-social objects associated with each reward history in real life. Thus, sequential behaviors to multiple objects are often required for attaining goals. For instance, making eye contact can lead to the discernment of social context, which in turn can inform subsequent behavior as warranted by the social goals. In the experiments described here, the monkeys' eye movements dwelled on the central position as well as the eye regions (**Figure 2**). This central gaze fixation was longer for Rich faces than for Poor faces (**Figure 2D**). In our task procedure, the center of the face was the region where the active or passive cue would appear after the free viewing stage (**Figure 1A**). Thus, it would be one of the significant anticipatory gazes for the upcoming targets (i.e., active or passive cue). Indeed, the monkeys' gaze acquired the central cue faster in the Rich contexts than in Poor contexts, implying higher motivation on the former trials (**Supplementary Figures 1D–H**).

In summary, the present study found that LHb neuronal activities represent the reward value of each social and non-social object sequentially and phasically. We thus suggest that the sequential LHb neuronal activities and the gazes of monkeys around the eye region and other prominent reward features in this study might similarly contribute to monkeys' interactions with other animals like a monkey and human interaction as a sequential goal-directed behavior.

MATERIALS AND METHODS

Two adult rhesus monkeys (both male, 8-years-old, 10-12 kg) were used for this study. All animal care and experiment procedures were approved by Animal Care and Use Committee of the National Eye Institute and complied with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. We recorded 54 single neurons in LHb region around + 7 mm anterior to the interaural plane and + 1 mm from midline using a plastic recording chamber and grid with 1 mm spacing. The recording sites were identified by MRI (4.7 T, Bruker). We then found 33 neurons in the LHb that were sensitive to reward prediction error. The neuronal activities were inhibited by unexpected reward outcomes and excited by reward omission and punishment outcomes as shown in the previous study (Matsumoto and Hikosaka, 2009). The LHb neurons were recorded from only one grid hole at each hemisphere. The LHb neurons were distinguished with the surrounding mediodorsal thalamus region (1 mm away from the LHb neurons) which showed unclear reward prediction error response.

The neurons in both monkeys were recorded with glass-coated electrodes (diameter 0.38 mm, 1 MΩ, Alpha-Omega). The chamber was tilted posteriorly by 8°. The electrode was advanced by an oil-driven micro-manipulator (MO-97A, Narishige). A microelectrode AC amplifier (model 1800; A-M Systems) was used to amplify the neuronal signals (10 k gain) and band-pass filtered from 0.1 to 10 kHz (model 3384; Krohn-Hite). Single neurons were isolated using an online custom voltage- and time-based window discriminator in the software

Blip (www.robilis.com/blip/) and collected at 1 kHz along with monkey's eye position (EyeLink 1000 Plus, SR Research).

The monkeys were trained to perform the face-based active/passive task (five blocks) (**Figure 1**). They were head-fixed during the task. At the start of a trial, a face (40° × 40°)[1] represented one of four contexts appeared (Rich-Safe, big reward and no punishment; Rich-Danger, big reward and punishment; Poor-Safe, small reward and no punishment; Poor-Danger, small reward and punishment) (**Figure 1B**). We used these human face images to investigate the effect of reward history on the social gaze of captive monkeys who are familiar with interaction with human caregivers. On half of the trials (192 out of 384 total trials), landscape scenes were used instead of faces[2,3] as non-social stimuli (**Supplementary Figure 1**). The total of 384 trials was conducted per cell. There was no difference between the tasks when the face stimuli were used and the tasks when scene stimuli were used. After 1 s free viewing of the face/scene stimulus, either an active (Magenta square) or a passive cue (Magenta circle) (2 × 2°) appeared at the center of the screen (**Figure 1A** middle). The face/scene stimuli stayed on the screen throughout the entire trial (**Figure 1A**). The shape of the cue indicated whether the trial would proceed as an active or passive task. In the active task, monkeys were required to fixate (700 ms) on the active cue. After the fixation on the active cue, either a good or a bad object appeared on the left or right side of the screen (15 degree). The monkeys could collect the reward by fixating on the good object that appeared for 500 ms. They were required to avoid any bad objects that appeared by not gazing at the object for more than 500 ms. The volume of the juice reward was adjusted depending on whether the trial was Rich (600 μl) or Poor (200 μl). In the active task, reward volume was the same in both the Safe and the Dangerous contexts (**Figure 1B**). The passive task was a Pavlovian conditioning procedure and required no particular behavior from the monkeys. The monkeys were not needed to fixate on any cue or object in this condition. Each trial of the passive task started with a 1 s free viewing epoch of the face/scene stimulus and was indistinguishable from the preamble to the active task. After the passive cue appeared at the center screen for 1 s, one of three objects (fractal images) appeared in the peripheral region of the face/scene background image. The identity of the fractal stimulus indicated the probability of the reward/punishment outcome (100, 50, and 0%). After 1.5 s, monkeys received a reward (either big or small) in the Rich-Safe and Poor-Safe contexts and received an airpuff (100 ms) in both Rich-Dangerous and Poor-Dangerous according to the probabilities previously foreshadowed by the fractal object. The objects were created using fractal geometry (Yamamoto et al., 2012). Data were analyzed using MATLAB (MathWorks) and Prism8 (GraphPad Software) and presented as mean ± standard error of the mean (SEM). Firing rates were presented by smoothening with a Gaussian kernel (σ = 10 ms). The statistical significances were tested using a one-way analysis of variance (ANOVA) with Tukey *post hoc* test. The statistical tests were performed on groups of cells and behaviors from both monkeys.

[1]https://fei.edu.br/~cet/facedatabase.html

[2]https://www.google.com/earth

[3]https://openaerialmap.org

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by National Eye Institute Animal Care and Use Committee. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HL and OH conceived, designed, performed the experiments, analyzed the data, and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by the Intramural Research Program at the National Institutes of Health, National Eye Institute (EY000415).

ACKNOWLEDGMENTS

We thank A. Ghazizadeh, H. Amita, D. BT. McMahon, K. Maeda, J. Kunimatsu, A. Gopal, G. Costello, A. Yoshida, X. Yu, M. K. Smith, A. M. Nichols, T. W. Ruffner, D. Yochelson, A. V. Hays, J. W. McClurkin, J. Fuller-Deets, and current and former colleagues of LSR for discussions and technical support. D. Parker, A. Lopez, D. O'Brien, V. L. McLean, and G. Tansey for animal facility assistance. S. Hong, S. Yamamoto, D. A. Leopold, K. Koyano, C.E. Thomaz, and his colleagues for gift of recording software and visual stimuli.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2022.815461/full#supplementary-material

Supplementary Figure 1 | Responses to high- and low-valued scenes. **(A)** Normalized eye scan patterns during the scene-based task procedure. Hot colors indicate a higher gaze probability. **(B)** Lateral habenula responses to the scenes at the scene onset. **(C)** Probability of gaze on the scene regions. **(D)** Start time of anticipatory gaze on the center region before task cue onset. **(E)** Start time of fixation on the active task cue after the cue onset. **(F)** The rates of trials that monkeys refused to fixate their gaze on the active cue. **(G)** Start time of fixation on the passive task cue after the cue onset. **(H)** The rates of trials that monkeys refused to fixate their gaze on the passive cue.

REFERENCES

Amita, H., and Hikosaka, O. (2019). Indirect pathway from caudate tail mediates rejection of bad objects in periphery. *Sci. Adv.* 5:eaaw9297. doi: 10.1126/sciadv.aaw9297

Arcaro, M. J., Schade, P. F., Vincent, J. L., Ponce, C. R., and Livingstone, M. S. (2017). Seeing faces is necessary for face-domain formation. *Nat. Neurosci.* 20, 1404–1412. doi: 10.1038/nn.4635

Brosnan, S. F., and De Waal, F. B. (2003). Monkeys reject unequal pay. *Nature* 425, 297–299. doi: 10.1038/nature01963

Burkart, J. M., and van Schaik, C. (2013). Group service in macaques (*Macaca fuscata*), capuchins (*Cebus apella*) and marmosets (Callithrix jacchus): a comparative approach to identifying proactive prosocial motivations. *J. Comp. Psychol.* 127, 212–225. doi: 10.1037/a0026392

Dettmer, A. M., Kaburu, S. S., Simpson, E. A., Paukner, A., Sclafani, V., Byers, K. L., et al. (2016). Neonatal face-to-face interactions promote later social behaviour in infant rhesus monkeys. *Nat. Commun.* 7:11940. doi: 10.1038/ncomms11940

Dölen, G., Darvishzadeh, A., Huang, K. W., and Malenka, R. C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* 501, 179–184. doi: 10.1038/nature12518

Ferrari, P. F., Paukner, A., Ionica, C., and Suomi, S. J. (2009). Reciprocal face-to-face communication between rhesus macaque mothers and their newborn infants. *Curr. Biol.* 19, 1768–1772. doi: 10.1016/j.cub.2009.08.055

Herkenham, M., and Nauta, W. J. (1979). Efferent connections of the habenular nuclei in the rat. *J. Comp. Neurol.* 187, 19–47. doi: 10.1002/cne.901870103

Hikosaka, O. (2010). The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.* 11, 503–513. doi: 10.1038/nrn2866

Hikosaka, O., and Kawakami, T. (1977). Inhibitory reticular neurons related to the quick phase of vestibular nystagmus–their location and projection. *Exp. Brain Res.* 27, 377–386. doi: 10.1007/BF00235511

Hikosaka, O., Maeda, M., Nakao, S., Shimazu, H., and Shinoda, Y. (1977). Presynaptic impulses in the abducens nucleus and their relation to postsynaptic potentials in motoneurons during vestibular nystagmus. *Exp. Brain Res.* 27, 355–376. doi: 10.1007/BF00235510

Hikosaka, O., Nakamura, K., and Nakahara, H. (2006). Basal ganglia orient eyes to reward. *J. Neurophysiol.* 95, 567–584. doi: 10.1152/jn.00458.2005

Hikosaka, O., Yasuda, M., Nakamura, K., Isoda, M., Kim, H. F., Terao, Y., et al. (2019). Multiple neuronal circuits for variable object-action choices based on short- and long-term memories. *Proc. Natl. Acad. Sci.U.S.A.*116:26313–26320 doi: 10.1073/pnas.1902283116

Hong, S., and Hikosaka, O. (2013). Diverse sources of reward value signals in the basal ganglia nuclei transmitted to the lateral habenula in the monkey. *Front. Hum. Neurosci.* 7:778. doi: 10.3389/fnhum.2013.00778.

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Igusa, Y., Sasaki, S., and Shimazu, H. (1980). Excitatory premotor burst neurons in the cat pontine reticular formation related to the quick phase of vestibular nystagmus. *Brain Res.* 182, 451–456. doi: 10.1016/0006-8993(80)91202-0

Izawa, Y., Sugiuchi, Y., and Shinoda, Y. (2007). Neural organization of the pathways from the superior colliculus to trochlear motoneurons. *J. Neurophysiol.* 97, 3696–3712. doi: 10.1152/jn.01073.2006

Katayama, M., Kubo, T., Yamakawa, T., Fujiwara, K., Nomoto, K., Ikeda, K., et al. (2019). Emotional Contagion From Humans to Dogs Is Facilitated by Duration of Ownership. *Front. Psychol.* 10:1678. doi: 10.3389/fpsyg.2019.01678

Kim, D. Y., Heo, G., Kim, M., Kim, H., Jin, J. A., Kim, H. K., et al. (2020). A neural circuit mechanism for mechanosensory feedback control of ingestion. *Nature* 580, 376–380. doi: 10.1038/s41586-020-2167-2

Kim, H. F., Ghazizadeh, A., and Hikosaka, O. (2015). Dopamine Neurons Encoding Long-Term Memory of Object Value for Habitual Behavior. *Cell* 163, 1165–1175. doi: 10.1016/j.cell.2015.10.063

Krach, S., Paulus, F. M., Bodden, M., and Kircher, T. (2010). The rewarding nature of social interactions. *Front. Behav. Neurosci.* 4:22. doi: 10.3389/fnbeh.2010.00022

Kunimatsu, J., Yamamoto, S., Maeda, K., and Hikosaka, O. (2021). Environment-based object values learned by local network in the striatum tail. *Proc. Natl. Acad. Sci.U.S.A.*118:e2013623118. doi: 10.1073/pnas.2013623118

Langer, T. P., and Kaneko, C. R. (1990). Brainstem afferents to the oculomotor omnipause neurons in monkey. *J. Comp. Neurol.* 295, 413–427. doi: 10.1002/cne.902950306

Li, Y. Q., Takada, M., and Mizuno, N. (1993). Demonstration of habenular neurons which receive afferent fibers from the nucleus accumbens and send their axons to the midbrain periaqueductal gray. *Neurosci. Lett.* 158, 55–58. doi: 10.1016/0304-3940(93)90611-n

Livingstone, M. S., Vincent, J. L., Arcaro, M. J., Srihasam, K., Schade, P. F., and Savage, T. (2017). Development of the macaque face-patch system. *Nat. Commun.* 8:14897. doi: 10.1038/ncomms14897

Magoun, H. W., Atlas, D., Ingersoll, E. H., and Ranson, S. W. (1937). Associated Facial, Vocal and Respiratory Components of Emotional Expression: An Experimental Study. *J. Neurol. Psychopathol.* 17, 241–255. doi: 10.1136/jnnp.s1-17.67.241

Matsumoto, M., and Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447, 1111–1115. doi: 10.1038/nature05860

Matsumoto, M., and Hikosaka, O. (2009). Representation of negative motivational value in the primate lateral habenula. *Nat. Neurosci.* 12, 77–84. doi: 10.1038/nn.2233

Mosher, C. P., Zimmerman, P.E. and Gothard, K.M. (2011). Videos of conspecifics elicit interactive looking patterns and facial expressions in monkeys. *Behav. Neurosci.* 125 639-652. doi: 10.1037/a0024264

Nakao, S., Curthoys, I. S., and Markham, C. H. (1980). Direct inhibitory projection of pause neurons to nystagmus-related pontomedullary reticular burst neurons in the cat. *Exp. Brain Res.* 40, 283–293. doi: 10.1007/BF00237793

Ninomiya, T., Noritake, A., Kobayashi, K., and Isoda, M. (2020). A causal role for frontal cortico-cortical coordination in social action monitoring. *Nat. Commun.* 11:5233. doi: 10.1038/s41467-020-19026-y

Norris, K. S., and Schilt, C. R. (1988). Cooperative Societies in 3-Dimensional Space - on the Origins of Aggregations, Flocks, and Schools, with Special Reference to Dolphins and Fish. *Ethol. Sociobiol.* 9, 149–179.

Optican, L. M., and Pretegiani, E. (2017). What stops a saccade? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 372:20160194. doi: 10.1098/rstb.2016.0194

Range, F., Horn, L., Viranyi, Z., and Huber, L. (2009). The absence of reward induces inequity aversion in dogs. *Proc. Natl. Acad. Sci.U.S.A.* 106, 340–345. doi: 10.1073/pnas.0810957105

Schneier, F. R., Rodebaugh, T. L., Blanco, C., Lewin, H., and Liebowitz, M. R. (2011). Fear and avoidance of eye contact in social anxiety disorder. *Compr. Psychiatry* 52, 81–87. doi: 10.1016/j.comppsych.2010.04.006

Stander, P. E. (1992). Cooperative Hunting in Lions - the Role of the Individual. *Behav. Ecol. Sociobiol.* 29, 445–454. doi: 10.1002/ece3.7807

Stoeger, A. S., Mietchen, D., Oh, S., de Silva, S., Herbst, C. T., Kwon, S., et al. (2012). An Asian elephant imitates human speech. *Curr. Biol.* 22, 2144–2148. doi: 10.1016/j.cub.2012.09.022

Sugiuchi, Y., Izawa, Y., Takahashi, M., Na, J., and Shinoda, Y. (2005). Physiological characterization of synaptic inputs to inhibitory burst neurons from the rostral and caudal superior colliculus. *J. Neurophysiol.* 93, 697–712.

Szatmari, P., Chawarska, K., Dawson, G., Georgiades, S., Landa, R., Lord, C., et al. (2016). Prospective Longitudinal Studies of Infant Siblings of Children With Autism: Lessons Learned and Future Directions. *J. Am. Acad. Child Adolesc. Psychiatry* 55, 179–187. doi: 10.1016/j.jaac.2015.12.014

Takahashi, M., Sugiuchi, Y., and Shinoda, Y. (2014). Convergent synaptic inputs from the caudal fastigial nucleus and the superior colliculus onto pontine and pontomedullary reticulospinal neurons. *J. Neurophysiol.* 111, 849–867. doi: 10.1152/jn.00634.2013

Takahashi, M., Sugiuchi, Y., Izawa, Y., and Shinoda, Y. (2005). Synaptic inputs and their pathways from fixation and saccade zones of the superior colliculus to inhibitory burst neurons and pause neurons. *Ann. N.Y.Acad. Sci.* 1039, 209–219. doi: 10.1196/annals.1325.020

Trezza, V., Baarendse, P. J., and Vanderschuren, L. J. (2010). The pleasures of play: pharmacological insights into social reward mechanisms. *Trends Pharmacol. Sci.* 31, 463–469. doi: 10.1016/j.tips.2010.06.008

Tuber, D. S., Sanders, S., Hennessy, M. B., and Miller, J. A. (1996). Behavioral and glucocorticoid responses of adult domestic dogs (Canis familiaris) to companionship and social separation. *J. Comp. Psychol.* 110, 103–108. doi: 10.1037/0735-7036.110.1.103

Uono, S., and Hietanen, J. K. (2015). Eye contact perception in the West and East: a cross-cultural study. *PLoS One* 10:e0118094. doi: 10.1371/journal.pone.0118094

Wieser, M. J., Pauli, P., Alpers, G. W., and Muhlberger, A. (2009). Is eye to eye contact really threatening and avoided in social anxiety?–An eye-tracking and psychophysiology study. *J. Anxiety Disord.* 23, 93–103. doi: 10.1016/j.janxdis.2008.04.004

Yamamoto, S., Monosov, I. E., Yasuda, M., and Hikosaka, O. (2012). What and where information in the caudate tail guides saccades to visual objects. *J. Neurosci.* 32, 11005–11016. doi: 10.1523/JNEUROSCI.0828-12.2012

Yoshida, K., Iwamoto, Y., Chimoto, S., and Shimazu, H. (2001). Disynaptic inhibition of omnipause neurons following electrical stimulation of the superior colliculus in alert cats. *J. Neurophysiol.* 85, 2639–2642. doi: 10.1152/jn.2001.85.6.2639

Yoshida, K., McCrea, R., Berthoz, A., and Vidal, P. P. (1982). Morphological and physiological characteristics of inhibitory burst neurons controlling horizontal rapid eye movements in the alert cat. *J. Neurophysiol.* 48, 761–784. doi: 10.1152/jn.1982.48.3.761

Zhang, S., Liu, X., Yang, X., Shu, Y., Liu, N., Zhang, D., et al. (2021). The Influence of Key Facial Features on Recognition of Emotion in Cartoon Faces. *Front. Psychol.* 12:687974. doi: 10.3389/fpsyg.2021.687974

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 09 May 2022
doi: 10.3389/fnbeh.2022.817859

Activation of Estrogen Receptor β in the Lateral Habenula Improves Ovariectomy-Induced Anxiety-Like Behavior in Rats

Xiaofeng Liu[1], Meiying Song[1], Xiaowei Chen[2], Yanfei Sun[2], Renfei Fan[2], Liping Wang[2], Weihong Lin[1], Zheng Hu[1,3] and Hua Zhao[1,2]**

[1] Key Laboratory of Organ Regeneration and Transplantation of Ministry of Education, Neuroscience Research Center, The First Hospital of Jilin University, Changchun, China, [2] Department of Physiology, College of Basic Medical Sciences, Jilin University, Changchun, China, [3] National-Local Joint Engineering Laboratory of Animal Models for Human Diseases, Changchun, China

Background: Loss of estrogen due to menopause or ovarian resection is involved in the development of anxiety, which negatively impacts work productivity and quality of life. Estrogen modulates mood by binding to estrogen receptors in the brain. Estrogen receptor beta (ERβ) is highly expressed in the lateral habenula (LHb), a key site for controlling the activities of dopaminergic neurons in the ventral tegmental area (VTA) and serotoninergic neurons in the dorsal raphe nucleus (DRN) that are known to be involved in anxiety.

Methods: In this study, we examined the role of LHb in the anxiolytic-like effect of estrogen in ovariectomized (OVX) rats. The establishment of OVX anxiety model was validated in behavioral tests, including elevated plus maze (EPM) and mirror chamber maze (MCM) tasks. The expression of c-Fos in the LHb neurons was analyzed by immunohistochemistry, and monoamine neurotransmitter levels in related nuclei were analyzed using high-performance liquid chromatography (HPLC).

Results: Estrogen-treated OVX rats showed a lower degree of anxiety-like behavior than OVX rats. OVX rats showed anxiety-like behavior and low monoamine levels in the DRN and VTA compared with sham operated and estrogen-treated OVX rats. c-Fos expression in the LHb was higher than that in the sham operated and estrogen-treated OVX rats. Intra-LHb injection of the ERβ-selective agonist diarylprepionitrile (DPN) reduced expression of c-Fos (a neuronal activity marker) and anxiety-like behavior in OVX rats, but not in normal rats, as evidenced by increased time spent in EPM open areas and the MCM mirror chamber. These changes coincided with higher levels of serotonin and dopamine in the DRN and higher dopamine levels in the VTA in OVX rats receiving intra-LHb DPN compared with those receiving vehicle injection.

Conclusion: These results suggest that OVX-induced anxiety-like behavior may be associated with increased LHb activity. DPN may inhibit LHb activity to improve anxiety-like behavior in OVX rats by increasing monoamine neurotransmitter levels in the DRN and VTA.

Keywords: ovariectomy, estrogen, anxiety-like behavior, lateral habenula, c-Fos, estrogen receptor beta, monoamine neurotransmitters

OPEN ACCESS

Edited by:
Manuel Mameli, The University of Lausanne, Switzerland

Reviewed by:
Joaquin Piriz, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
Juan Francisco Rodríguez-Landa, Universidad Veracruzana, Mexico

***Correspondence:**
Zheng Hu
zhenghu@jlu.edu.cn
Hua Zhao
zhua@jlu.edu.cn

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *18 November 2021*
Accepted: *01 April 2022*
Published: *09 May 2022*

Citation:
Liu X, Song M, Chen X, Sun Y, Fan R, Wang L, Lin L, Hu Z and Zhao H (2022) Activation of Estrogen Receptor β in the Lateral Habenula Improves Ovariectomy-Induced Anxiety-Like Behavior in Rats. Front. Behav. Neurosci. 16:817859. doi: 10.3389/fnbeh.2022.817859

INTRODUCTION

Loss of estrogen during menopause or consequent ovarian resection can lead to endocrine disorders and neuropsychiatric symptoms, such as memory impairment, insomnia, anxiety, and depression symptoms (Schiller et al., 2016; Soares, 2019; Sovijit et al., 2019). Indeed, 38% of menopausal women reported experiencing anxiety-like symptoms and 36% reported experiencing depressive symptoms (Heidari et al., 2017). Women suffer from anxiety almost twice as much as men (Craske and Stein, 2016). Menopausal women generally have lower levels of monoamine neurotransmitters (Zárate et al., 2002), which may explain why medication (selective serotonin-reuptake inhibitors and serotonin–norepinephrine reuptake inhibitors) for anxiety disorders are effective in the treatment of menopause anxiety (Pinkerton, 2020). These disorders have a profoundly negative impact on quality of life (Craske and Stein, 2016), and it is of great clinical significance to investigate how altered levels of estrogen, in particular estradiol (E2), influence the pathogenesis of neuropsychiatric disorders. Such information will help develop specific therapies for menopausal anxiety disorder.

Studies have shown that anxiety-related behavior is associated with reduced levels of monoamine neurotransmitters, including serotonin (5-HT) and dopamine (DA), and the synthesis and metabolism of these neurotransmitters are influenced by estrogen. For example, ovariectomy-induced anxiety in rats is associated with monoamine levels (Pandaranandaka et al., 2006), and estrogen receptor beta (ERβ) knockout mice exhibit lower monoamine levels in the brain and anxiety symptoms (Krezel et al., 2001; Imwalle et al., 2005). Thus, monoamine neurotransmitters may play an important role in linking anxiety-like behaviors to the loss of estrogen signaling.

The lateral habenula (LHb), located in the epithalamus, is one of the few brain regions that control both dopaminergic and serotonergic systems, and has attracted great attention due to its critical role in regulating mood disorders (Hu et al., 2020). Habenular connectivity with the monoaminergic nervous system may predict treatment responses in depressive psychosis (Gosnell et al., 2019). The dorsal raphe nucleus (DRN) and ventral tegmental area (VTA), the key brain regions for the synthesis and release of 5-HT and DA, are controlled by the LHb (Lecourtier and Kelly, 2007; Zhao et al., 2015; Metzger et al., 2017, 2021). The LHb projects to dopaminergic neurons in the VTA and serotonergic neurons in the DRN (Lecourtier and Kelly, 2007; Baker et al., 2016) and excitation of the LHb inhibits firing of dopaminergic neurons in the VTA (Christoph et al., 1986) and serotonergic neurons in the DRN (Stern et al., 1979).

Anxiety-like behavior in postpartum mice, which is generated by removal of their pups, has been shown to be associated with increased Fos-immunoreactive cell counts in the LHb, suggesting an association between LHb activation and maternal anxiety-like behavior (Smith and Lonstein, 2008). Similarly, increased *c-fos* mRNA expression has been found in the habenula of anxious larval zebrafish (Chen et al., 2015). We previously reported that ovariectomized (OVX) rats had significantly increased *c-fos* mRNA and protein levels in the LHb, and elevated T-type calcium currents in the LHb (Li et al., 2015; Song et al., 2018). Together, these studies suggest that LHb neurons may be activated in an estrogen reduction-induced anxiety state.

Although enrichment of ERβ-expressing cells is found in the LHb (Shughrue et al., 1997; Mitra et al., 2003; Milner et al., 2010) but not in the medial habenula (Simerly et al., 1990; Wagner et al., 1998), the LHb has not been identified as a target of estrogen. In this study, using an OVX rat model, we tested whether estrogen exerts anti-anxiety effects by impacting the LHb and explored the underlying mechanisms involved. Anxiety behavior was assessed using an elevated plus maze (EPM) and a mirror chamber maze (MCM), and neuronal activity was assessed by immunohistochemistry. Monoamine neurotransmitters in the DRN and VTA were determined using an electrochemical high-performance liquid chromatography (HPLC) method to investigate this mechanism.

MATERIALS AND METHODS

Ethics Statement

All animal procedures were carried out in compliance with international ethical guidelines based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Council, 2011) and the Management and Use of Experimental Animals published by the National Science and Technology Foundation of China (He et al., 2016). The study was approved by the Committee on the Ethics of Animal Experiments of the First Hospital of Jilin University (ethical code: 2019024). Experimental rats were decapitated under anesthesia for brain sampling, and every effort was made to minimize the pain experienced by the animals.

Animals

Female Wistar rats (200–220 g) were purchased from Changchun Yisi Experimental Animal Technology Co., Ltd. The 105 animals were divided into different groups. The rats were housed (four rats per cage in the same experimental group) under controlled temperature, humidity, and light conditions with *ad libitum* access to food and water. The lights were programmed on a 12-h light/dark cycle (lights on at 7:00 a.m.). The rats were randomly divided into three groups: non-OVX rats (Sham + Oil group), OVX group (OVX + Oil group), and estrogen supplementation group (OVX + E2 group). Animals with intra-LHb injection of diarylprepionitrile (DPN) were randomly divided into four groups: non-OVX rats and OVX rats were injected with DPN in the LHb (Sham + DPN/OVX + DPN group) or an artificial cerebrospinal fluid (Sham + CSF/OVX + CFS) vehicle. The cannula administration groups were single-caged after cannula installation to allow a full recovery environment and to prevent the destruction of cannula from affecting subsequent experiments. The rats were subjected to fasting and water deprivation for 12 h prior to surgery. Animals were handled daily for at least 3 days prior to behavioral testing to reduce their stress during behavioral testing.

Drug and Microinjection

17β-Estradiol (E2) (180 μg/ml, E8875) was dissolved in sesame oil (S3547). E2 dosage and administration schedule were based on previous studies (Strom et al., 2008). The ERβ-selective agonist DPN (H5915) was dissolved in dimethyl sulfoxide (DMOS; D2650) as the stock solution and diluted in artificial CSF as a treatment solution (2 μg DPN/μl) (Walf and Frye, 2007; Bastos et al., 2015) to meet the physiological osmotic pressure requirements for brain injection. Rats were injected with the DPN treatment solution (DPN groups) or an equivalent dose of DMSO artificial CSF vehicle (CFS groups). All reagents were purchased from Sigma–Aldrich.

The rats were gently wrapped in a towel during drug microinjection. A dual injection tube connected with a polyethylene tubing (outer diameter (OD), 0.85 mm/inner diameter (ID), 0.42 mm) filled with solution was inserted into the dual guide cannula (OD, 0.48 mm/ID, 0.34 mm). Each polyethylene tube was connected to a 1-μl microsyringe and the solution was injected into the LHb (200 nl/side) using a syringe pump (LSP02-1B, Longer Precision Pump Co., Ltd., China). The needle was left in place for 1 min before injection. The injection lasted for 1 min, and the needle was left in the injection position for 1 min in order to fully diffuse the solution. Behavioral tests were conducted 10 min after the DPN injection (Campos et al., 2018).

Surgery Procedures

Rats were anesthetized with pentobarbital (intraperitoneal injection, 50 mg/kg). Body temperature was maintained using a thermostatically controlled electric heating pad. Bilateral ovariectomy was performed as previously described (Deurveilher et al., 2009) with minor modifications. OVX rats were administered with E2 to maintain normal E2 stimulation (Li et al., 2015). Under anesthesia, a longitudinal incision was made along the dorsal midline. The muscle layer was drawn from the abdomen through an incision using sterilized hemostatic forceps. The oviducts were clamped using hemostatic forceps and the ovaries were removed. The muscle layer was closed, and then silicone rubber capsules containing E2 dissolved in sesame oil (Dow Corning, United States, 1.57 mm inner diameter × 3.18 mm outer diameter; 20 mm in length), which had been sealed with Type A biogum and pre-incubated in sesame oil for 16 h, were implanted subcutaneously through the incision. Plain sesame oil capsules were implanted under the abdominal skin of the control rats. After surgery, the rats were returned to the housing facilities for 4 weeks until they were evaluated in the behavioral tests. The animals received intraperitoneal injections of 40,000 units of penicillin daily for 3 days.

Stereotaxic Surgery

Injection sites were determined using a rat brain atlas (Paxinos and Watson, 2007) and verified in a pre-experiment. The LHb injection site coordinates were: −3.5 mm (posterior to bregma), ± 0.7 mm (lateral of bregma), and 4.8 mm (ventral to bregma).

After anesthesia with pentobarbital (intraperitoneal injection, 50 mg/kg), when the rats showed no movement reaction to a foot pinch and had a stable breath rate, they were fixed in a stereotaxic instrument with a rat adaptor (RWD Life Science Co., Ltd., Shenzhen, China), with the head being held still with ear bars. The body temperature was maintained using a thermostatically controlled electric heating pad. The hair on top of the head was shaved with an infant hair clipper and the scalp skin was sterilized with povidone-iodine solution. The scalp was opened with a midline incision and the underlying skull was cleaned with saline to create a clear field. The adapter was adjusted to place the skull at the level position, and holes (diameter 0.6 mm) were drilled with a cranial drill. Dual guide cannula (OD 0.48 mm, ID 0.34 mm, RWD Life Science Co., Ltd., Shenzhen, China) were implanted aiming at the LHb (coordinates above), but the cannula tip was placed lowered to 4.6 mm ventral to the skull surface instead of 4.8 mm leaving space for the injector needle (OD 0.30 mm, ID 0.14 mm, RWD Life Science Co., Ltd., Shenzhen, China) to extend 0.2 mm beyond the tip of the guide cannula. The guide cannula was fixed to the skull surface by using screws and dental cement. The animals received daily intraperitoneal injections of 40,000 units of penicillin for 3 days to prevent infection. The location of the agonist or CFS injected into the LHb was identified by tracing the tip of inserted cannula. The cannula was inserted above the LHb and the inner tube was inserted into the LHb for the injection.

Immunohistochemistry

The rats were anesthetized with pentobarbital (intraperitoneal injection, 50 mg/kg), and their brains were isolated quickly and immersed in 10% formalin solution for 24 h, but for no more than a week. According to the position of the LHb, the brain was trimmed to contain the LHb and transected to a thickness of 3 mm. The brains were dehydrated, embedded in paraffin, and cut into 5 μm sections using a Leica microtome (RM2245). The sections were deparaffinized with xylene and rehydrated with gradient alcohol, and then treated with sodium citrate at 95°C for 15 min of antigen repair and washed with phosphate-buffered saline (PBS, 0.01 M) for three times. An immunehistochemical kit was used according to the manufacturer's protocol (MX, Fuzhou, China; catalog no: KIT- 9710). Sections were then incubated with anti-c-Fos (1:1,000, Abcam, Cambridge, United Kingdom, catalog no: ab190289) at 4°C overnight. After completing the staining steps according to the kit instructions, the sections were placed in a color-developing agent (MXB; Fuzhou, China; catalog no: DAB-0031) and the color reaction was terminated with 0.01 M PBS when the color reached an appropriate saturation, and the sections were sealed with neutral resin. The sections were examined under a light microscope (Olympus IX71), and c-Fos immunopositive neurons were counted as an index of neuronal excitability.

Behavior

The experimental rats were subjected to behavioral tests in numbered order, regardless of the group. Behavioral experiments were performed between 5:00 and 7:00 p.m.

Elevated Plus Maze

The EPM is a behavioral test used to assess anxiety in rodents by exploiting the conflict between their natural urge to explore and their aversion to an elevated open environment (Montgomery, 1955; Handley and Mithani, 1984). Our plus-maze apparatus was a cross-shaped black iron plate 70 cm above the ground, consisting of two pairs of opposite-facing arms at right angles to each other. Both arms in one opposite-facing pair were enclosed by 22.5-cm-high iron plate walls with a low light level (10 lx). The other two sides were not enclosed by walls (open arms) and were exposed to moderately bright light (140 lx). Each arm was 15 cm wide and 42.5 cm long, excluding the center 15 cm × 15 cm intersection region of the plus maze.

During the EPM experiment, rats were placed in the middle of the arm intersection. Each animal was monitored for 5 min. A trial was considered invalid if the rat left the maze. The time spent in the maze and the number of entries into the open arms were used as anxiety index parameters. All parameters were measured using a video tracker (EthoVision XT, Noldus, Netherlands). The anxiety index was calculated as follows: 1 - [(open arm time/test duration) + (open arm entries/total entries)]/2. Anxiety index values range from 0 to 1, with a higher anxiety index indicating greater anxiety (Cohen et al., 2008a,b; Mazor et al., 2009). After each trial, the maze was cleaned thoroughly with 75% alcohol to prevent smell interference between the trials.

Mirror Chamber Maze

A mirror chamber was used to assess anxious behavior based on the idea that conflict avoidance behaviors are triggered in animals when they see their reflections. The MCM apparatus, which consisted of an open field chamber (76 cm × 57 cm × 35 cm) with four mirrored walls connected to a non-mirrored alleyway (57 cm × 12.5 cm × 35 cm) (Walf et al., 2009), is a modified version of the original mouse apparatus (Toubas et al., 1990).

The rats were placed individually in a mirror chamber, and their movements were recorded for 5 min. A shorter duration of time spent in the mirrored part of the chamber was considered indicative of anxiety. A central area was drawn between the mirror and alley to allow the rats to choose whether to enter the mirror or alley. The anxiety index (modified from our EPM anxiety index) was calculated as 1 −[(Time in the mirror chamber/test duration) + (entries in the mirror chamber/total entries)]/2. The anxiety index values ranged from 0 to 1, with a higher anxiety index indicating greater anxiety. After each trial, the maze was cleaned thoroughly with 75% alcohol to prevent smell interference between the trials.

High-Performance Liquid Chromatography

Rats were decapitated under deep anesthesia (pentobarbital, 50 mg/kg, intraperitoneal injection) for brain sampling. For the acquisition of brain tissues, rats were anesthetized and decapitated 10 min after DPN or CSF was injected into the LHb. The brain tissue sample needed for separation in the cerebrospinal fluid on ice was stored in a −80℃ freezer for later use. Monoamine neurotransmitter concentrations were determined using reverse-phase HPLC coupled with an electrochemical detector (Waters 2,465; Waters Corporation, United States). Each brain tissue sample was weighed, placed in 0.1 M perchloric acid, and then homogenized in a pre-sterilized pestle (PES-15-B-SI, Axygen). The homogenates were subjected to ultrasonication for 10 s (cycle 0.5, amplitude 40%), placed on ice for 30 min, and centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were filtered through a membrane (0.22 μm) and used immediately for neurotransmitter measurement or stored at −80°C for later use. The monoamine neurotransmitters 5-HT and DA were separated on a Sunfire® C18 column, with a mobile phase containing 120 mM disodium hydrogen orthophosphate (pH 3.2), 250 mg/L sodium octane sulfonate, 80 mg/L EDTA-2Na, 2 mM KCl, and 16% (v/v) methanol. Standard curves were used to quantify the neurotransmitter levels in each sample by calculating the area under the curve. The measured monoamine neurotransmitter concentrations in the brain homogenate were corrected for tissue weight and expressed as ng/mg.

Statistical Analysis

Rats were randomly assigned to groups. Analyses were performed in a manner blinded to the treatment assignments in all behavioral experiments. Statistical analysis was performed using Prism 8 (GraphPad Software, San Diego, CA, United States). Values were excluded if the drug delivery sites were outside the LHb (pre-established criteria). Statistical differences were determined using one-way analysis of variance (ANOVA) for more than three groups. Data that failed the normality test were analyzed using the Kruskal-Wallis test. When the results of ANOVAs were significant, Tukey's multiple-comparison *post-hoc* test was conducted. Results are presented as mean ± standard error (SEM), and group sizes are indicated in the figure legends, with "n" representing the number of animals. Correlations between behavioral variables and neurotransmitters in all experimental groups were investigated using Pearson's correlation.

RESULTS

Estrogen Supplementation Alleviated Ovariectomy-Induced Anxiety-Like Behavior

To study the behavior of rats after ovariectomy, we divided the rats into three groups and tested the elevated plus maze and mirror chamber maze after the different treatments (**Figure 1A**). **Figure 1B** showed the movement tracks of the elevated plus maze of rats. In the EPM, the results showed that compared with the Sham + Oil group, OVX + Oil rats spent less time in the open arms ($p < 0.01$; **Figure 1C**), more time in the closed arms ($p < 0.05$; **Figure 1C**), and showed a higher anxiety index ($p < 0.05$; **Figure 1E**). The OVX + E2 group had a lower anxiety index than the OVX + Oil group ($p < 0.05$; **Figure 1E**), which improved this anxiety state. There was no statistical difference in the number of open and closed arm explorations among the three groups (**Figure 1D**).

The movement tracks in mirror chamber maze are shown in **Figure 1F**. Compared to the Sham + Oil group, the OVX + Oil group spent significantly less time exploring the mirror chamber ($p < 0.05$; **Figure 1G**) and had fewer entries into the mirror chamber ($p < 0.05$; **Figure 1H**), showing a higher mean anxiety index value ($p < 0.05$; **Figure 1I**). The OVX + E2 group spent more time exploring the mirror chamber and had a lower anxiety index than the OVX + Oil group, which improved this anxiety state.

Estrogen Supplementation Alleviated Ovariectomy-Induced the Activation of c-Fos Expression and Changes in Neurotransmitters

Immunohistochemistry results showed that the number of c-Fos in the LHb increased significantly in the OVX + Oil group compared to that in the Sham + Oil group ($p < 0.05$, **Figures 2A,B**). c-Fos expression in the OVX + E2 group was significantly lower than that in the OVX + Oil group ($p < 0.05$, **Figures 2A,B**).

Results of the HPLC analysis showed that OVX + Oil group had significantly lower levels of 5-HT ($p < 0.05$) and marginally lower DA ($p = 0.06$) in the DRN compared with the Sham + Oil group. The 5-HT and DA levels in the VTA were significantly lower than those in the Sham + Oil group ($p < 0.05$ for 5-HT and DA). Compared with the OVX + Oil group, the OVX + E2 group showed higher levels of 5-HT and DA ($p < 0.01$) in the DRN and DA ($p < 0.05$) in the VTA (**Figure 2D**), indicating that the reduction in monoamine neurotransmitters may be improved after estrogen supplementation (**Figures 2C,D**).

Intra-Lateral Habenula Diarylprepionitrile Attenuated Anxiety-Like Behavior in Ovariectomized Rats

To test whether estrogen could improve anxiety-like behavior in OVX rats through the LHb, we investigated behavioral changes in OVX rats by injecting DPN into the LHb. A schematic of the experimental design (**Figure 3A**), and anxiety behavioral data obtained from the LHb-microinjected OVX rats are shown in **Figure 3**. In the EPM, compared with OVX + CSF rats, OVX + DPN rats spent significantly more time in the open arms and less time in the closed arms ($p < 0.05$; **Figure 3C**), had significantly more open arm entries ($p < 0.01$) and closed arm entries ($p < 0.05$; **Figure 3D**), and had a significantly reduced mean EPM anxiety index value ($p < 0.05$; **Figure 3E**). Compared with the OVX + CSF rats, the OVX + DPN rats spent significantly more time in the mirror chamber ($p < 0.01$; **Figure 3G**), showed significantly more entries into the mirror chamber ($p < 0.05$; **Figure 3H**), and had a significantly reduced mean MCM anxiety index value ($p < 0.05$; **Figure 3I**). There was no significant difference between the Sham + CFS group and the Sham + DPN group in any behavioral change.

Intra-Lateral Habenula Injection of Diarylprepionitrile Can Reduce c-Fos Expression in the Lateral Habenula and Increase Monoamine Levels in the Dorsal Raphe Nucleus and Ventral Tegmental Area of Ovariectomized Rats

The locations for microinjection of DPN and CSF to the LHb in the OVX rats are shown in **Figure 4A**. We quantitatively evaluated the number of c-Fos positive cells in LHb after DPN was injected to the LHb. Immunohistochemical images suggested that the number of c-Fos cells in the LHb was reduced in response to DPN microinjection into the LHb (**Figure 4B**). Marked differences were detected in the LHb. There was a decrease in the number of c-Fos in the LHb of rats in the OVX + DPN group when compared to OVX + CFS group ($p < 0.01$; **Figure 4C**) suggesting that DPN reduced neuronal excitability. There was no change in the number of c-Fos in the LHb following CFS and DPN injections in sham rats.

In the DRN, the 5-HT ($p < 0.01$; **Figure 4D**) and DA ($p < 0.05$; **Figure 4E**) concentrations were significantly higher in the OVX + DPN group than in the OVX + CSF group. In the VTA, DA concentrations were significantly higher in the OVX + DPN group than in the OVX + CSF group ($p < 0.05$; **Figure 4E**). CFS and DPN injections in sham rats did not change the concentrations of DRN or VTA neurotransmitters.

Correlation Between Behavioral Variables and Neurotransmitters in the Ventral Tegmental Area and Dorsal Raphe Nucleus

We analyzed the correlations between the behavioral variables and the levels of monoamine neurotransmitters in the VTA and DRN of OVX + Oil and OVX + E2 groups (**Table 1**) and intra-LHb injection of OVX + CFS and OVX + DPN groups (**Table 2**). In the systemic estrogen experimental groups, Pearson's correlation coefficient indicated that DA in the VTA and 5-HT in the DRN were significantly and positively correlated with the time spent on the open arms in the EPM and the time/entries in the mirror chamber, but a significant negative correlation was found with the anxiety index in the EPM/MCM and the time spent on the closed arms in the EPM. In the MCM, the DA in the DRN is positively correlated with the exploration into the mirror and negatively correlated with the anxiety index. Correlations similar to those found in the systemic administration groups were also observed in the OVX + CFS and OVX + DPN groups.

DISCUSSION

It has been reported that the loss of estrogen can induce anxiety behavior, and its pathogenesis involves abnormal function of the estrogen β receptor. The LHb plays an important role in the development of depression and anxiety (Metzger et al., 2021) and

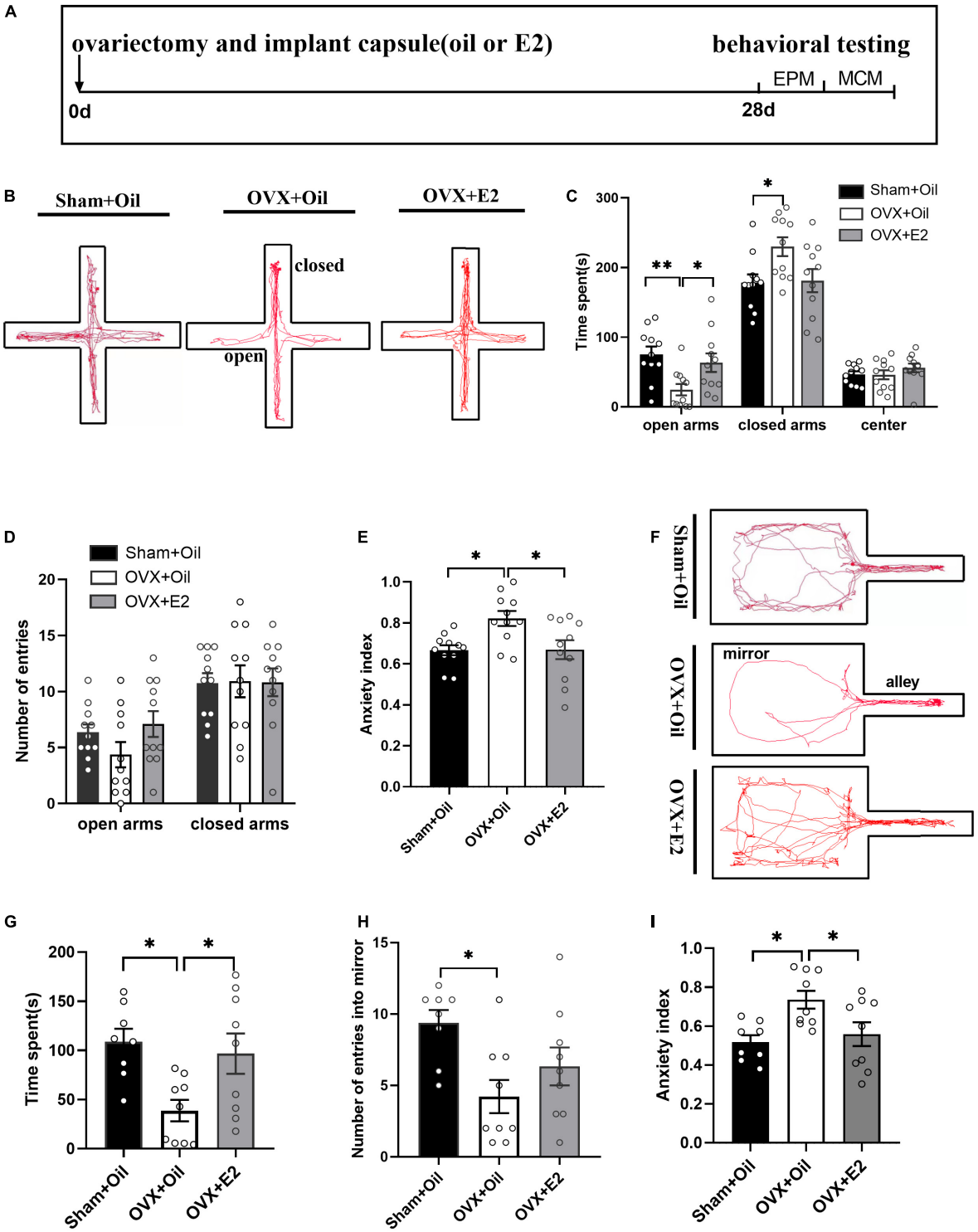

FIGURE 1 | E2 supplementation alleviates anxiety behavior in OVX rats. The timeline of experimental design **(A)**, trajectory maps of EPM **(B)**, times spent in the open arms, closed arms, and center **(C)**, numbers of open-arm entries **(D)**, and mean EPM anxiety index values **(E)** were compared between the Sham + Oil (n = 11), OVX + Oil (n = 11), and OVX + E2 (n = 11) groups during a 5-min EPM test period. The trajectory maps of MCM **(F)**, times spent in the mirror chamber **(G)**, numbers of mirror chamber entries **(H)**, and mean MCM anxiety index values **(I)** were compared among the Sham + Oil (n = 8), OVX + Oil (n = 9), and OVX + E2 (n = 9) groups. *$p < 0.05$, **$p < 0.01$.

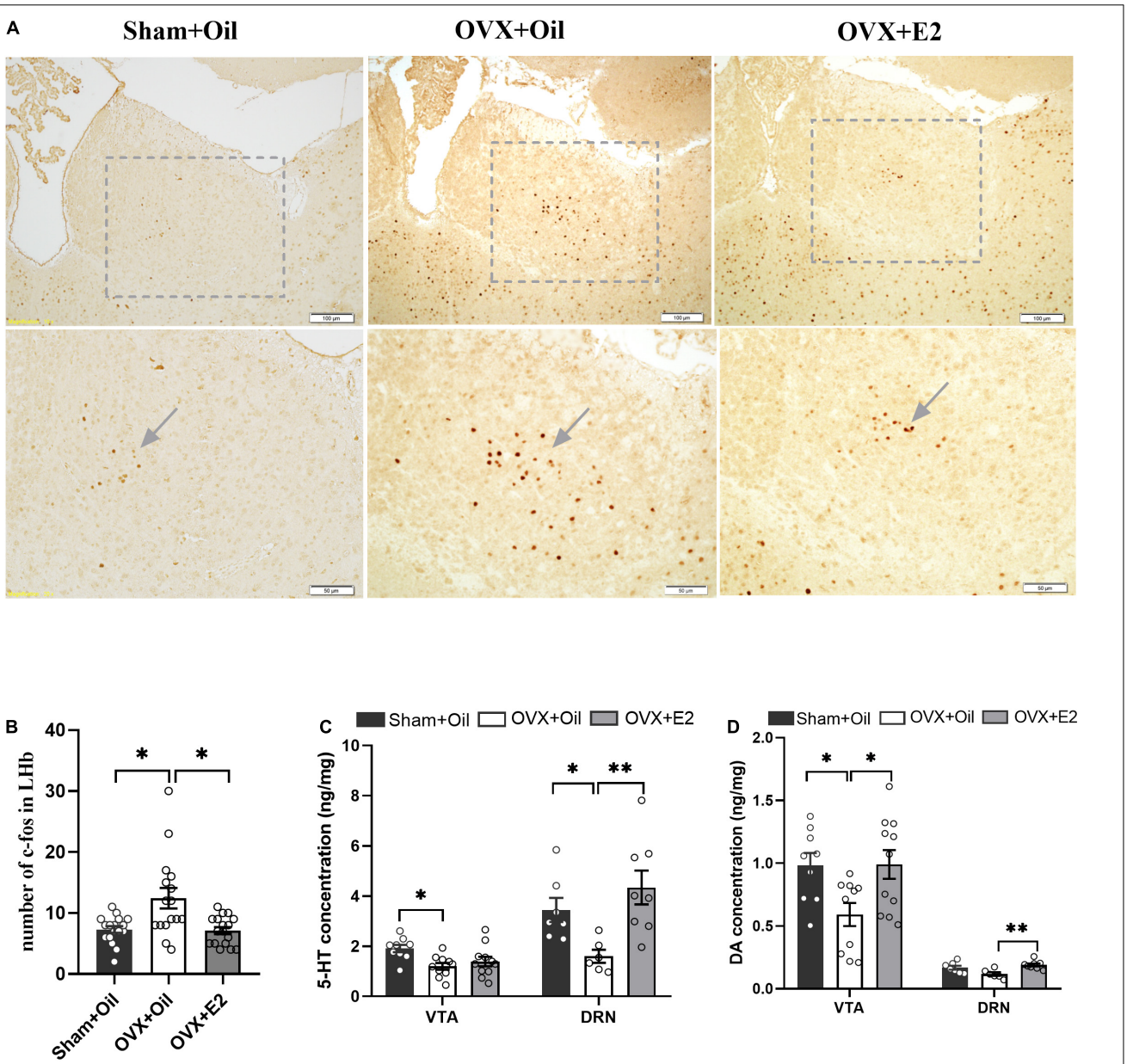

FIGURE 2 | Systemic administration of estrogen reduces c-Fos expression in the LHb and increases the concentrations of monoamine neurotransmitters in the DRN and VTA of OVX rats. The representative images of c-Fos expression in the Sham + Oil, OVX + Oil and OVX + E2 rats **(A)**. Systemic administration of estrogen induced decreased density of c-Fos cells in the LHb. Data are presented as the quantitative analysis of c-Fos positive neurons in the Sham + Oil, OVX + Oil, and OVX + E2 groups **(B)**. Comparison of 5-HT **(C)** and DA **(D)** concentrations in the VTA and DRN among the Sham + Oil (n = 9, 7), OVX + Oil (n = 10, 6), and OVX + E2 (n = 11, 8) groups. *$p < 0.05$, **$p < 0.01$.

has a large distribution of estrogen β receptors (Milner et al., 2010). However, it is still unclear whether LHb mediates the anti-anxiety effects of estrogen.

In the present study, we showed that ovariectomy-induced anxiety behavior could be attenuated with E2 supplementation, as evidenced by improved performance in the exploration of the open spaces of the EPM and the mirror chamber of the MCM. The LHb showed increased c-Fos expression in ovariectomized rats, and these changes were reversed by estrogen supplementation. We further found that intra-LHb injection of the ERβ-selective agonist DPN in OVX rats reduced the number of c-Fos positive cells and increased monoamine neurotransmitter levels in the DRN and VTA, suggesting that reduced neuronal activity of DPN in the LHb may relieve anxiety-like behavior induced by the loss of estrogen, possibly through increasing the levels of monoamine neurotransmitters in the DRN and VTA. The significant correlations between the behavioral variables and the levels of

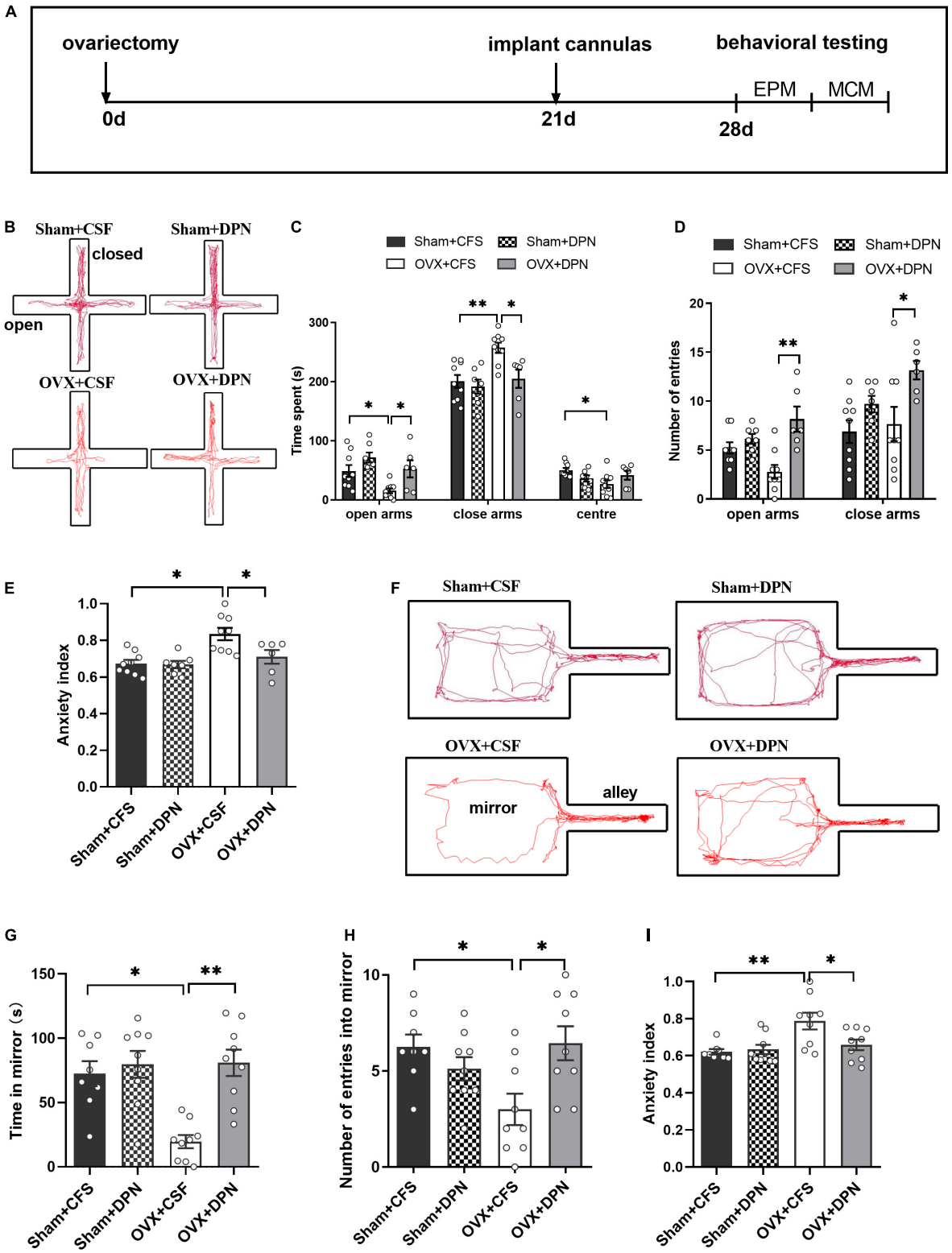

FIGURE 3 | Intra-LHb injection of DPN improves anxiety behavior in sham and OVX rats. Timeline of experimental design **(A)**. The trajectory maps of EPM **(B)**, times spent in the open arms, closed arms, and center **(C)**, numbers of open-arm entries **(D)**, and mean EPM anxiety index values **(E)** were compared among the Sham + CFS (n = 9), Sham + DPN (n = 7), OVX + CFS (n = 9), and OVX + DPN (n = 6) groups during a 5-min EPM test. The trajectory maps of MCM **(F)**, times spent in the mirror chamber **(G)**, numbers of mirror chamber entries **(H)**, and mean MCM anxiety index values **(I)** were compared between the Sham + CFS (n = 8), Sham + DPN (n = 9), OVX + CFS (n = 9), and OVX + DPN (n = 9) groups. *$p < 0.05$; **$p < 0.01$.

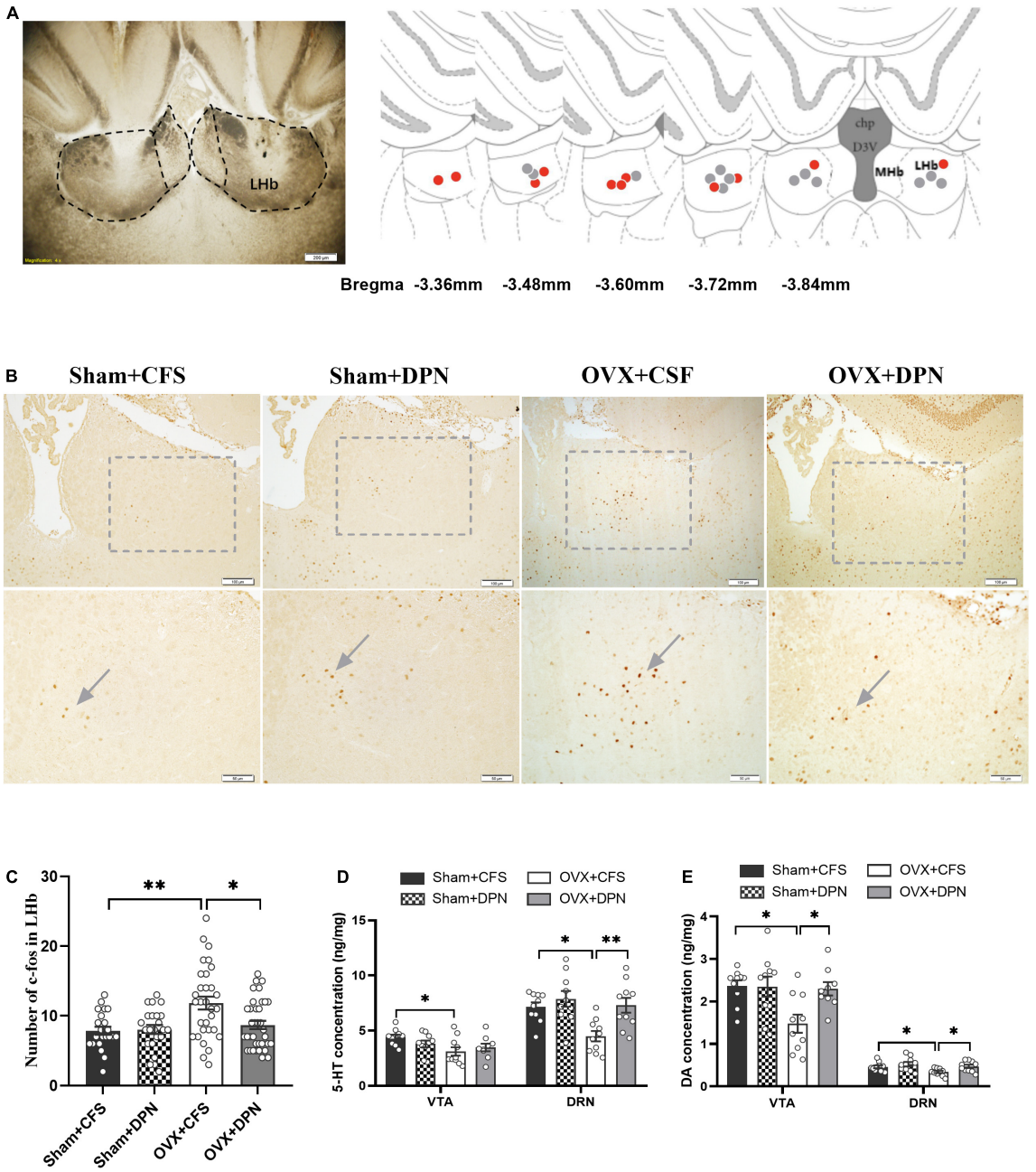

FIGURE 4 | Intra-LHb injection of DPN reduces c-Fos expression in the LHb and increases concentrations of monoamine neurotransmitters in the DRN and VTA of the Sham and OVX rats. Location of the cannula in the LHb (**A**, left). Coronal sections are 3.36–3.84 mm posterior to bregma. Red and gray circles represent injection sites in the LHb of OVX + DPN and OVX + CFS rats in the LHb, respectively (**A**, right). The representative images of c-Fos expression in the Sham + CFS, Sham + DPN, OVX + CSF, and OVX + DPN groups **(B)**. Intra-LHb injection of DPN induced decreased in the density of c-Fos cells in LHb **(C)**. Data are presented as quantitative analysis of c-Fos positive neurons in the LHb. Comparison of 5-HT **(D)** and DA concentrations **(E)** in the VTA and DRN among the Sham + CFS (n = 10, 10), Sham + DPN (n = 10, 10), OVX + CSF (n = 10, 10), and OVX + DPN (n = 9, 10) groups. *$p < 0.05$; **$p < 0.01$.

monoamine neurotransmitters in the VTA and DRN in all intra-LHb injections of DPN experimental groups further supports the above hypothesis.

We conducted animal behavioral experiments 4 weeks after ovariectomy in rats. The rats with 4 weeks of ovariectomy exhibited anxious behavior in the EPM and MCM tasks, and estrogen supplementation improved the anxious behavior. These results are consistent with previous reports showing that loss of estrogen (Pandaranandaka et al., 2006) or OVX rats exhibited increased anxiety-like behaviors in the light-dark shuttle box and locomotor activity tests (Fedotova et al., 2017; Puga-Olguín et al., 2019). Thus, we are confident that, in this study, the model

TABLE 1 | Corrections between behavioral variables and neurotransmitters in the VTA and DRN of OVX + Oli and OVX + E2 groups.

Behavioral variables	VTA				DRN			
	DA (ng/mg)		5-HT (ng/mg)		DA (ng/mg)		5-HT (ng/mg)	
	r	*p*	R	*p*	*r*	*p*	*r*	*p*
Elevated plus maze								
Open arms (s)	0.694	0.006	0.276	0.338	0.117	0.689	0.718	0.004
Closed arm (s)	–0.624	0.017	–0.266	0.358	–0.088	0.762	–0.625	0.017
Entries open arms (*n*)	0.472	0.088	0.258	0.372	–0.164	0.574	0.465	0.093
Entries closed arms (*n*)	–0.192	0.509	0.079	0.788	–0.301	0.295	–0.315	0.272
Anxiety index	–0.690	0.006	–0.232	0.424	–0.097	0.741	–0.738	0.003
Mirror chamber maze								
Mirror (s)	0.592	0.026	0.190	0.515	0.548	0.042	0.671	0.009
Entries mirror (*n*)	0.760	0.002	0.468	0.091	0.430	0.124	0.585	0.028
Anxiety index	–0.627	0.016	–0.300	0.297	–0.607	0.021	–0.650	0.012

TABLE 2 | Corrections between behavioral variables and neurotransmitters in the VTA and DRN of OVX + CFS and OVX + DPN groups.

Behavioral variables	VTA				DRN			
	DA (ng/mg)		5-HT (ng/mg)		DA (ng/mg)		5-HT (ng/mg)	
	r	*p*	R	*p*	*r*	*p*	*r*	*p*
Elevated plus maze								
Open arms (s)	0.586	0.022	–0.109	0.699	0.188	0.502	0.671	0.006
Closed arm (s)	–0.660	0.007	0.011	0.968	–0.320	0.230	–0.733	0.002
Entries open arms (*n*)	0.590	0.020	0.044	0.876	0.384	0.157	0.668	0.007
Entries closed arms (*n*)	0.414	0.124	–0.121	0.666	0.391	0.146	0.557	0.030
Anxiety index	–0.560	0.030	–0.144	0.607	–0.298	0.281	–0.576	0.024
Mirror chamber maze								
Mirror (s)	0.688	0.005	–0.137	0.624	0.491	0.063	0.782	0.001
Entries mirror (*n*)	0.655	0.008	–0.089	0.751	0.592	0.020	0.689	0.004
Anxiety index	–0.670	0.006	–0.093	0.740	–0.541	0.037	–0.647	0.009

(s), time in seconds; (n), number; r, Pearson's correlation coefficient; VTA, ventral tegmental area; DRN, dorsal raphe nucleus; DA, dopamine; 5-HT, serotonin.

was successful, and the results generated from using this model should be reliable.

The LHb in OVX rats showed an increased expression of c-Fos, which was inhibited by estrogen supplementation, which was consistent with our previous results showing increased c-Fos expression in the LHb 1 week after ovariectomy (Li et al., 2015). We previously demonstrated that estrogen supplementation not only reversed the increase in c-Fos expression in the LHb of OVX rats but also reversed the expression of Cav3.3 T-type calcium channels and T-type calcium currents in the LHb of OVX rats (Song et al., 2018), which is critical for regulating neuronal excitability (Arbogast, 2008). More importantly, estradiol suppressed spontaneous firing activity in the LHb neurons compared with firing rates prior to estradiol treatment using whole-cell recording (Song et al., 2018). These results suggest that ovariectomy-induced anxiety behavior may be related to changes in LHb neuronal activity mediated by T-type calcium channels and that the LHb may be a potential target for estradiol action.

In the present study, we found that the ERβ-selective agonist DPN microinjected into the LHb of OVX rats alleviated ovariectomy-induced anxiety behavior, as evidenced by improved performance in the exploration of the open arms of the EPM and the mirror chamber of the MCM. DPN also decreased c-Fos expression in the LHb of OVX rats and increased monoamine neurotransmitter levels in the DRN and the VTA. Estrogen regulates brain function by affecting the release of monoamine neurotransmitters (Pandaranandaka et al., 2006; Long et al., 2018). In the present study, rats showing anxiety-like behavior following ovariectomy had lower levels of 5-HT/DA in the DRN and DA in the VTA. 5-HT and DA, which play important roles in anxiety-like behaviors (Liu et al., 2018; Schanzer et al., 2019), are synthesized and released into the DRN and VTA, respectively. It has been suggested A decrease in dopaminergic neuronal activity in the VTA may be the basis of anxiety-related behavior (Coque et al., 2011; Rincón-Cortés et al., 2018; Rincon-Cortes and Grace, 2020) and the inhibition of 5-HT neurons in the DRN could induce anxiety-related behaviors in mice and rats (Nishitani et al., 2019). Correlation analysis showed that DA in the VTA and 5-HT

in the DRN were correlated with behavioral variables in the EPM and MCM in all systemic estrogen experimental groups, which supports the hypothesis that the DRN and VTA are involved in the development of anxiety-like behavior.

The LHb plays an important role in the pathogenesis of anxiety and depression (Yang et al., 2008; Zhao et al., 2015) and is a key site for controlling neuronal activity in the DRN and VTA. Activation of the LHb inhibits neuronal activity in the DRN and VTA. OVX rats exhibited hyperactivity of neurons in the LHb in the present and previous studies (Li et al., 2015). Therefore, we hypothesized that OVX-induced anxiety-like behavior may be attributed to increased neuronal activity of the LHb, leading to reduced neuronal activity of the DRN and VTA, which reduces the release of 5-HT and DA neurotransmitters. DPN microinjected into the LHb of OVX rats suppressed the activity of LHb neurons, as evidenced by decreased c-Fos expression and increased levels of 5-HT and DA in the DRN and DA in the VTA. We suggest that DPN in the LHb suppresses its activity, which weakens the inhibitory effect on the DRN and VTA, leading to elevated DRN and VTA neuronal activity and alleviation of anxiety-like behavior.

Ovariectomy has been reported to reduce gamma-aminobutyric acid (GABA) levels and $GABA_A$ receptor expression in the hippocampus, amygdala, and other brain structures (Herbison and Fénelon, 1995; Tominaga et al., 2001). Binding of estrogen to receptors can increase the synthesis of the inhibitory neurotransmitter GABA in the brain (McCarthy, 2008). Therefore, we hypothesized that DPN injection into the LHb may activate GABA neurons and thus inhibit the neuronal activity of the LHb neurons, leading to improved anxiety behavior in ovariectomized rats.

It has been reported that c-Fos expression decreases in the lateral septal nucleus of ovariectomized rats and is restored when E2 is administered to ovariectomized rats (Puga-Olguín et al., 2019). About 75% of GABAergic neurons in the septal nucleus project to the LHb and inhibit its activity (Golden et al., 2016). Therefore, we hypothesized that the reduced activity of GABA neurons projected from the lateral septal nucleus to the LHb weakened its inhibitory effect on the LHb leading to increased LHb activity and further inhibition of the activity of 5-HT neurons in the DRN and DA neurons in the VTA, which may be involved in the development of ovariectomy-induced anxiety behavior. There is a morphological and functional connection between VTA and DRN neurons, thus the interaction between them during ovariectomy-induced anxiety behavior needs to be further studied.

In summary, estrogen loss can cause anxiety-related behaviors, and the LHb, which highly expresses ERβ, appears to be associated with the development of anxiety-related behaviors. In this study, we demonstrated the effects of estrogen deficiency induced by ovariectomy in rats, including increased anxiety-like behaviors, as well as decreased levels of 5-HT and DA in the DRN and DA in the VTA. It was further found that systemic estrogen supplementation or local ERβ agonism within the LHb could improve anxiety-like behavior and reverse estrogen-deficiency-induced decreases in monoamine neurotransmitter levels of the DRN and VTA. These results support our hypothesis that the LHb plays an important role in anxiety associated with estrogen deficiency owing to its influence on 5-HT and DA levels in the DRN and DA levels in the VTA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics of Animal Experiments of the First Hospital of Jilin University.

AUTHOR CONTRIBUTIONS

XL conducted the experiments and wrote the manuscript. MS and XC assisted in conducting the experiments. YS, RF, LW, and WL assisted in preparing materials for the experiment. ZH reviewed and edited the manuscript. HZ designed the study, supervised all aspects of the study, and wrote the manuscript. All authors approved the final version of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (32171145) and the Program for JLU Science and Technology Innovative Research Team (JLUSTIRT:2017TD-30).

ACKNOWLEDGMENTS

We gratefully acknowledge Li Jicheng and Zhang Zifeng in the college of Basic Medical Sciences of Jilin University for their technical assistance in conducting the experiments.

REFERENCES

Arbogast, L. A. (2008). Estrogen genomic and membrane actions at an intersection. *Trends Endocrinol. Metab.* 19, 1–2. doi: 10.1016/j.tem.2007.10.002

Baker, P. M., Jhou, T., Li, B., Matsumoto, M., Mizumori, S. J., Stephenson-Jones, M., et al. (2016). The lateral habenula circuitry: reward processing and cognitive control. *J. Neurosci.* 36, 11482–11488. doi: 10.1523/JNEUROSCI.2350-16.2016

Bastos, C. P., Pereira, L. M., Ferreira-Vieira, T. H., Drumond, L. E., Massensini, A. R., Moraes, M. F. D., et al. (2015). Object recognition memory deficit and depressive-like behavior caused by chronic ovariectomy can be transitorialy recovered by the acute activation of hippocampal estrogen receptors. *Psychoneuroendocrinology* 57, 14–25. doi: 10.1016/j.psyneuen.2015.03.020

Campos, G. V., de Noronha, S. R., de Souza, A. A., Lima, P. M., Abreu, A. R., Chianca-Jr, D., et al. (2018). Estrogen receptor beta activation within dorsal

raphe nucleus reverses anxiety-like behavior induced by food restriction in female rats. *Behav. Brain Res.* 357-358, 57–64. doi: 10.1016/j.bbr.2018.03.029

Chen, F., Chen, S., Liu, S., Zhang, C., and Peng, G. (2015). Effects of lorazepam and WAY-200070 in larval zebrafish light/dark choice test. *Neuropharmacology* 95, 226–233. doi: 10.1016/j.neuropharm.2015.03.022

Christoph, G. R., Leonzio, R. J., and Wilcox, K. S. (1986). Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rat. *J Neurosci.* 6, 613–619. doi: 10.1523/JNEUROSCI.06-03-00613.1986

Cohen, H., Geva, A. B., Matar, M. A., Zohar, J., and Kaplan, Z. (2008a). Post-traumatic stress behavioural responses in inbred mouse strains: can genetic predisposition explain phenotypic vulnerability? *Int. J. Neuropsychopharmacol.* 11, 331–349. doi: 10.1017/S1461145707007912

Cohen, H., Matar, M. A., Buskila, D., Kaplan, Z., and Zohar, J. (2008b). Early post-stressor intervention with high-dose corticosterone attenuates posttraumatic stress response in an animal model of posttraumatic stress disorder. *Biol. Psychiatry* 64, 708–717. doi: 10.1016/j.biopsych.2008.05.025

Coque, L., Mukherjee, S., Cao, J. L., Spencer, S., Marvin, M., Falcon, E., et al. (2011). Specific role of VTA dopamine neuronal firing rates and morphology in the reversal of anxiety-related, but not depression-related behavior in the ClockDelta19 mouse model of mania. *Neuropsychopharmacology* 36, 1478–1488. doi: 10.1038/npp.2011.33

Council, N. R. (2011). *Guide for the Care and Use of Laboratory Animals*, 8 Edn. Washington, DC: The National Academies Press.

Craske, M. G., and Stein, M. B. (2016). Anxiety. *Lancet* 388, 3048–3059. doi: 10.1016/s0140-6736(16)30381-6

Deurveilher, S., Rusak, B., and Semba, K. (2009). Estradiol and progesterone modulate spontaneous sleep patterns and recovery from sleep deprivation in ovariectomized rats. *Sleep* 32, 865–877.

Fedotova, J., Pivina, S., and Sushko, A. (2017). Effects of chronic vitamin D(3) hormone administration on anxiety-like behavior in adult female rats after long-term ovariectomy. *Nutrients* 9:28. doi: 10.3390/nu9010028

Golden, S. A., Heshmati, M., Flanigan, M., Christoffel, D. J., Guise, K., Pfau, M. L., et al. (2016). Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature* 534, 688–692. doi: 10.1038/nature18601

Gosnell, S. N., Curtis, K. N., Velasquez, K., Fowler, J. C., Madan, A., Goodman, W., et al. (2019). Habenular connectivity may predict treatment response in depressed psychiatric inpatients. *J. Affect. Disord.* 242, 211–219. doi: 10.1016/j.jad.2018.08.026

Handley, S. L., and Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch. Pharmacol.* 327, 1–5. doi: 10.1007/BF00504983

He, Z. M., Li, G. P., Zhu, D. S., and Lu, S. M. (2016). *Guidelines for the Management and Use of Laboratory Animals*. Beijing: Science Press.

Heidari, M., Ghodusi, M., and Rafiei, H. (2017). Sexual self-concept and its relationship to depression, stress and anxiety in postmenopausal women. *J. Menopausal. Med.* 23, 42–48. doi: 10.6118/jmm.2017.23.1.42

Herbison, A. E., and Fénelon, V. S. (1995). Estrogen regulation of GABAA receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. *J. Neurosci.* 15(3 Pt 2), 2328–2337. doi: 10.1523/jneurosci.15-03-02328.1995

Hu, H., Cui, Y., and Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nat. Rev. Neurosci.* 21, 277–295. doi: 10.1038/s41583-020-0292-4

Imwalle, D. B., Gustafsson, J. A., and Rissman, E. F. (2005). Lack of functional estrogen receptor beta influences anxiety behavior and serotonin content in female mice. *Physiol. Behav.* 84, 157–163. doi: 10.1016/j.physbeh.2004.11.002

Krezel, W., Dupont, S., Krust, A., Chambon, P., and Chapman, P. F. (2001). Increased anxiety and synaptic plasticity in estrogen receptor beta -deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12278–12282. doi: 10.1073/pnas.221451898

Lecourtier, L., and Kelly, P. H. (2007). A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. *Neurosci. Biobehav. Rev.* 31, 658–672. doi: 10.1016/j.neubiorev.2007.01.004

Li, C.-Y., Song, M.-Y., Huang, M. I. N., Li, J.-C., Xiao, J.-Y., and Zhao, H. U. A. (2015). Estradiol suppresses neuronal firing activity and c-Fos expression in the lateral habenula. *Mol. Med. Rep.* 12, 4410–4414. doi: 10.3892/mmr.2015.3942

Liu, Y., Zhao, J., and Guo, W. (2018). Emotional roles of mono-aminergic neurotransmitters in major depressive disorder and anxiety disorders. *Front. Psychol.* 9:2201. doi: 10.3389/fpsyg.2018.02201

Long, T., Yao, J. K., Li, J., Kirshner, Z. Z., Nelson, D., Dougherty, G. G., et al. (2018). Comparison of transitional vs surgical menopause on monoamine and amino acid levels in the rat brain. *Mol. Cell. Endocrinol.* 476, 139–147. doi: 10.1016/j.mce.2018.05.003

Mazor, A., Matar, M. A., Kaplan, Z., Kozlovsky, N., Zohar, J., and Cohen, H. (2009). Gender-related qualitative differences in baseline and post-stress anxiety responses are not reflected in the incidence of criterion-based PTSD-like behaviour patterns. *World J. Biol. Psychiatry* 10(4 Pt 3), 856–869. doi: 10.1080/15622970701561383

McCarthy, M. M. (2008). Estradiol and the developing brain. *Physiol. Rev.* 88, 91–124. doi: 10.1152/physrev.00010.2007

Metzger, M., Bueno, D., and Lima, L. B. (2017). The lateral habenula and the serotonergic system. *Pharmacol. Biochem. Behav.* 162, 22–28. doi: 10.1016/j.pbb.2017.05.007

Metzger, M., Souza, R., Lima, L. B., Bueno, D., Goncalves, L., Sego, C., et al. (2021). Habenular connections with the dopaminergic and serotonergic system and their role in stress-related psychiatric disorders. *Eur. J. Neurosci.* 53, 65–88. doi: 10.1111/ejn.14647

Milner, T. A., Thompson, L. I., Wang, G., Kievits, J. A., Martin, E., Zhou, P., et al. (2010). Distribution of estrogen receptor beta containing cells in the brains of bacterial artificial chromosome transgenic mice. *Brain Res.* 1351, 74–96. doi: 10.1016/j.brainres.2010.06.038

Mitra, S. W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H. A., Hayashi, S., et al. (2003). Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. *Endocrinology* 144, 2055–2067. doi: 10.1210/en.2002-221069

Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory behavior. *J. Comp. Physiol. Psychol.* 48, 254–260. doi: 10.1037/h0043788

Nishitani, N., Nagayasu, K., Asaoka, N., Yamashiro, M., Andoh, C., Nagai, Y., et al. (2019). Manipulation of dorsal raphe serotonergic neurons modulates active coping to inescapable stress and anxiety-related behaviors in mice and rats. *Neuropsychopharmacology* 44, 721–732. doi: 10.1038/s41386-018-0254-y

Pandaranandaka, J., Poonyachoti, S., and Kalandakanond-Thongsong, S. (2006). Anxiolytic property of estrogen related to the changes of the monoamine levels in various brain regions of ovariectomized rats. *Physiol. Behav.* 87, 828–835. doi: 10.1016/j.physbeh.2006.02.002

Paxinos, G., and Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates*, 6th Edn. Amsterdam: Elsevier.

Pinkerton, J. V. (2020). Hormone therapy for postmenopausal women. *N. Engl J. Med.* 382, 446–455. doi: 10.1056/NEJMcp1714787

Puga-Olguín, A., Rodríguez-Landa, J. F., Rovirosa-Hernández, M. J., Germán-Ponciano, L. J., Caba, M., Meza, E., et al. (2019). Long-term ovariectomy increases anxiety- and despair-like behaviors associated with lower Fos immunoreactivity in the lateral septal nucleus in rats. *Behav. Brain Res.* 360, 185–195. doi: 10.1016/j.bbr.2018.12.017

Rincon-Cortes, M., and Grace, A. A. (2020). Postpartum changes in affect-related behavior and VTA dopamine neuron activity in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 97:109768. doi: 10.1016/j.pnpbp.2019.109768

Rincón-Cortés, M., Gagnon, K. G., Dollish, H. K., and Grace, A. A. (2018). Diazepam reverses increased anxiety-like behavior, social behavior deficit, and dopamine dysregulation following withdrawal from acute amphetamine. *Neuropsychopharmacology* 43, 2418–2425. doi: 10.1038/s41386-018-0123-8

Schanzer, B., Rivas-Grajales, A. M., Khan, A., and Mathew, S. J. (2019). Novel investigational therapeutics for generalized anxiety disorder (GAD). *Expert Opin. Investig. Drugs* 28, 1003–1012. doi: 10.1080/13543784.2019.1680638

Schiller, C. E., Johnson, S. L., Abate, A. C., Schmidt, P. J., and Rubinow, D. R. (2016). Reproductive Steroid Regulation of Mood and Behavior. *Compr. Physiol.* 6, 1135–1160. doi: 10.1002/cphy.c150014

Shughrue, P. J., Lane, M. V., and Merchenthaler, I. (1997). Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J. Comp. Neurol.* 388, 507–525. doi: 10.1002/(sici)1096-9861(19971201)388:4<507::aid-cne1>3.0.co;2-6

Simerly, R. B., Chang, C., Muramatsu, M., and Swanson, L. W. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an *in situ* hybridization study. *J. Comp. Neurol.* 294, 76–95. doi: 10.1002/cne.902940107

Smith, C. D., and Lonstein, J. S. (2008). Contact with infants modulates anxiety-generated c-fos activity in the brains of postpartum rats. *Behav. Brain Res.* 190, 193–200. doi: 10.1016/j.bbr.2008.02.030

Soares, C. N. (2019). Depression and menopause: an update on current knowledge and clinical management for this critical window. *Med. Clin. North Am.* 103, 651–667. doi: 10.1016/j.mcna.2019.03.001

Song, M. Y., Li, C. Y., Liu, X. F., Xiao, J. Y., and Zhao, H. (2018). Effect of 17beta-oestradiol on T-type calcium channels in the lateral habenula. *J. Neuroendocrinol.* 30:e12629. doi: 10.1111/jne.12629

Sovijit, W. N., Sovijit, W. E., Pu, S., Usuda, K., Inoue, R., Watanabe, G., et al. (2019). Ovarian progesterone suppresses depression and anxiety-like behaviors by increasing the *Lactobacillus* population of gut microbiota in ovariectomized mice. *Neurosci. Res.* 168, 76–82. doi: 10.1016/j.neures.2019.04.005

Stern, W. C., Johnson, A., Bronzino, J. D., and Morgane, P. J. (1979). Effects of electrical stimulation of the lateral habenula on single-unit activity of raphe neurons. *Exp. Neurol.* 65, 326–342. doi: 10.1016/0014-4886(79)90102-x

Strom, J. O., Theodorsson, E., and Theodorsson, A. (2008). Order of magnitude differences between methods for maintaining physiological 17beta-oestradiol concentrations in ovariectomized rats. *Scand. J. Clin. Lab. Invest.* 68, 814–822. doi: 10.1080/00365510802409703

Tominaga, K., Yamauchi, A., Shuto, H., Niizeki, M., Makino, K., Oishi, R., et al. (2001). Ovariectomy aggravates convulsions and hippocampal gamma-aminobutyric acid inhibition induced by cyclosporin A in rats. *Eur. J. Pharmacol.* 430, 243–249. doi: 10.1016/s0014-2999(01)01377-2

Toubas, P. L., Abla, K. A., Cao, W., Logan, L. G., and Seale, T. W. (1990). Latency to enter a mirrored chamber: a novel behavioral assay for anxiolytic agents. *Pharmacol. Biochem. Behav.* 35, 121–126. doi: 10.1016/0091-3057(90)90215-4

Wagner, C. K., Silverman, A. J., and Morrell, J. I. (1998). Evidence for estrogen receptor in cell nuclei and axon terminals within the lateral habenula of the rat: regulation during pregnancy. *J. Comp. Neurol.* 392, 330–342. doi: 10.1002/(sici)1096-9861(19980316)392:3<330::aid-cne4>3.0.co;2-2

Walf, A. A., and Frye, C. A. (2007). Administration of estrogen receptor beta-specific selective estrogen receptor modulators to the hippocampus decrease anxiety and depressive behavior of ovariectomized rats. *Pharmacol. Biochem. Behav.* 86, 407–414. doi: 10.1016/j.pbb.2006.07.003

Walf, A. A., Paris, J. J., and Frye, C. A. (2009). Chronic estradiol replacement to aged female rats reduces anxiety-like and depression-like behavior and enhances cognitive performance. *Psychoneuroendocrinology* 34, 909–916. doi: 10.1016/j.psyneuen.2009.01.004

Yang, L. M., Hu, B., Xia, Y. H., Zhang, B. L., and Zhao, H. (2008). Lateral habenula lesions improve the behavioral response in depressed rats *via* increasing the serotonin level in dorsal raphe nucleus. *Behav. Brain Res.* 188, 84–90. doi: 10.1016/j.bbr.2007.10.022

Zárate, A., Fonseca, E., Ochoa, R., Basurto, L., and Hernández, M. (2002). Low-dose conjugated equine estrogens elevate circulating neurotransmitters and improve the psychological well-being of menopausal women. *Fertil. Steril.* 77, 952–955. doi: 10.1016/s0015-0282(02)03077-7

Zhao, H., Zhang, B. L., Yang, S. J., and Rusak, B. (2015). The role of lateral habenula-dorsal raphe nucleus circuits in higher brain functions and psychiatric illness. *Behav. Brain Res.* 277, 89–98. doi: 10.1016/j.bbr.2014.09.016

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership