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A neural pathway controlling motivation to exert effort

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The neural mechanisms conferring reduced motivation, as observed in depressed individuals, is poorly understood. Here, we examine in rodents if reduced motivation to exert effort is controlled by transmission from the lateral habenula (LHb), a nucleus overactive in depressed-like states, to the rostromedial tegmental nucleus (RMTg), a nucleus that inhibits dopaminergic neurons. In an aversive test wherein immobility indicates loss of effort, LHb→RMTg transmission increased during transitions into immobility, driving LHb→RMTg increased immobility, and inhibiting LHb→RMTg produced the opposite effects. In an appetitive test, driving LHb→RMTg reduced the effort exerted to receive a reward, without affecting the reward's hedonic property. Notably, LHb→RMTg stimulation only affected specific aspects of these motor tasks, did not affect all motor tasks, and promoted avoidance, indicating that LHb→RMTg activity does not generally reduce movement but appears to carry a negative valence that reduces effort. These results indicate that LHb→RMTg activity controls the motivation to exert effort and may contribute to the reduced motivation in depression.

lateral habenula | rostromedial tegmental nucleus | motivation | optogenetics | fiber photometry

Depressive disorders cause significant morbidity and mortality in the human population (1). A number of potentially aberrant neural mechanisms have been characterized, which may reflect the multiplicity of depressive symptoms (2–9). While recent studies in humans (10–14) and rodents (15–23) suggest that excessive lateral habenula (LHb) activity may contribute to depression, the impact of LHb hyperactivity on an individual's level of motivation has not been examined.

Motivation can be defined as the propensity of an organism to exert effort to move toward a rewarding, or away from an aversive, stimulus (24). The amount of effort an individual exerts to achieve a goal is believed to depend on a complex calculation of the cost required to perform a defined action and the perceived benefit gained from that action (24). Maladaptive dysfunction in neural pathways encoding such information (e.g., if the cost of performing an action is overvalued or if the perceived benefit is undervalued) can lead to behavioral deficits such as the reduced motivation seen in depression (25, 26). The specific neural pathways underlying such motivational deficits in depression are unknown.

The LHb, a predominantly glutamatergic nucleus, receives inputs from several limbic nuclei associated with motivational states (27–31). It provides a major disynaptic inhibitory output, through the midbrain GABAergic rostromedial tegmental nucleus (RMTg) to monoaminergic centers (32). In particular, the RMTg transmits reward-related signals from the LHb to dopamine neurons, which are suggested to play a central role in reinforcing or discouraging ongoing action (33, 34). LHb→RMTg signals have been shown to promote active, passive, and conditioned behavioral avoidance (35). However, the relation between these signals and motivation has not been examined.

It is notable that monoaminergic output has been associated with increased motivated behavior and positive affective states (36, 37). Increasing dopaminergic cell activity in the ventral tegmental area increases motivated behavioral responses in a challenging task, and their negative modulation decreases such responses (38). The impact of reduced mesolimbic system activity has been characterized as an inflation in the perceived cost of exerting effort, leading to immobility (24). We thus reasoned that the LHb→RMTg pathway, by inhibiting monoaminergic centers, could control motivated behavior; in particular, we hypothesized that this pathway controls the motivation to exert effort.

Results

LHb→RMTg Activity Increases with Transitions to Immobility in the Forced Swim Test. To examine LHb→RMTg activity in a behaving animal, the LHb of rats was injected with an adeno-associated virus (AAV) encoding the calcium indicator GCaMP6s (AAV-hSyn-GCaMP6s). An optical fiber was implanted over the RMTg to measure the activity of axon terminals from LHb→RMTg (Fig. 1*A*, Fig. S1, and *SI Experimental Procedures*). Control rats received the same surgery but were injected with an AAV encoding GFP (AAV-hSyn-GFP). Four weeks later, GCaMP6s expression was detected in cell bodies of the LHb and at the axon terminals of those fibers in the RMTg (Fig. 1*A*). Changes in fluorescence, indicating changes in neural activity (39), were recorded with a custom-built fiber photometry system (*SI Experimental Procedures* and ref. 40). To assess motivation, rats were subjected to the forced swim test (FST) (41), an aversive inescapable environment in which a rat's effort is indicated by the persistence of its movement (Fig. 1*B*). Over the course of the test, rats spend a larger fraction of time immobile. These periods of immobility can be used as a measure of reduced motivation in rodents (29, 38, 42). The rats' behavior was captured using a digital camera, and immobility bouts were determined using an

Significance

The lateral habenula, a brain region that has been implicated in depression, receives inputs from brain nuclei associated with basic emotions and drives. In this report, using fiber photometry and optogenetics on behaving rats, we show that one major lateral habenula output pathway controls the motivation to exert effort in both aversive and appetitive contexts. Overactivity of this pathway could contribute to the reduced motivation seen in human depression.

Author contributions: C.D.P., S.A., and R.M. designed research; C.D.P., S.A., D.M., C.M., A.L., and S.J.S. performed research; C.D.P., S.A., and S.J.S. contributed new reagents/analytic tools; C.D.P., S.A., B.M., and R.M. analyzed data; and C.D.P., S.A., and R.M. wrote the paper.

Reviewers: O.H., NIH; and B.L., Cold Spring Harbor Laboratory.

Conflict of interest statement: C.D.P., R.M., and Okihide Hikosaka are coauthors on a review article published in 2014. S.A. founded the company Neurophotometrics Ltd., which manufactures fiber photometry systems.

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Data deposition: All relevant data and code supporting the findings of this study have been deposited on GitHub and are available at https://github.com/NotAnHerb/LHbRMTg.

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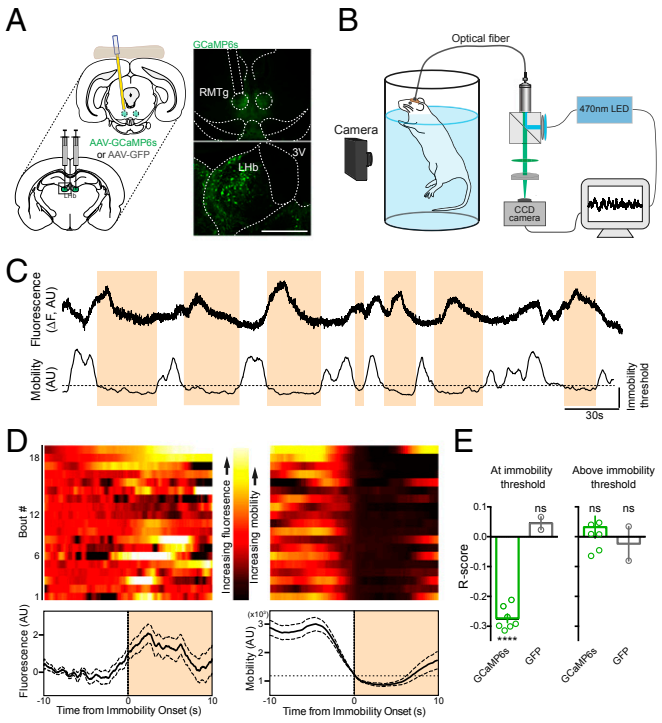

Fig. 1. Activity of LHb→RMTg is coincident with immobility bouts in the FST. (*A*, *Left*) AAV encoding GCaMP6s were injected into LHb, and a 400-μm optical fiber was implanted over the RMTg. (*A*, *Right*) Diagram and fluorescence images. (Scale bar, 500 μm.) 3V, third ventricle. (*B*) Diagram of recording setup. Video and CCD cameras captured rat mobility and changes in fluorescence in RMTg, respectively. (*C*) Representative example of change in fluorescence (*Top*) and mobility (*Bottom*) during FST; immobility threshold is indicated. (*D*) Representative examples (*Top*) and mean ± SEM (*Bottom*) of change in fluorescence (*Left*) and mobility (*Right*), aligned to onset of immobility bout. (*E*) Graph of correlation (Pearson's) between fluorescence and mobility for rats (circles indicate individuals; bar indicates mean) expressing indicated constructs, aligned about the onset of immobility bouts (*Left*) or during periods of mobility (*Right*). ****$P < 0.0001$. AU, arbitrary unit; ns, not significant.

unbiased MATLAB script that was validated against human scorers (Fig. S2 and *SI Experimental Procedures*).

The onset of immobility bouts coincided with increased fluorescence signal (Fig. 1 *C* and *D*), as indicated by a significant negative correlation between the rat's movement about the onset of an immobility bout and the neural activity of the LHb→RMTg (Fig. 1*E*, *Left*; $r = -0.27 \pm 0.01$, $P < 0.0001$; rat $n = 7$, test $n = 12$, bout $n = 298$; see *SI Experimental Procedures* for measurements and statistical tests). Such correlation was not observed in rats injected with AAV-GFP ($r = 0.05 \pm 0.02$; rat $n = 2$, test $n = 5$, bout $n = 167$). When the same analysis was made at periods of mobility (above threshold), no correlation was measured in rats expressing GCaMP6s (Fig. 1*E*, *Right*; $r = 0.03 \pm 0.04$, $P > 0.05$; rat $n = 7$, test $n = 12$, bout $n = 424$) or GFP ($r = -0.02 \pm 0.06$, $P > 0.05$; rat $n = 2$, test $n = 5$, bout $n = 101$). These findings support the view that increased neural activity in the LHb→RMTg correlates with reduced motivation to exert effort in an aversive context; furthermore, this correlation is not generally related to movement, as it occurs only during specific periods (i.e., at immobility threshold, when an animal would be expected to have low motivation).

Driving LHb→RMTg Increases Immobility in the FST. To test the hypothesis that the activity of the LHb→RMTg is sufficient to reduce the motivation to exert effort, the LHb was injected with an AAV encoding a light-dependent excitatory opsin, oChIEF (43), fused to the red fluorescent protein tdTomato (AAV-hSyn-oChIEF-tdTomato) or a control fluorophore (AAV-hSyn-tdTomato), and an optical fiber was implanted over the RMTg (Fig. 2*A* and Fig. S3). Single-unit recordings in anesthetized rats confirmed that brief, 5-ms pulses of blue light (~15 mW at optical fiber tip) delivered at 25 Hz was sufficient to drive postsynaptic activity in RMTg neurons (Fig. S4).

Rats were subjected to 20-min FST sessions, as with the fiber photometry recordings, with each session consisting of alternating 2-min epochs with and without unilateral 25-Hz optical stimulation (Fig. 2*B*). Stimulation of the LHb→RMTg was sufficient to decrease a rat's mobility during 2-min epochs, compared with the average mobility of the preceding and proceeding nonstimulation epochs [Fig. 2*C*; 3,300 ± 100 significant motion pixels (smp) (44)] without light vs. 2,700 ± 100 smp with light, $P < 0.001$). Notably, during stimulation periods, an animal's average mobility (when the animal was above the immobility threshold) was unaffected (Fig. 2*D*; 3,500 ± 100 smp without light vs. 3,400 ± 100 smp with light, $P > 0.05$). This suggests that stimulation did not affect a rat's general ability to move. Rather, the overall effect of stimulation on mobility was mostly due to an increase in the fraction of time a rat spent below the mobility threshold (Fig. 2*E*; 0.15 ± 0.01 without light vs. 0.32 ± 0.02 with light, $P < 0.001$). We observed that these immobility events occurred more often (18 ± 3 events without light vs. 33 ± 1 events with light, $P < 0.001$) and were longer in duration (3.7 ± 0.6 s without light vs. 5.6 ± 0.3 s with light, $P < 0.05$) during activation of the LHb→RMTg. Stimulation had no significant effect on the mobility of control rats expressing tdTomato (Fig. 2 *C–E*; mobility: 3,300 ± 70 smp without light vs. 3,300 ± 80 smp with light, $P > 0.05$; mobility above threshold: 3,600 ± 60 smp without light vs. 3,600 ± 80 smp with light, $P > 0.05$; fraction immobility: 0.19 ± 0.01 without light vs. 0.17 ± 0.02 with light, $P > 0.05$), with no change in the number (30 ± 4 events without light vs. 26 ± 3 events with light, $P > 0.05$) or the duration (3.4 ± 0.3 s without light vs. 7 ± 3 s with light, $P > 0.05$) of immobility events. These results support the view that stimulation of the LHb→RMTg neural pathway is sufficient to reduce motivation to exert effort in an aversive context.

Inhibiting LHb→RMTg Reduces Immobility in the FST. To test the hypothesis that activity in the LHb→RMTg is necessary to drive immobility in the same aversive swim test, rats were injected in the LHb with an AAV encoding the light-dependent proton pump eArch3.0 fused to a yellow fluorescent protein (AAV-hSyn-eArch3.0-eYFP) or with GFP (AAV-EF1α-GFP) as a control. Optical fibers (200-μm diameter) were implanted over the RMTg (Fig. 3*A* and Fig. S5). As above, rats were examined during a 20 min swim session with alternating 2-min epochs of no light and constant green light. When light was applied, a rat's mobility in an epoch with light was significantly higher than the average mobility of the flanking epochs without light (Fig. 3 *B* and *C*; 3,620 ± 90 smp without light vs. 3,770 ± 90 smp with light, $P < 0.05$). Furthermore, light delivery decreased the fraction of time the rat spent immobile (Fig. 3*E*; 0.25 ± 0.02 without light vs. 0.17 ± 0.02 with light, $P < 0.01$). We observed that these immobility events occurred less often (33 ± 1 events without light vs. 22 ± 2 events with light, $P < 0.05$) and were shorter in duration (4.7 ± 0.2 s without light vs. 3.8 ± 0.1 s with light, $P < 0.05$) when light was delivered to the RMTg. No significant change was observed in rats expressing GFP (Fig. 3 *C–E*; mobility: 3,360 ± 90 smp without light vs. 3,400 ± 100 smp with light, $P > 0.05$; mobility above threshold: 3,710 ± 80 smp without light vs. 3,700 ± 100 smp with light, $P > 0.05$; fraction immobility: 0.21 ± 0.02 without light vs. 0.20 ± 0.01 with light, $P > 0.05$), with no change in the number of events (33 ± 2 events without light vs. 31 ± 4 events with light, $P > 0.05$) or their duration (3.8 ± 0.3 s without light vs. 3.4 ± 0.2 s with light, $P > 0.05$).

These results are generally opposite compared with those observed with blue light activation of oChIEF, supporting the views that green light delivery to eArch3.0-expressing terminals inhibits their activity (45, 46) and that LHb→RMTg activity is necessary to produce the normal level of immobility in the aversive context of the FST.

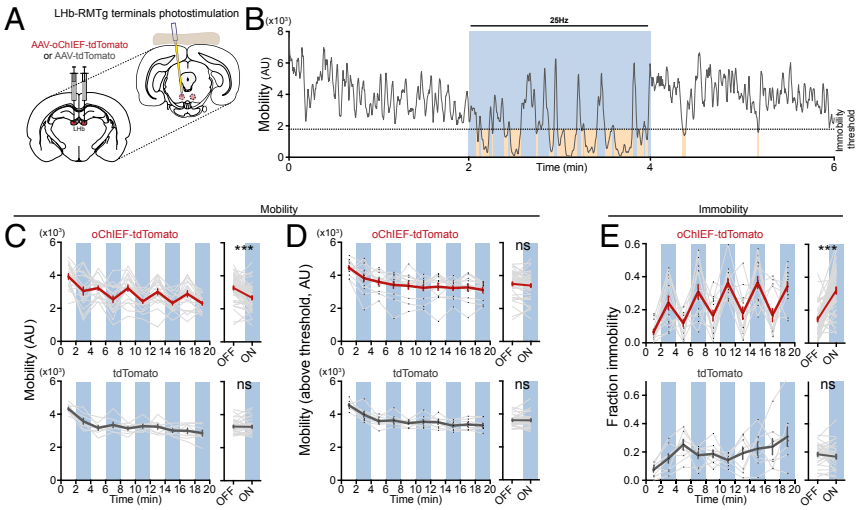

Fig. 2. Stimulation of LHb→RMTg increases immobility in FST. (*A*) AAV encoding the light-sensitive cation channel oChIEF-tdTomato ($n = 13$) (or tdTomato alone, $n = 7$) was injected into the LHb, and a 200-μm optical fiber was implanted over the RMTg. (*B*) Representative example of change in mobility during light delivery (blue); brown shading indicates periods of immobility. (*C–E*, *Top*): (*C*, *Left*) Plot of mean mobility (gray indicates individual rats; red indicates mean ± SEM) during periods of light (blue) or no light (white). (*C*, *Right*) Mean ± SEM for indicated periods. (*D*) Same as *C*, for mobility values above immobility threshold. (*E*) Same as *C*, for time spent immobile. (*C–E*, *Bottom*) Same as *C–E*, *Top*, for rats expressing tdTomato. ***$P < 0.001$; paired Student's *t* test. AU, arbitrary unit; ns, not significant.

Activating LHb→RMTg Reduces Effort Exerted to Gain Rewards in an Appetitive Test. To examine whether increased activity at the LHb→RMTg was sufficient to decrease motivation in an appetitive context, rats were tested in a progressive ratio (PR) operant task, which is commonly used to evaluate motivation in rodents (47). In this test, increasing work (more lever presses) is required to receive a reward as trials proceed. The maximal work a rat exerts to receive a reward, the breaking point (BP), is used as a measure of its motivation (Fig. 4*A*).

We trained rats injected and implanted as in Fig. 2 to press a lever to obtain a sucrose reward. After training, we tested rats with a PR schedule of reinforcement (*SI Experimental Procedures*). In alternating sessions (one session per day), rats were or were not exposed to blue light through an optical fiber (trains of 25 Hz for 1 s every 2 s) during the entire session. With stimulation, rats' BPs were significantly reduced by more than 40% compared with nonstimulation sessions (Fig. 4 *B*, *i*; BP without light 35 ± 4 vs. BP with light 21 ± 3, $P < 0.001$). This indicates that driving LHb→RMTg activity is sufficient to reduce the work performed by a rat to receive a reward. LHb→RMTg stimulation significantly increased the time between receiving a reward and the subsequent lever press (Fig. 4 *B*, *ii*; 36 ± 5 s without light vs. 67 ± 12 s with light, $P < 0.05$). Interestingly, during a bout of lever presses before a reward, the time interval between lever presses was unaffected (Fig. 4 *B*, *iii*; 0.85 ± 0.04 s without light vs. 0.88 ± 0.05 s with light, $P > 0.05$), suggesting that once a threshold motivation to work is achieved, the vigor of a rat's performance is not modified. Stimulation did not affect a rat's preference for sucrose water over plain water [sucrose preference test (SPT)], indicating that the hedonic value of the reward is unaffected by stimulation (Fig. 4*C*; 84 ± 4% of water consumed contained sucrose without light vs. 73 ± 6% with light, $P > 0.05$). Additionally, LHb→RMTg activation did not reduce thirst as revealed by the total liquid consumed (Fig. 4*C*; 0.037 ± 0.002 g of liquid per gram of body weight without light vs. 0.032 ± 0.003 g of liquid per gram of body weight with light, $P > 0.05$). These results indicate that the motivation to exert effort to receive a reward, rather than the value of the reward or the ability to perform the task, is affected by LHb→RMTg activation.

Activating LHb→RMTg During an Open Field Test. We next tested animals in the open field (OF) (38), normally used to determine if a manipulation has nonspecific effects on movement. Rats expressing oChIEF-tdTomato or the control fluorophore tdTomato were placed in an OF, and movement was monitored during a 10-min period during which alternating 2-min epochs with and without unilateral 25-Hz optical stimulation were delivered (Fig. 5). When light was applied, a rat's mobility in an epoch with light was significantly lower than that of the flanking epochs without light (Fig. 5). Both low and high levels of movement were affected

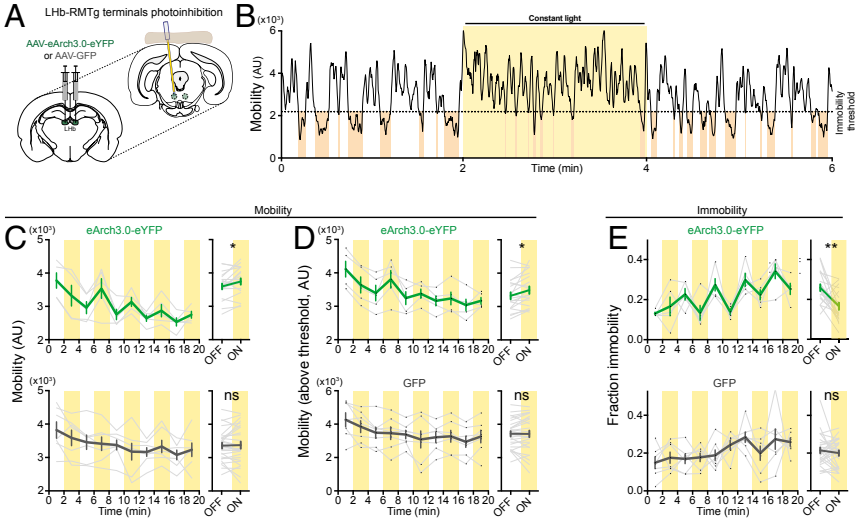

Fig. 3. Reducing LHb→RMTg activity decreases immobility in FST. (*A*) AAV encoding the hyperpolarizing proton pump eArch3.0 ($n = 5$) or GFP ($n = 7$) was injected into the LHb, and a 200-μm optical fiber was implanted over the LHb. (*B*) Representative example of change in mobility during light delivery (yellow); brown shading indicates periods of immobility. (*C–E*, *Top*): (*C*, *Left*) Plot of mean mobility (gray indicates individual rats; green indicates mean ± SEM) during periods of light (yellow) or no light (white). (*C*, *Right*) Mean ± SEM for indicated periods. (*D*) Same as *C*, for mobility values above immobility threshold. (*E*) Same as *C*, for time spent immobile. (*C–E*, *Bottom*) Same as *C–E*, *Top*, for rats expressing GFP. *$P < 0.05$, **$P < 0.01$; paired Student's *t* test. AU, arbitrary unit; ns, not significant.

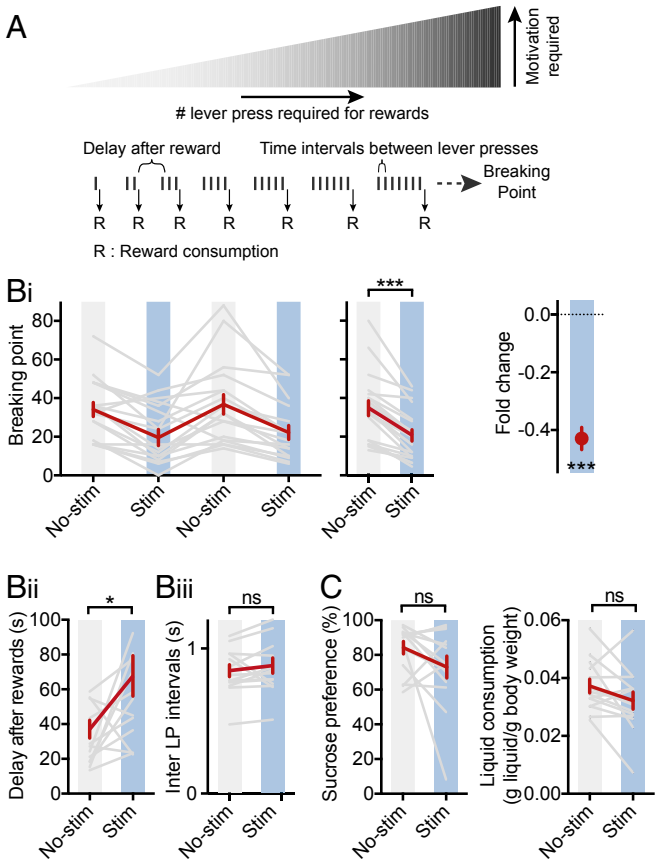

Fig. 4. Stimulation of LHb→RMTg reduces motivation to receive reward, but not reward value. (*A*) Diagram of PR reinforcement schedule; see *SI Experimental Procedures*. (*B i–iii*) Plots of BP (*i*), delay after rewards (*ii*), and inter–lever-press (LP) intervals (*iii*) for mean of daily trials (*i*, *Left*), indicated conditions (*i*, *Middle*; *ii*; and *iii*), and fold change by stimulation (*i*, *Right*, one sample Student's *t* test); gray indicates individual rats; red indicates mean ± SEM. (*C*) Plots of sucrose preference (*Left*) and total liquid consumed (*Right*) for indicated conditions during SPT; see *SI Experimental Procedures*. Same rats as used in Fig. 2. $*P < 0.05$, $***P < 0.001$; paired Student's *t* test, unless otherwise indicated. ns, not significant; stim; stimulation.

by light (Fig. 5; mobility: 3,600 ± 100 smp without light vs. 2,200 ± 200 smp with light, $P < 0.001$; mobility above threshold: 4,400 ± 100 smp without light vs. 3,300 ± 200 smp with light, $P < 0.01$; fraction immobility: 0.23 ± 0.02 without light vs. 0.46 ± 0.05 with light, $P < 0.001$). Stimulation had no significant effect on the mobility of control rats expressing tdTomato (Fig. 5; mobility: 2,300 ± 200 smp without light vs. 2,400 ± 200 smp with light, $P > 0.05$; mobility above threshold: 3,500 ± 100 smp without light vs. 3,500 ± 200 smp with light, $P > 0.05$; fraction immobility: 0.44 ± 0.05 without light vs. 0.40 ± 0.05 with light, $P > 0.05$).

While the OF test has been used to measure basic motor function, this test is also a measure of a rat's motivation to explore an environment (48, 49). Since there was no effect of LHb→RMTg stimulation on suprathreshold mobility in the FST nor on the inter–lever-press interval in the PR test, the more parsimonious interpretation is that the effect of such stimulation on the OF test is due to reduced motivation to explore (50, 51) and is not an effect on general motor function.

Activating LHb→RMTg Has No Effect on Motor Coordination and Is Aversive. As a further test to distinguish between an effect of LHb→RMTg activity on the motivation to move rather than an effect on general motor function, rats injected with AAV-oChIEF-tdTomato, which had already been tested on the FST (Fig. 2), were tested on the rotarod, a task requiring quick and coordinated movements. After an initial training period, rats' latencies to fall were measured under a variety of conditions (slow or fast constant speed and slow or fast ramping speed) (Fig. 6*A*). Under no condition did LHb→RMTg stimulation have a significant effect on rats' latencies to fall [Fig. 6*B*; 20 rpm (rotations per minute): 180 ± 20 s without light vs. 150 ± 20 s with light, $P > 0.05$; 30 rpm: 60 ± 20 s without light vs. 50 ± 20 s with light, $P > 0.05$; ramp from 2 to 15 rpm: 18 ± 1 s without light vs. 17 ± 1 s with light, $P > 0.05$; ramp from 2 to 30 rpm: 26 ± 2 s without light vs. 26 ± 2 s with light, $P > 0.05$]. Notably, these animals had shown specific deficits in the FST. This indicates that LHb→RMTg stimulation does not have a generalized effect on motor activity, but rather has an effect only under specific conditions.

Lastly, we asked whether stimulation of the LHb→RMTg was aversive, as would be expected for the experience of reduced motivational states (52). In this test (the real-time place preference test), rats are placed in a box with two compartments. After an initial habituation period, rats receive optical stimulation (25 Hz) only when they were present in one compartment. Rats expressing oChIEF actively avoided the compartment paired with stimulation (Fig. 6C; preference score: 0.16 ± 0.03 without light vs. −0.6 ± 0.1 with light, $P < 0.001$). Stimulation had no significant effect on control rats expressing tdTomato (Fig. 6*D*; preference score: 0.03 ± 0.04 without light vs. 0.08 ± 0.07 with light, $P > 0.05$). These results indicate that overactivity of the LHb→RMTg is aversive (Fig. 6*C*) (35).

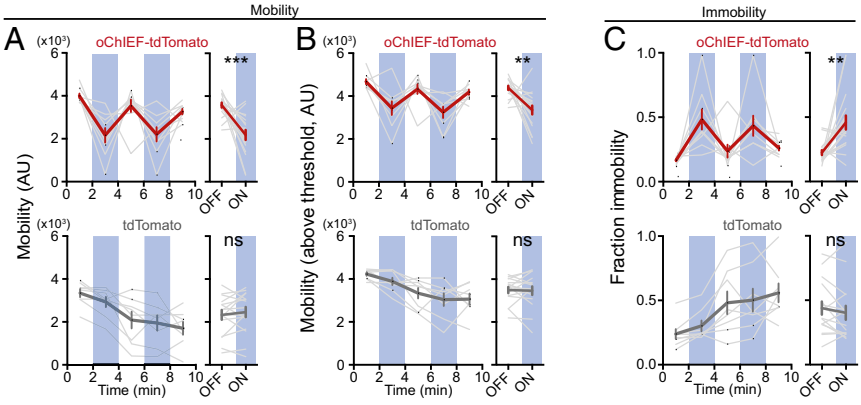

Fig. 5. Stimulation of LHb→RMTg increases immobility in the OF. (*A–C*, *Top*): (*A*, *Left*) Plot of mean mobility for rats expressing oChIEF (gray indicates individual rats; red indicates mean ± SEM) during periods of light (blue) or no light (white). (*A*, *Right*) Mean ± SEM for indicated periods. (*B*) Same as *A*, for mobility values above immobility threshold. (*C*) Same as *A*, for time spent immobile. (*A–C*, *Bottom*) Same as *A–C*, *Top*, for rats expressing tdTomato. Same rats as used in Figs. 2 and 4. $**P < 0.01$, $***P < 0.001$; paired Student's *t* test. AU, arbitrary unit; ns, not significant.

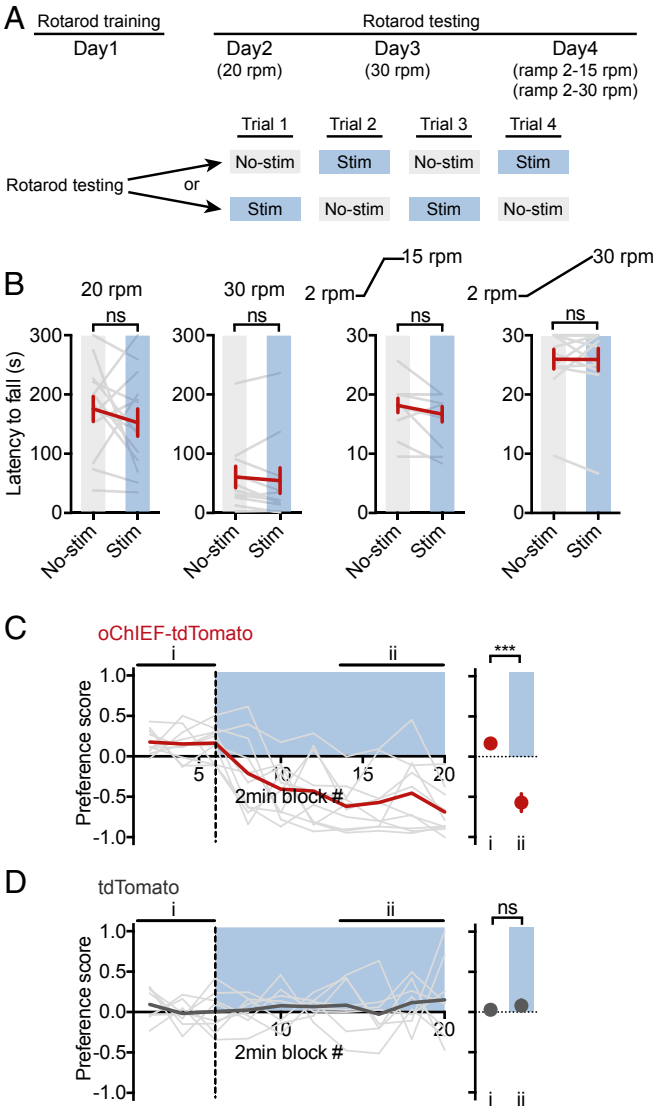

Fig. 6. Stimulation of the LHb→RMTg had no effect on motor coordination and is aversive. (*A*) Diagram of rotarod test (*SI Experimental Procedures*) schedule. (*B*) Plot of latency to fall for indicated conditions. Real-time place preference test for rats expressing oChIEF (*C*) and tdTomato (*D*). (*C* and *D*, *Left*) Plots of preference score [light gray indicates individual rats; red (*C*) or dark gray (*D*) indicates mean] for the indicated conditions. (*C* and *D*, *Right*) Mean for values during indicated periods (*i* and *ii*); blue indicates period of light stimulation while rat is in the side of box unpaired (above *x* axis) or paired (below *x* axis) with stimulation. Same rats as used in Figs. 2 and 4. ***$P < 0.001$; paired Student's *t* test. ns, not significant; stim; stimulation.

Discussion

Major depression is a leading cause of disability worldwide (1). It encompasses a heterogeneous set of disorders with distinct features (e.g., depressed mood, anhedonia, altered weight and/or sleep, fatigue, guilt, etc.). Abnormalities in several brain regions, such as the ventral tegmental area, the nucleus accumbens, and, more recently, the LHb, have been linked to depression (2–4, 53).

A deficit in motivation is a central symptom of depression that can impact behavior in aversive and rewarding environments (54, 55). This deficit could be the result of inflating the perceived cost, or undervaluing the potential benefit, of avoiding punishment or receiving a reward (24–26). In any of these cases, a deficit in motivation suggests an abnormality in the functional link between brain regions that evaluate reward-related events and those that control motivated behavior. Anatomically, the LHb is well suited to fill this role, as it receives afferents from brain nuclei signaling reward-related events and provides signals to monoaminergic centers known to control motivated behaviors (27–31, 56–58). Functionally, the activity of LHb neurons is affected by stimuli that contain motivational value; for example, they are excited by stimuli that predict punishment or the absence of rewards (31, 56, 57). The LHb is thus well positioned, both anatomically and functionally, to have an impact on motivated behaviors based on expected outcomes. In light of these findings and the proposed role of the LHb in depression, we tested whether the projection from the LHb to the RMTg, an important LHb output that inhibits dopaminergic centers, can control motivation in aversive and appetitive contexts.

In an initial set of experiments, we examined whether the motivation to exert effort in an aversive environment is controlled by the LHb→RMTg. We found that this pathway displays increased activity coinciding with the animal's entry into states of immobility in the FST—states that are classically described as "behavioral despair" and are a measure of reduced motivation in an aversive and energetically demanding environment (29, 38, 42). Notably, activity of this pathway did not correlate with fluctuations in mobility that do not contain an immobility bout, suggesting that this pathway does not simply control movement. Driving or inhibiting this pathway increased or decreased, respectively, a rat's mobility, specifically by changing the frequency and duration of inactive states, with no effect on its ability to move vigorously. Combined, these data establish a causal relationship between activity of the LHb→RMTg pathway and immobility, supporting the view that this pathway controls the motivation to sustain effort in an aversive context. Interestingly, stimulation of medial prefrontal cortex (mPFC) axons terminating in the LHb also reduced mobility in the FST (29). Our results suggest that mPFC→LHb inputs may target LHb neurons that project to the RMTg.

We also tested whether the motivation to exert effort in a rewarding environment is controlled by the LHb→RMTg. Given the complexity of the brain circuitry known to encode reward and aversion, where specific circuit components have been identified for each (58), the motivation to exert effort in these different contexts could be mediated by separate or overlapping mechanisms. Here, we show that activation of the LHb→RMTg pathway reduces the motivation to work for a reward in an appetitive operant task, suggesting that at least some overlapping neural pathways control motivation in both aversive and appetitive contexts.

The effects observed in our behavioral tasks measuring the impact of LHb→RMTg stimulation on motivation are unlikely to be caused by a general reduction in mobility, as suggested by its effect in the OF test. This conclusion is supported by several observations: LHb→RMTg stimulation (*i*) produced no change in suprathreshold mobility in the FST; (*ii*) did not affect the inter–lever-press interval in the PR task; (*iii*) did not affect the total liquid consumed in the SPT; (*iv*) had no effect in the rotarod test; and (*v*) drove, rather than inhibited, movement in the real-time place preference test. Furthermore, LHb→RMTg activity correlated with transitions into immobility, but not generally with movement. Together, these findings support the view that increasing LHb→RMTg activity does not have a general effect on motor behavior, but rather controls motivation to exert effort. The effects in the OF test are more likely due to a reduced motivation to explore (50, 51). In this regard, it will be interesting to compare the effects of LHb→RMTg stimulation on an animal's OF behavior in a familiar or novel environment.

Our results are consistent with pharmacological and dopamine-depletion manipulations, which have been shown to reduce the amount of effort made in challenging or energetically demanding conditions, and to decrease spontaneous (non–goal-oriented) locomotor function (e.g., OF) while leaving intact some motor behaviors (24, 25, 50). Other studies examining inputs to, or outputs from, the LHb (27–30), are also consistent with LHb→RMTg activity reducing motivation.

In summary, our data indicate that activity of neurons that project from the LHb to the RMTg control the motivation to perform behaviors requiring effort, irrespective of whether those

behaviors are directed toward escaping an aversive condition or toward acquiring a reward. Aberrant overactivity of this pathway, previously examined in other contexts (35, 59), may be responsible for some of the motivational deficits seen in depression.

Experimental Procedures

All procedures involving animals were approved by the Institutional Animal Care and Use Committees of the University of California, San Diego. Detailed methods describing stereotaxic injections of AAVs and optic fiber cannula implantation, fiber photometry in vivo calcium imaging, behavioral assays, and statistical methods are described in *SI Experimental Procedures*.

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