

RESEARCH ARTICLE SUMMARY

NEUROSCIENCE

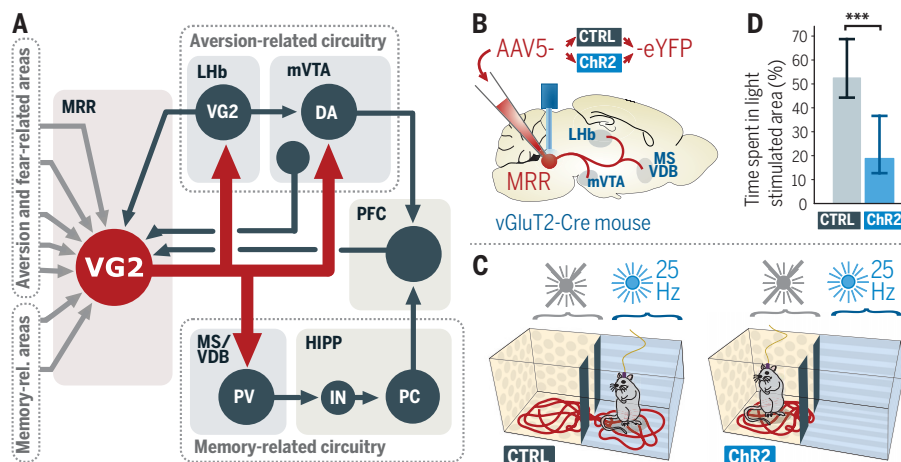
Median raphe controls acquisition of negative experience in the mouse

András Szőnyi*, Krisztián Zichó*, Albert M. Barth, Roland T. Gönczi, Dániel Schlingloff, Bibiána Török, Eszter Sipos, Abel Major, Zsuzsanna Bardóczy, Katalin E. Sos, Attila I. Gulyás, Viktor Varga, Dóra Zelena, Tamás F. Freund, Gábor Nyiri†

INTRODUCTION: Coping with negative experience is essential for survival. Animals must quickly recognize a harmful situation, produce an adequate response, and learn its context, so that they can predict the reoccurrences of similar experiences. This process requires the lateral habenula (LHb) and the medial ventral tegmental area (mVTA) for evaluating and predicting aversive stimuli. LHb neurons promote encoding of aversive behavior; learn to respond to cues that predict aversive stimuli, and activate negative experience-processing mVTA dopaminergic (DA) neurons. Overexcitation of LHb neurons leads to depression-like symptoms, whereas their inactivation has an antidepressant effect. Coping with negative experience also requires the septohippocampal system to record and recall contextual memories of events. This process necessitates increased firing of pacemaker parvalbumin (PV)-positive neurons in the medial septum and the vertical limbs of the diagonal bands of Broca (MS/VDB) and subsequent theta oscillations in the hip-

pocampus. However, how all these brain centers coordinate their activity during adverse events is poorly understood.

RATIONALE: Because the LHb does not project directly to the septohippocampal system, the brainstem median raphe region (MRR) has been proposed to coordinate their activity. Although MRR plays an important role in regulating mood, fear, and anxiety, and neuronal projections from it have been extensively studied for decades, it is still unclear how MRR neurons process these negative experiences. Using cell type-specific neuronal tract tracing, monosynaptic rabies tracing, block-face scanning immunoelectron microscopy, and in vivo and in vitro electrophysiological methods, we investigated the neurons of mouse MRR that are responsible for these functions. We used in vivo optogenetics combined with behavioral experiments or electrophysiological recordings to explore the role of MRR neurons responsible for the acquisition of negative experience.



MRR vGluT2 neurons serve as a key hub for aversive behavior. (A) MRR vGluT2 (VG2) neurons process aversive events by activating LHb and mVTA neurons and hippocampus (HIPP)-projecting memory acquisition-promoting PV-positive cells in MS/VDB. PFC, prefrontal cortex; IN, interneuron; PC, pyramidal cell. After viruses made MRR vGluT2 neurons light-sensitive (B), mice were light-stimulated in a specific area (C), which caused significant avoidance of that area compared with response in control mice (D). Medians and interquartile ranges; *** $P = 0.00034$; Mann-Whitney U test. AAV5, adeno-associated virus serotype 5; CTRL, control; ChR2, channelrhodopsin 2; eYFP, enhanced yellow fluorescent protein.

RESULTS: We discovered that the MRR harbors a vesicular glutamate transporter 2 (vGluT2)-positive cell population that gives rise to the largest ascending output of the MRR. These neurons received extensive inputs from negative sensory experience-related brain centers, whereas their excitatory fibers projected to the LHb, mVTA, and MS/VDB (see figure). MRR vGluT2 neurons mainly innervated MRR- or mVTA-projecting cells in the medial (limbic) LHb, creating a direct feedback in the MRR-LHb-mVTA axis. MRR vGluT2 neurons were selectively activated by aversive but not rewarding stimuli in vivo. Stimulation of MRR vGluT2 neurons induced strong aversion (see figure), agitation, and aggression and suppressed reward-seeking behavior; whereas their chronic activation induced depression-related anhedonia. The latter can at least partly be explained by our three-dimensional electron microscopy data showing highly effective synaptic targeting of LHb neurons and by our in vitro data showing that MRR vGluT2 terminals can trigger depressive behavior-related bursting activity of LHb neurons.

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MRR vGluT2 neurons seem to be involved in active responses to negative experience, therefore inducing aggression or avoidance, classical fight-or-flight responses. Suppression of MRR vGluT2 neurons precisely at the moment of the aversive stimulus presentation strongly disrupted the expression of both contextual and cued fear memories and prevented fear generalization. MRR vGluT2 neurons could facilitate the learning of negative experience, because their LHb-projecting axons bifurcated and selectively innervated pacemaker MS/VDB PV-positive neurons that projected to the hippocampus. Consequently, in vivo stimulation of MRR vGluT2 neurons instantly evoked memory acquisition-promoting hippocampal theta oscillations in mice.

CONCLUSION: Our results revealed that the MRR harbors a previously unrecognized brainstem center that serves as a key hub for the acquisition of negative experience. MRR vGluT2 neurons could activate the aversion- and negative prediction-related LHb-mVTA axis and could swiftly transform the state of the septohippocampal system for immediate acquisition of episodic memories of the negative experience. Maladaptations in processing negative experience form the basis of several types of mood disorders, which have a huge social and economic impact on individuals and society. Selective targeting of this neural hub may form the basis of new therapies. ■

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Median raphe controls acquisition of negative experience in the mouse

András Szőnyi^{1*†‡}, Krisztián Zichó^{1*}, Albert M. Barth¹, Roland T. Gönczi¹, Dániel Schlingloff^{1,2}, Bibiána Török^{2,3}, Eszter Sipos³, Abel Major¹, Zsuzsanna Bardóczi¹, Katalin E. Sos^{1,2}, Attila I. Gulyás¹, Viktor Varga¹, Dóra Zelena³, Tamás F. Freund¹, Gábor Nyiri^{1§}

Adverse events need to be quickly evaluated and memorized, yet how these processes are coordinated is poorly understood. We discovered a large population of excitatory neurons in mouse median raphe region (MRR) expressing vesicular glutamate transporter 2 (vGluT2) that received inputs from several negative experience-related brain centers, projected to the main aversion centers, and activated the septohippocampal system pivotal for learning of adverse events. These neurons were selectively activated by aversive but not rewarding stimuli. Their stimulation induced place aversion, aggression, depression-related anhedonia, and suppression of reward-seeking behavior and memory acquisition-promoting hippocampal theta oscillations. By contrast, their suppression impaired both contextual and cued fear memory formation. These results suggest that MRR vGluT2 neurons are crucial for the acquisition of negative experiences and may play a central role in depression-related mood disorders.

To survive, animals must quickly recognize a harmful situation, produce an adequate response, and learn its context to help predict the occurrence of similar negative experiences in the future (1–6). This process requires the lateral habenula (LHb) and medial ventral tegmental area (mVTA) for evaluating and predicting aversive stimuli and also requires the septohippocampal system to record and recall memories of these adverse events. Yet how these brain centers coordinate their activity during adverse events is poorly understood. Because the LHb does not project directly to the septohippocampal system, the brainstem median raphe region (MRR) has been proposed to coordinate their activity (7–14). Although the MRR plays an important role in regulating mood, fear, and anxiety, its role in processing negative experience remains elusive (13, 15, 16). It contains projection neurons expressing serotonin [5-hydroxytryptamine (5-HT)] and/or type 3 vesicular glutamate transporter (vGluT3), yet after decades of studies, it is still unclear how MRR neurons can support these functions (17–19). Although projections from the MRR to the LHb, mVTA,

and medial septum and the vertical limbs of the diagonal bands of Broca (MS/VDB) must be crucial to understanding negative experience-related behavior, the identity of key MRR neurons responsible for these connections remains unknown (20, 21).

Most MRR projection neurons are vGluT2-positive

In mice, the transmitter phenotypes and targets of almost 25% of MRR neurons are unknown (22). In this study, injections of the Cre-dependent tracer virus AAV5-eYFP (AAV5, adeno-associated virus serotype 5; eYFP, enhanced yellow fluorescent protein) into the MRR of vGluT2-Cre mice, together with complete stereological measurements, revealed that at least 20% of the MRR neurons are vGluT2-positive (Fig. 1, A to C; table S5; and supplementary materials). MRR vGluT2-positive neurons were evenly distributed both in the median and the paramedian part of the MRR. Fluorescent immunohistochemistry demonstrated that this group of cells was distinct from 5-HT- and/or vGluT3-positive neurons in the MRR (Fig. 1B). Terminals of eYFP-expressing MRR neurons of vGluT2-Cre mice were positive for vGluT2 (fig. S1, A and B), but they do not express the plasma membrane serotonin transporter, vesicular GABA transporter (vGAT), or vGluT3 (fig. S1C).

MRR vGluT2 neurons are linked to negative experience-related brain regions

Viral labeling of vGluT2 neurons with Cre-dependent AAV5-eYFP in vGluT2-Cre mice revealed that they strongly innervate the LHb and the mVTA (Fig. 1, E and F), as well as other neurons locally (fig. S1D). We did not observe similar innervation patterns after

injecting surrounding brain areas in vGluT2-Cre mice, nor after injecting AAV5-eYFP into the MRR of tryptophan hydroxylase (TpH)-Cre, vGluT3-Cre, or vGAT-Cre mice [labeling serotonergic, vGluT3-positive, and γ -aminobutyric acid-releasing (GABAergic) MRR neurons, respectively] (fig. S3, A to I).

Injections of Cre-dependent AAV5-eYFP into the MRR of vGluT2-Cre mice showed that MRR vGluT2 neuronal projections avoided positive reinforcement-related lateral VTA dopaminergic (DA) cells (Fig. 1G). Instead, they innervated mVTA DA neurons (Fig. 1, G and H). Glutamatergic LHb neurons also innervate mVTA DA cells to regulate negative reward predictions and aversive behavior (1–3, 23). Indeed, when we simultaneously injected AAV5-mCherry into the LHb and AAV5-eYFP into the MRR of the same vGluT2-Cre mice, we detected that both LHb and MRR vGluT2 neurons targeted the mVTA specifically (Fig. 1, G and H).

Aversion-related mVTA DA cells target the medial prefrontal cortex (mPFC) (23, 24). We injected the retrograde tracer cholera toxin B subunit (CTB) into the mPFC and Cre-dependent AAV5-eYFP into the MRR of vGluT2-Cre mice (fig. S1E). vGluT2-positive MRR terminals established synaptic contacts with those mVTA DA neurons that project to the mPFC (fig. S1, F and G), showing that vGluT2-positive MRR neurons target DA cells related to negative reward predictions.

Glutamatergic LHb neurons (primarily in the medial part of the LHb) also innervate the MRR (25), but the identity of their target cells is unknown. We injected Cre-dependent AAV5-eYFP into the MRR and AAV5-mCherry into the LHb of vGluT2-Cre mice and found that MRR vGluT2 neurons primarily targeted the medial part of the LHb (Fig. 1E), whereas at least 39% of LHb terminals innervated vGluT2-positive neurons in the MRR (Fig. 1, I to L). Serotonergic and vGluT3-positive MRR neurons were also targeted by LHb vGluT2 neurons (for the exact ratios, see fig. S2, F and G). Using combined anterograde and retrograde tracing, we also found that there is a direct reciprocal connection between the LHb-projecting vGluT2-positive MRR neurons and the MRR-projecting vGluT2-positive LHb neurons (Fig. 1I) (for measured ratios, see figs. S1, H to J, and S2, A to E, and supplementary materials). vGluT2-positive MRR neurons also innervate LHb neurons that project to the mVTA (fig. S1, H to J). These results indicate an excitatory positive-feedback loop between the vGluT2 neurons of the MRR and LHb. Both of these neuronal populations project to the aversion-encoding mVTA as well.

To identify upstream brain areas that synaptically target the MRR vGluT2-positive neurons, we used mono-transsynaptic rabies tracing (26). We used a Cre-dependent helper

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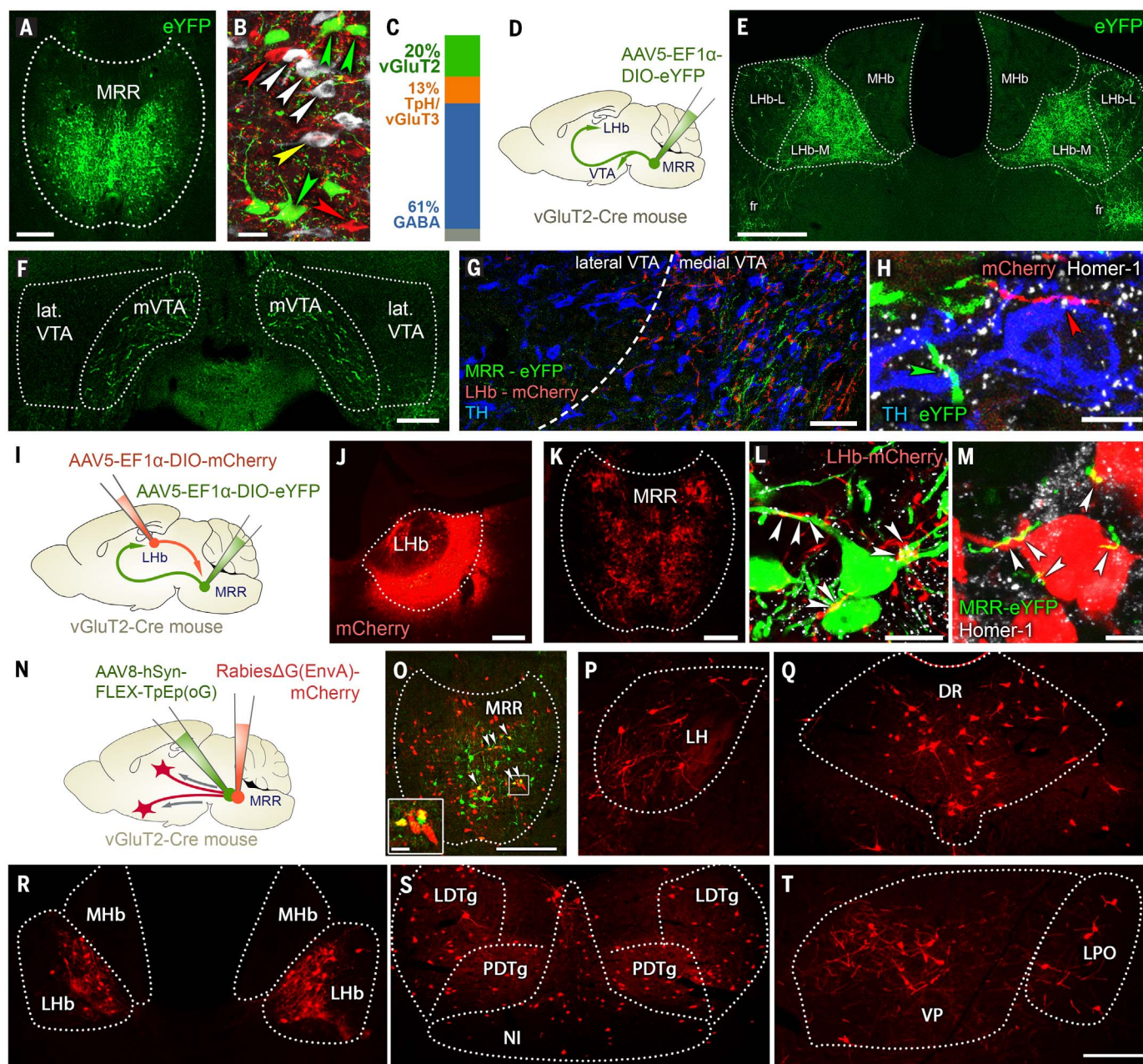


Fig. 1. vGluT2 neurons are the largest population of projection cells in the MRR. (A) AAV5-eYFP labeling in the MRR in vGluT2-Cre mice. Scale bar: 200 μ m. (B) eYFP-labeled vGluT2-positive (green arrows), immunolabeled TpH-positive (white arrows), vGluT3-positive (red arrows), and TpH/vGluT3 double positive (yellow arrow) cells in MRR. Scale bar: 20 μ m. (C) At least 20% of the MRR neurons are vGluT2-positive. (For stereological statistical details, see table S5). (D) AAV2/5-EF1 α -DIO-eYFP was injected into the MRR of vGluT2-Cre mice ($n = 3$). (E and F) Virally labeled vGluT2-positive MRR fibers innervate the Lhb (primarily the medial part) and mVTA. fr, fasciculus retroflexus; Lhb-L, lateral habenula, lateral division; Lhb-M, lateral habenula, medial division; MHb, medial habenula; VTA, ventral tegmental area. Scale bars: 200 μ m. (G) vGluT2-positive fibers from MRR (green) and Lhb (red) innervate the same medial VTA but not the lateral VTA. DA cells were labeled with anti-tyrosine hydroxylase (TH, blue). Scale bar: 50 μ m. Statistical details for all figures are provided in the supplementary text section of the supplementary materials. (H) vGluT2-positive MRR axon terminal (green) and an Lhb terminal (red) establish Homer-1 (white) positive synaptic contacts with the same dopaminergic

cell (TH, blue) in mVTA. Scale bar: 5 μ m. (I) AAV2/5-EF1 α -DIO-eYFP was injected into the MRR and AAV2/5-EF1 α -DIO-mCherry was injected into the Lhb of vGluT2-Cre mice bilaterally ($n = 2$). (J and K) Injection site in the Lhb (J) and its vGluT2-positive fibers in the MRR (K). Scale bar: 200 μ m. (L) vGluT2-positive Lhb fibers (red) establish Homer-1 (white) positive synaptic contacts (white arrowheads) with vGluT2-positive MRR neurons (green). Scale bar: 10 μ m. (M) vGluT2-positive MRR fibers (green) establish Homer-1 (white) positive synaptic contacts (white arrowheads) with vGluT2-positive Lhb neurons (red). Scale bar: 5 μ m. (N) A helper AAV2/8-hSyn-FLEX-TpEp(oG) was injected into the MRR of vGluT2-Cre mice, followed by an injection of Rabies(Δ G)-EnvA-mCherry 2 weeks later ($n = 3$ mice). (O) Injection site of helper (green) and rabies (red) viruses into the MRR of vGluT2-Cre mice. Inset shows some starter neurons expressing both viruses. Scale bar: 100 μ m (main image), 10 μ m (inset). (P to T) Rabies-labeled neurons in different brain areas establish synapses on vGluT2-positive MRR neurons. Scale bar: 100 μ m. LDTg, laterodorsal tegmental nucleus; LPO, lateral preoptic area; PDTg, posterodorsal tegmental nucleus; VP, ventral pallidum. For detailed analysis, see table S6.

virus encoding both an avian tumor virus receptor A (TVA) and an optimized rabies glycoprotein, and we used a TVA-receptor-dependent and glycoprotein-deleted rabies virus in vGluT2-Cre mice (supplementary materials and Fig. 1, N and O)—the specificity of this virus combination was validated in our previous study (27). Brain areas that play an essential role in negative experience-related behavior, including the dorsal raphe (DR), lateral hypothalamus (LH), periaqueductal gray, and zona incerta, showed a strong convergence onto vGluT2-positive MRR cells (Fig. 1, P to T; for details, see table S6). The LHb accounts for most of the monosynaptically labeled input cells (Fig. 1R). Areas related to the encoding of aversive memories [such as the mammillary areas, the pontine reticular nucleus, or the nucleus incertus (NI)] also sent

strong projections onto vGluT2-positive MRR neurons (table S6).

MRR vGluT2 neurons establish multiple burst-promoting synapses on LHb neurons

Negative experience-related behavior and subsequent depression-like symptoms are strongly promoted by the excitatory inputs of LHb neurons (28–30). Using block-face scanning electron microscopy, we revealed that MRR vGluT2 neurons provide an extensive synaptic coverage on LHb neurons (Fig. 2, A, B, E, and F), and most of the axon terminals of MRR vGluT2 neurons established more than one synapse on the same or different target cells (Fig. 2D). *N*-methyl-D-aspartate (NMDA) receptor-dependent burst-firing in LHb neurons play a key role in the development of depression (28, 30). We found that MRR

vGluT2 neurons established NMDA receptor-containing excitatory glutamatergic synapses on LHb neurons (Fig. 2C). In the LHb, astroglia cooperate with excitation to regulate neuronal bursting and depression-like symptoms (28). These glial processes enwrapped most synapses of MRR vGluT2 terminals in the LHb (Fig. 2, B, E, and F).

To test the physiological effect of MRR vGluT2 neurons on the activity of the LHb neurons, we selectively activated MRR vGluT2 fibers in the LHb in vitro using channelrhodopsin 2 (ChR2)-containing Cre-dependent AAV5-ChR2-eYFP in optogenetic experiments (Fig. 3A). Light stimulation of ChR2-containing MRR vGluT2-positive fibers reliably evoked glutamatergic excitatory postsynaptic currents (EPSCs) in voltage-clamped neurons of the LHb (Fig. 3B and fig. S4, A and B), which

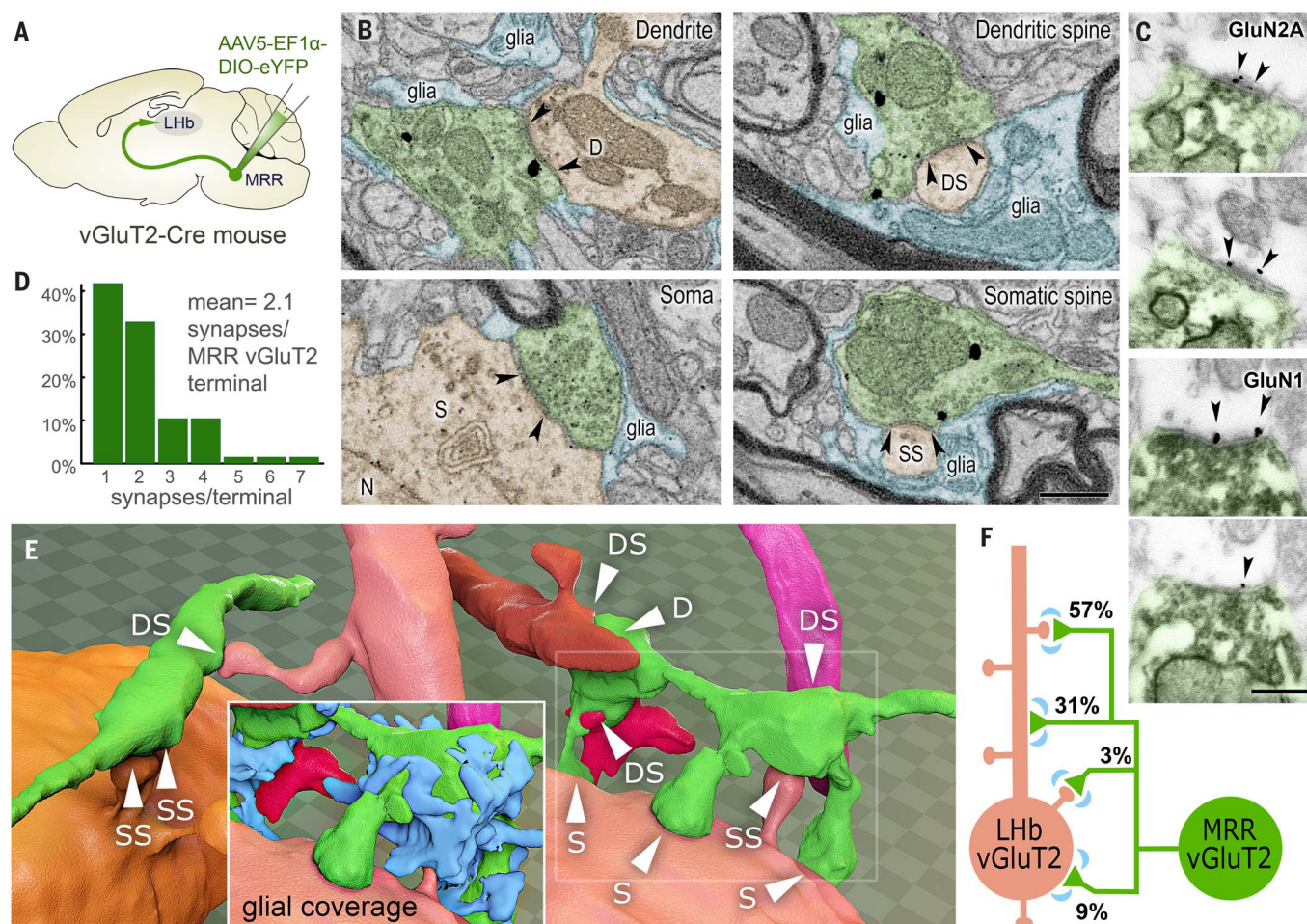


Fig. 2. MRR vGluT2 neurons establish glia-enwrapped glutamatergic synapses on LHb neurons. (A) AAV5/5-EF1 α -DIO-eYFP was injected into the MRR of vGluT2-Cre mice. (B) Scanning electron micrographs represent different types of synaptic contacts established by eYFP-positive MRR terminals (immunogold labeling, black spheres) on different subcellular compartments of LHb neurons. Arrows indicate synaptic edges. D, dendrite; DS, dendritic spine; N, nucleus; S, soma; SS, somatic spine. Scale bar: 500 nm. (C) AAV-eYFP positive terminals [serial sections of immunoperoxidase labeling, dark 3,3' diaminobenzidine (DAB) precipi-

tate] establish synapses on LHb neurons that contain the GluN2A (top two images) or GluN1 (bottom two images) NMDA-receptor subunits (immunogold particles indicated with arrowheads). Scale bar: 300 nm. (D) MRR vGluT2 terminals establish more than one synapse on LHb neurons (from two mice). (E) 3D reconstruction of MRR vGluT2 fibers (green) shows their synapses with different membrane domains of LHb neurons. Inset shows the abundant glial coverage around MRR vGluT2 terminals (blue). (F) Schematic illustration of the proportion of MRR vGluT2 synapses on different membrane domains of LHb neurons (from two mice).

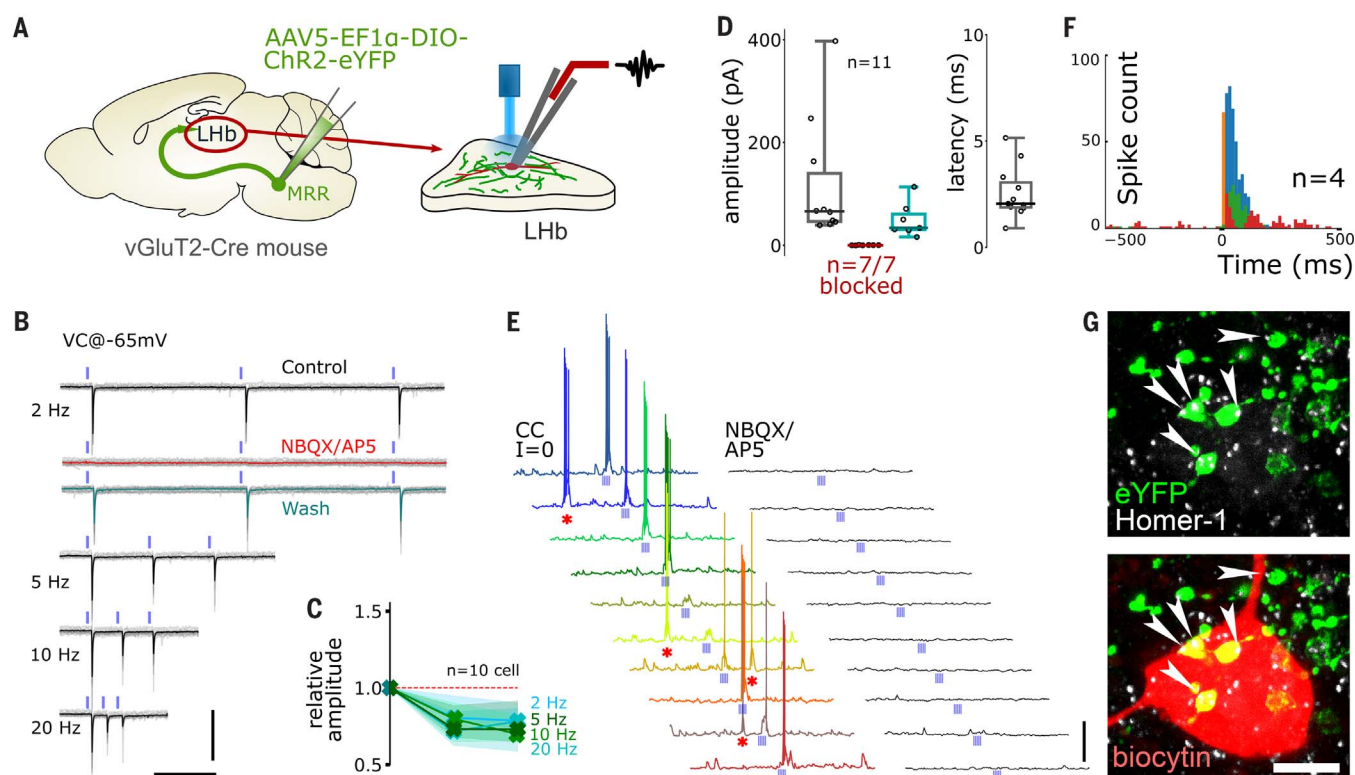


Fig. 3. Fibers of MRR vGluT2 neurons evoke burst firing in LHB neurons.

(A) AAV2/5-EF1 α -DIO-ChR2-eYFP was injected into the MRR of vGluT2-Cre mice ($n = 3$). Six weeks later, 300- μ m-thick horizontal in vitro slices were prepared from the LHB. Neurons in the LHB were whole-cell patch clamped in voltage-clamp mode (see supplementary methods). (B) Light stimulations of MRR vGluT2 fibers evoked EPSCs in LHB neurons. The average responses to 2-ms light pulses are shown above individual gray traces. Responses are strong in control conditions (black); they were completely abolished (red trace) by 20- μ M NBQX (AMPA/kainite receptor antagonists) and 50- μ M AP5 (NMDA antagonist) and partially recovered after washout (teal). Scale bars: 200 ms, 200 pA. (C) Cells displayed short-term depression of EPSC amplitudes at all stimulation frequencies (averages from 10 cells). (D) EPSC amplitude

and latency distributions from all 11 recorded neurons (in gray). Excitatory postsynaptic potentials were sensitive to glutamatergic antagonists in 7 out of 7 tested cells (red) and partially recovered after washout (teal). (E) Traces show representative current-clamped ($I = 0$) LHB neurons displaying spontaneous (asterisks) and light-evoked (pale blue lines, 5 pulses at 20 Hz) bursts. Ten consecutive stimulations are shown. Both spontaneous and light-evoked bursts were abolished by application of NBQX and AP5 (traces on the right). Scale bars: 1 s, 20 mV. (F) Peristimulus time histogram shows action potential distributions upon light stimuli ($n = 4$ cells). (G) A representative, recorded LHB cell (biocytin labeling, red) receives Homer-1-positive (white) synaptic contacts (white arrowheads) from eYFP-positive MRR fibers (green). Scale bar: 5 μ m.

received MRR vGluT2 terminals (Fig. 3G). These EPSCs showed strong short-term depression and were abolished by the simultaneous blockade of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and NMDA-type glutamate receptors (Fig. 3, B to D). After activating ChR2-expressing vGluT2-positive MRR terminals in the LHB, we frequently observed the induction of burst-firing in LHB neurons (Fig. 3, E and F), whereas some spontaneous burst-firing was also present in these neurons. Bursting disappeared after the combined blockade of AMPA- and NMDA-type glutamate receptors (Fig. 3E).

MRR vGluT2 neurons are selectively activated by aversive stimuli in vivo

We explored the in vivo response of identified MRR vGluT2 neurons to aversive and rewarding stimuli. We used a combination of multi-channel recording and optogenetic tagging in

the MRR of Cre-dependent, AAV-ChR2-eYFP-injected vGluT2-Cre mice. Awake mice were head-fixed on top of an air-supported spherical treadmill, while multiple single units were simultaneously recorded from the MRR using a multichannel silicone probe. Light pulses that were used to tag vGluT2 neurons were delivered through an optic fiber positioned above the MRR (Fig. 4, A and B). Tagged neurons reliably responded to brief blue laser light pulses with short latency and small jitter (Fig. 4C). A large set of optogenetically identified vGluT2-positive MRR neurons were robustly activated by strongly aversive air puffs (Fig. 4, D to F). By contrast, MRR vGluT2 neurons were practically never affected by rewarding stimuli (water drops) (Fig. 4, D to F). Mildly aversive light-emitting diode (LED) flashes triggered a slight, transient elevation of activity in a small subgroup of vGluT2-positive MRR neurons, partially overlapping with the air-puff

activated group (fig. S4, G and H), but this effect was significantly lower than that of air-puff stimulation (Fig. 4, D to F).

Optogenetic activation of MRR vGluT2 neurons causes strongly aversive behavior

The rapid, adverse experience-specific in vivo activity of MRR vGluT2 neurons suggests that they specifically process negative experience. To light-activate MRR vGluT2 neurons selectively in vivo, we injected ChR2-containing Cre-dependent AAV5-ChR2-eYFP into the MRR of vGluT2-Cre mice ("ChR2 mice"). Control mice were injected with a control virus that expressed no ChR2 ("CTRL mice"). Then, we implanted an optic fiber over the MRR (supplementary materials, Fig. 5A, and fig. S5). After handling, mice were placed in a chamber containing two different areas with different visual cues (Fig. 5A). In the habituation session, mice did not show a preference for one

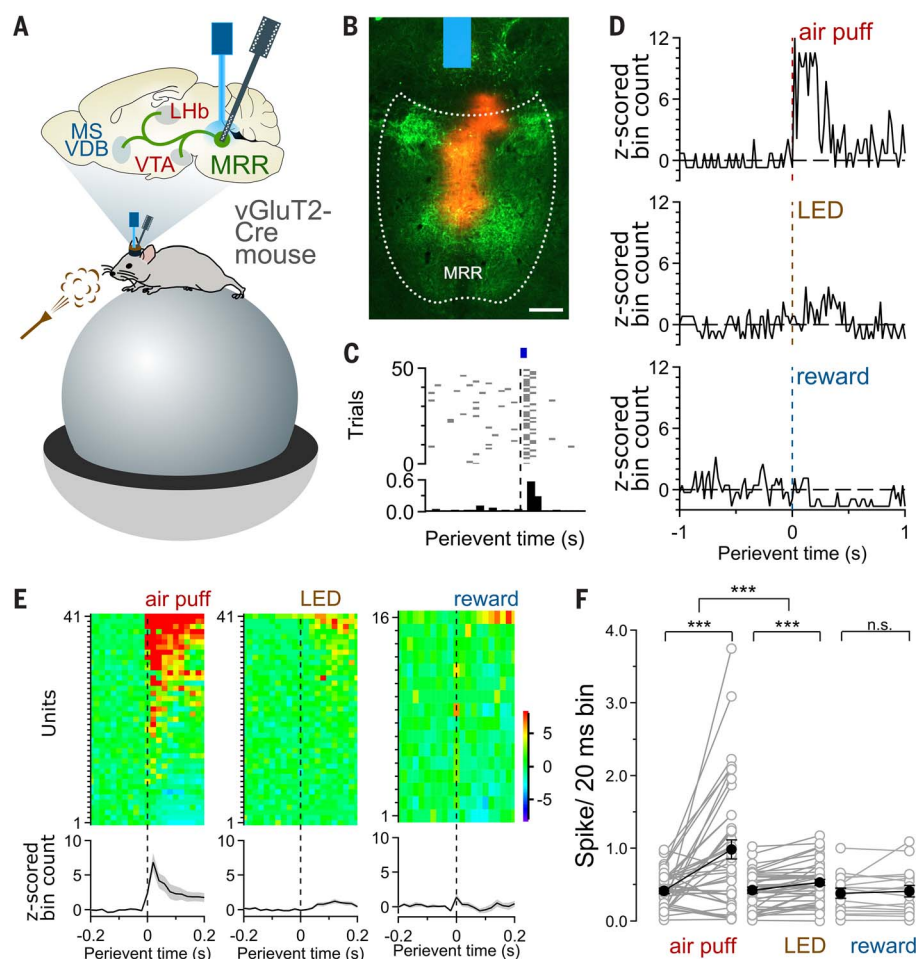


Fig. 4. In vivo, MRR vGluT2 neurons selectively respond to aversive stimuli. (A) AAV2/5-EF1 α -DIO-ChR2-eYFP was injected into the MRR of vGluT2-Cre mice. A silicone probe was inserted into and an optical fiber was placed above the MRR. Mice were head-fixed on top of an air-supported spherical treadmill, and strongly aversive (air puff), mildly aversive (LED flash), and rewarding (water drops) stimuli were randomly presented to the awake animals (eight mice). (B) A representative image of virus expression (green) and the position of the implanted optic fiber (blue) and silicone probe (red Dil labeling) in the MRR of a vGluT2-Cre mouse. Scale bar: 200 μ m. (C) A raster plot and a peristimulus time histogram, corresponding to an optogenetically tagged vGluT2 neuron in the MRR, shows short latency, high success rate, and low jitter of the responses. The blue square indicates the light pulse. (D) A representative MRR vGluT2 neuron was robustly activated by an aversive air puff, was only weakly activated by a slightly aversive LED stimulus, and showed no response to water reward. (E) Top matrices show the reaction of individual MRR vGluT2 neurons to different stimuli (each row is one neuron's z-scored peristimulus time histogram), sorted by descending response magnitudes. Bottom plots show average response across all tagged units (from eight animals, mean $\pm$ SEM). (F) Paired plots of cells [same as those displayed in (E)] show the baseline versus stimulus-evoked firing activity of MRR vGluT2 neurons (gray, individual tagged neurons; black, mean $\pm$ SEM). Air puffs evoked significantly larger responses than did LED flashes. Reward induced no significant population response.

area over the other (fig. S6, A and B). Twenty-four hours later, mice were returned to the same chamber and received optogenetic stimulation (25 Hz) only in one area of the chamber (area selection was systematically random). ChR2 mice, but not CTRL mice, showed a powerful and immediate real-time place aversion to the stimulation-linked area (Fig. 5, A and B, and fig. S6, A and B). The following day, previously stimulated ChR2 mice, but

not CTRL mice, showed a strong conditioned aversion to the area that was previously linked to stimulation (Fig. 5, A and B, and fig. S6, A and B).

We investigated whether the activity of MRR vGluT2 neurons is powerful enough to counteract the strongly positive expectation of a motivated, food-seeking animal. The above-mentioned ChR2 and CTRL mice were food-restricted and trained for 10 days in an

operant conditioning task to nose-poke for food rewards (Fig. 5C and supplementary materials). On the last day of their training, both groups of mice reached a plateau in the total number of nose-pokes during the 30-min-long operant conditioning session (Fig. 5C). Twenty-four hours later, mice received blue laser stimulation that started after each rewarded nose-poke during the entire 30-min-long session. ChR2 mice, but not CTRL mice, poked significantly less for reward pellets during the day of the stimulation experiment compared with the frequency of poking on the last day of their training (Fig. 5, D and E).

We also tested whether the activation of these cells can induce passive coping. We performed an optogenetic contextual fear conditioning experiment with the same cohort of mice that participated in the operant conditioning task (fig. S6C). Mice were allowed 3 days to rest and regain their original weight, and on the 25th day they were placed in a novel environment, where they received 15-s-long laser stimulation 10 times. Twenty-four hours later, mice were tested in the same environment. Neither ChR2 mice nor CTRL mice showed any sign of freezing behavior during light stimulation or 24 hours later in the same environment (fig. S6C).

Activation of MRR vGluT2 neurons induces aggression and depressive symptoms

Neuronal activity in the LHb is related to aggressive behavior (31). Therefore, we investigated whether activation of MRR vGluT2 neurons induces the prediction of negative experience in a social context and whether it promotes an aggressive behavior in mice during these social interactions. We used a DREADD (designer receptors exclusively activated by designer drugs)-containing AAV-based chemogenetic tool that expresses mutant receptors that activates cells upon binding to clozapine-N-oxide (CNO). We injected either Cre-dependent excitatory DREADD virus AAV8-h3MDq-mCherry ("hM3Dq mice") or Cre-dependent AAV8-mCherry virus ("CTRL mice") into the MRR of vGluT2-Cre mice. Then, both hM3Dq and CTRL mice received intraperitoneal injections of CNO (Fig. 6A and supplementary materials) and performed two behavioral tests. In the social interaction test, mice were placed in a novel environment 30 min after CNO injections, together with an unfamiliar mouse. CTRL mice showed mostly prosocial behavior toward the conspecific (Fig. 6B). In contrast, hM3Dq mice became highly aggressive (Fig. 6B and fig. S4, C to F). Five days later, we carried out a resident-intruder test. Thirty minutes after CNO injections, a subordinate intruder mouse was placed into the home cage of the CTRL or hM3Dq mice. Again, hM3Dq mice became highly aggressive

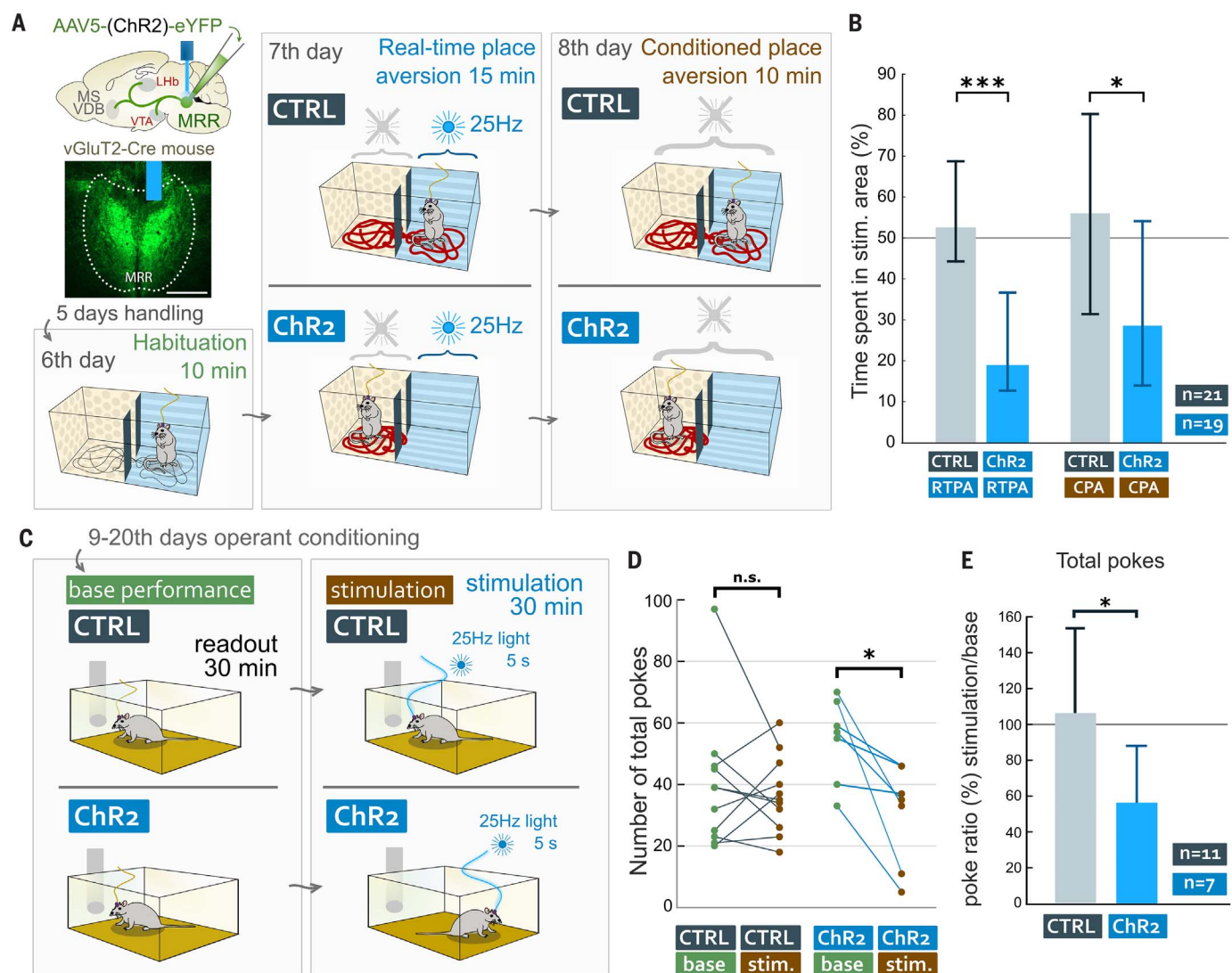


Fig. 5. MRR vGluT2 neurons induce active avoidance of negative experience. (A) After injecting ChR2-containing AAV2/5-EF1 α -DIO-ChR2-eYFP ("ChR2 mice") or control (only eYFP-containing) Cre-dependent AAV5 ("CTRL mice") into the MRR of vGluT2-Cre mice, we implanted an optic fiber over the MRR. Image represents an injection site in MRR and the position of the optic fiber (blue). Scale bar: 500 μ m. Four weeks later, mice were habituated to a chamber with two areas. On day 7, mice received 25-Hz light illumination in one of the areas of the chamber. ChR2 mice showed immediate, significant real-time place aversion (RTPA) of the stimulated area. On day 8, no light was presented, but ChR2 mice displayed a significant conditioned place aversion (CPA), suggesting

that the activity of MRR vGluT2 neurons can directly induce real-time and learned active contextual avoidance (see also fig. S6A). (B) RTPA and CPA tests show significant effects (medians and interquartile ranges). (C) Between days 9 and 21, mice were food-restricted and learned to nose-poke for reward pellets. On day 22, mice received 5-s 25-Hz light stimulation on nose-pokes. Compared with base performance (on day 21), ChR2 mice performed significantly fewer nose-pokes when nose-pokes were paired with laser stimulation (on day 22). (D) The total number of nose-pokes for rewards during the base and stimulation performance in the operant conditioning task. (E) Nose-poke ratios of stimulation/base performance in the operant conditioning task (mean and SD).

toward the intruder (Fig. 6C and fig. S4, C to F). In a separate experiment, chemogenetic activation of AAV8-h3MDq-expressing MRR vGluT2 neurons by CNO injections also induced significantly higher motor activities in a Y-maze compared with CTRL mice (fig. S4I).

Aggressive behavior frequently accompanies the depressive state (32–34), which is also promoted by chronic overactivation and burst-firing of LHB neurons (28–31, 35). Using the above-mentioned types of CTRL and hM3Dq

mice, we tested whether chronic activation of MRR vGluT2 neurons promoted depression-like symptoms. Mice received intraperitoneal injections of the DREADD-agonist CNO three times a week for 3 weeks (Fig. 6A and supplementary materials). On day 19, mice were tested in a sucrose preference test, which can detect anhedonia, a classic symptom of depression (36). CTRL mice preferred the 1% sucrose solution over water significantly more than hM3Dq mice did (Fig. 6D). The postmortem weight of

the adrenal glands of hM3Dq mice was significantly higher than that of CTRL mice (fig. S4J).

MRR vGluT2 neurons activate memory acquisition—promoting MS/VDB neurons

Fast and effective processing of negative experience requires the immediate induction of memory acquisition, which necessitates a rapid change in the state of the MS/VDB-hippocampal system (37). After AAV5-eYFP labeling of MRR vGluT2 neurons, we observed

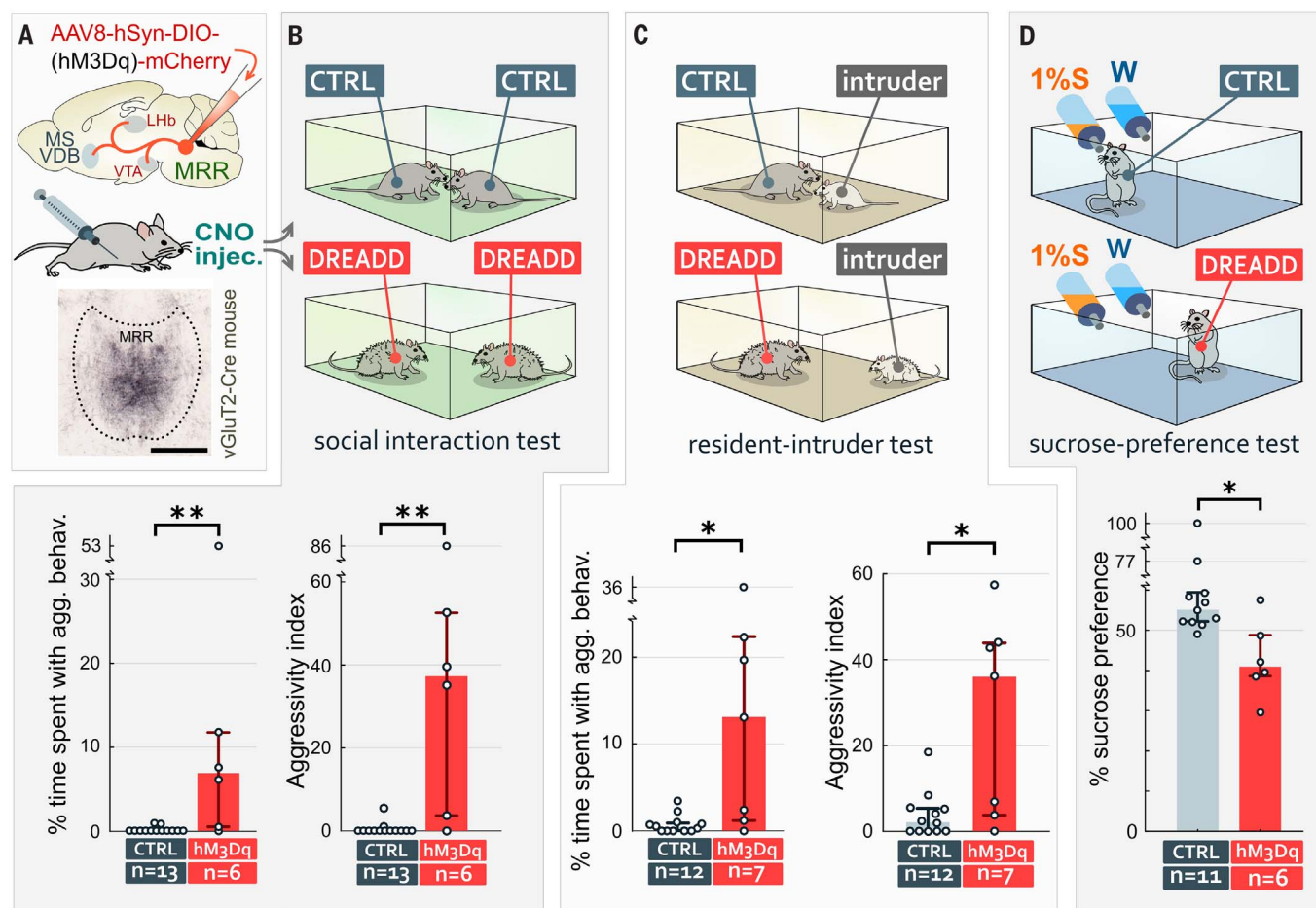


Fig. 6. Chronic overactivation of MRR vGluT2 neurons induces aggression and anhedonia. (A) AAV2/8-hSyn-DIO-hM3D(Gq)-mCherry or control AAV2/8-hSyn-DIO-mCherry was injected into the MRR of vGluT2-Cre mice (14 CTRL mice and 8 hM3Dq mice). All mice received hM3Dq-agonist clozapine-*N*-oxide (CNO, 1 mg 10 ml⁻¹ kg⁻¹ intraperitoneally, 30 min before testing). Image represents a DAB-labeled injection site in the MRR. Scale bar: 500 μ m. (B and C) Social interaction (B) and resident-intruder (C) tests. Percentage of time spent with aggressive

interactions and aggressivity index are shown at the bottom. Chemogenetic stimulation of MRR vGluT2 neurons led to highly aggressive behavior, suggesting the induction of active coping with a perceived strongly negative experience. (D) Testing anhedonia in a sucrose preference test (see supplementary methods for details). Chronic stimulation of MRR vGluT2 neurons in hM3Dq mice led to a significant difference in sucrose preference compared to CTRL mice, suggesting the induction of depression-related anhedonia. Graphs show medians and 25%–75% quartiles.

an abundant axonal labeling in the MS/VDB (Fig. 7, A and B). MRR vGluT2 neurons established NMDA receptor-containing excitatory synapses selectively with parvalbumin (PV)-positive GABAergic cells in the MS/VDB (Fig. 7, A to E; fig. S1K; and supplementary text). Using hippocampal injections of the retrograde tracer FluoroGold, in combination with AAV5-mCherry, into the MRR of vGluT2-Cre mice, we found that MRR vGluT2 neurons directly innervate hippocampus-projecting PV neurons in MS/VDB (Fig. 7, F to H). These PV neurons are the pacemakers of memory acquisition—promoting hippocampal theta rhythm (38, 39).

The theta-rhythmic activity of LHb neurons and the hippocampal network are phase-locked, and their concerted activity is necessary for proper memory formation (8, 14). Therefore, we investigated whether individual axons of

MRR vGluT2 neurons bifurcate and target both areas. We performed double retrograde tracing by injecting CTB into the LHb and FluoroGold into the MS/VDB of vGluT2-Cre mice, and we labeled vGluT2-positive MRR cells with AAV5-mCherry (Fig. 7, I and J). At least 61% of MRR vGluT2 neurons retrogradely labeled from the MS/VDB were retrogradely labeled from the LHb as well (Fig. 7K).

We also investigated the effect of activation of MRR vGluT2 neurons on hippocampal network activity in vivo. Using AAV5-ChR2-eYFP, we expressed ChR2 in MRR vGluT2 neurons and implanted an optic fiber over the MRR and a Buzsaki probe into the dorsal CA1 (Fig. 8A). Ten-second-long stimulation of MRR vGluT2 neurons by a 25-Hz light train triggered some movement (possibly related to the above-mentioned active avoidance of the stimuli)

and it triggered hippocampal theta oscillations (Fig. 8, B to D) known to facilitate the formation of memories.

MRR vGluT2 neurons are necessary for fear memory acquisition

MRR vGluT2 neurons are specifically excited by negative experience, which they relay to aversion centers and to the septohippocampal system, likely promoting memory encoding. We injected Cre-dependent Archetodopsin T-3 (ArchT 3.0)-containing AAV5-ArchT-GFP (ArchT mice) or control Cre-dependent AAV5-eYFP (CTRL mice) into the MRR of vGluT2-Cre mice and implanted an optic fiber over the MRR (Fig. 8E and fig. S5). After handling, mice were tested in a delay cued fear conditioning paradigm. First, we placed mice into a novel environment “A,” where they received three

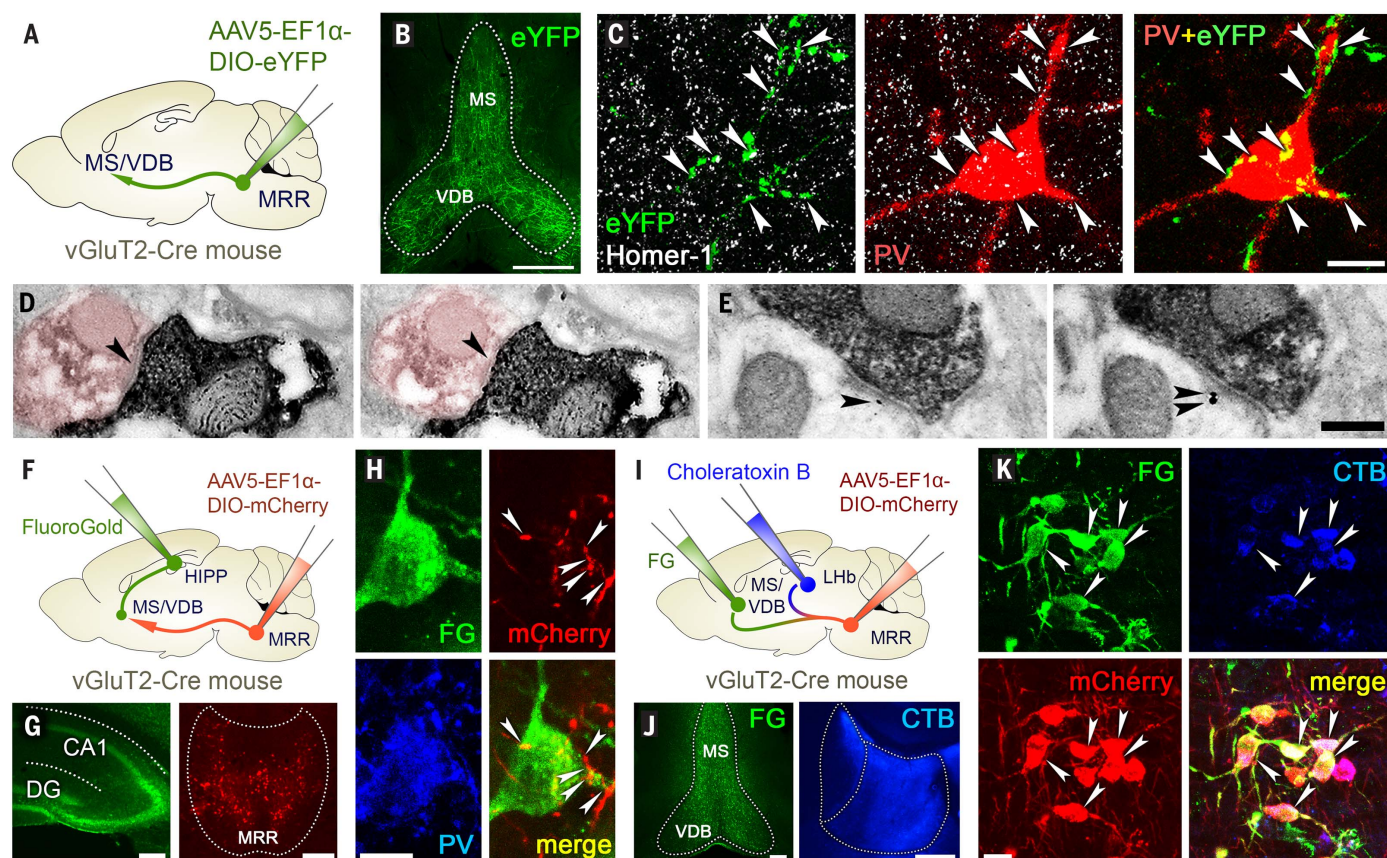


Fig. 7. MRR vGluT2 neurons selectively innervate PV-positive MS/VDB neurons. (A) AAV2/5-EF1 α -DIO-eYFP was injected into the MRR of vGluT2-Cre mice ($n = 3$). (B) MRR vGluT2 neuronal fibers innervate the MS/VDB. Scale bar: 200 μ m. (C) A representative MS/VDB PV-positive neuron (red) is innervated by basket-like multiple synapses of vGluT2-positive MRR fibers (green), establishing Homer 1-positive (white) synaptic contacts (white arrowheads). Scale bar: 10 μ m. (D and E) Electron microscopic images show serial sections of synapses of vGluT2-positive MRR terminals (dark SI-DAB). (D) PV-positive dendrite (false red colored, DAB-Ni precipitate) in the VDB received synaptic contacts (at least 12 synapses were reconstructed; black arrowheads). (E) Synapses contained GluN2A subunit of the NMDA receptors (immunogold labeling, black arrowheads) postsynaptically. Scale bar: 300 nm. (F) AAV2/5-EF1 α -DIO-mCherry was injected into the MRR and FluoroGold into the bilateral

hippocampi of vGluT2-Cre mice ($n = 2$). (G) Representative injection sites in the hippocampus [green FluoroGold (FG) labeling] and MRR (red AAV-mCherry). Scale bars: 200 μ m. (H) AAV-mCherry containing vGluT2-positive MRR terminals (red) establish several putative contacts (white arrowheads) with a FG-positive (green) septohippocampal PV-positive (blue) neuron. Scale bar: 10 μ m. (I) Double retrograde tracing was performed by injecting FG into the MS/VDB and CTB into the Lhb, bilaterally, and AAV2/5-EF1 α -DIO-mCherry was injected into the MRR to virally label vGluT2-positive neurons in vGluT2-Cre mice ($n = 2$). (J) Representative injection sites in the MS/VDB (FG labeling) and in the Lhb (CTB labeling). Scale bars: 200 μ m. (K) MRR vGluT2 neurons (red) contain both FG (green) and CTB retrograde labeling (blue), which shows that bifurcating axons of these neurons simultaneously target both the MS/VDB and Lhb. Scale bar: 20 μ m.

auditory tones, at the end of which they received foot shocks and light illuminations. Light delivery was precisely aligned to foot shocks (Fig. 8E). All mice displayed equally strong immediate reactions to foot shocks. In the tests that followed, mice received no light illumination. Twenty-four hours later, mice were placed into the same environment “A” to test their contextual memories. CTRL mice expressed strong contextual freezing behavior, whereas ArchT mice showed almost no freezing behavior (Fig. 8E). The next day, we placed mice into a different, neutral environment (environment “B”), where ArchT mice showed significantly lower generalized fear compared with CTRL mice (Fig. 8E). Then, in the same neutral environment,

we presented mice with an auditory cue. CTRL mice showed very high levels of freezing. In contrast, light-inhibited ArchT mice showed significantly diminished freezing, indicating impaired fear memory formation (Fig. 8E). Even after cue presentation was completed, the difference between the fear levels of ArchT and CTRL mice remained significant (fig. S6D).

MRR vGluT2 neurons serve as a key hub for the acquisition of negative experience

Animals must recognize adverse events quickly and decide whether to fight or flee, while at the same time efficiently learning the context of that event so that they can predict it in the future. The Lhb and mVTA are activated during the acquisition of negative experience

(7, 23, 24, 40), which initiates the encoding of “negative reward prediction errors” and aversion in these nuclei (5, 41). Activation of mVTA DA neurons is also aversive (23, 42), whereas the activity of lateral VTA neurons plays a role in positive reinforcement. The Lhb contains nearly exclusively glutamatergic neurons, which encode aversive behavior and activate DA neurons of the mVTA (23–25, 43). Lhb activity also indirectly inhibits encoding of positive reinforcement in the lateral VTA (23, 44). These processes fine-tune future strategies in similar situations (5, 41, 45–48). The Lhb and mVTA convey information related to negative predictions and learn to respond to cues that predict aversive stimuli (3, 42, 49–53).

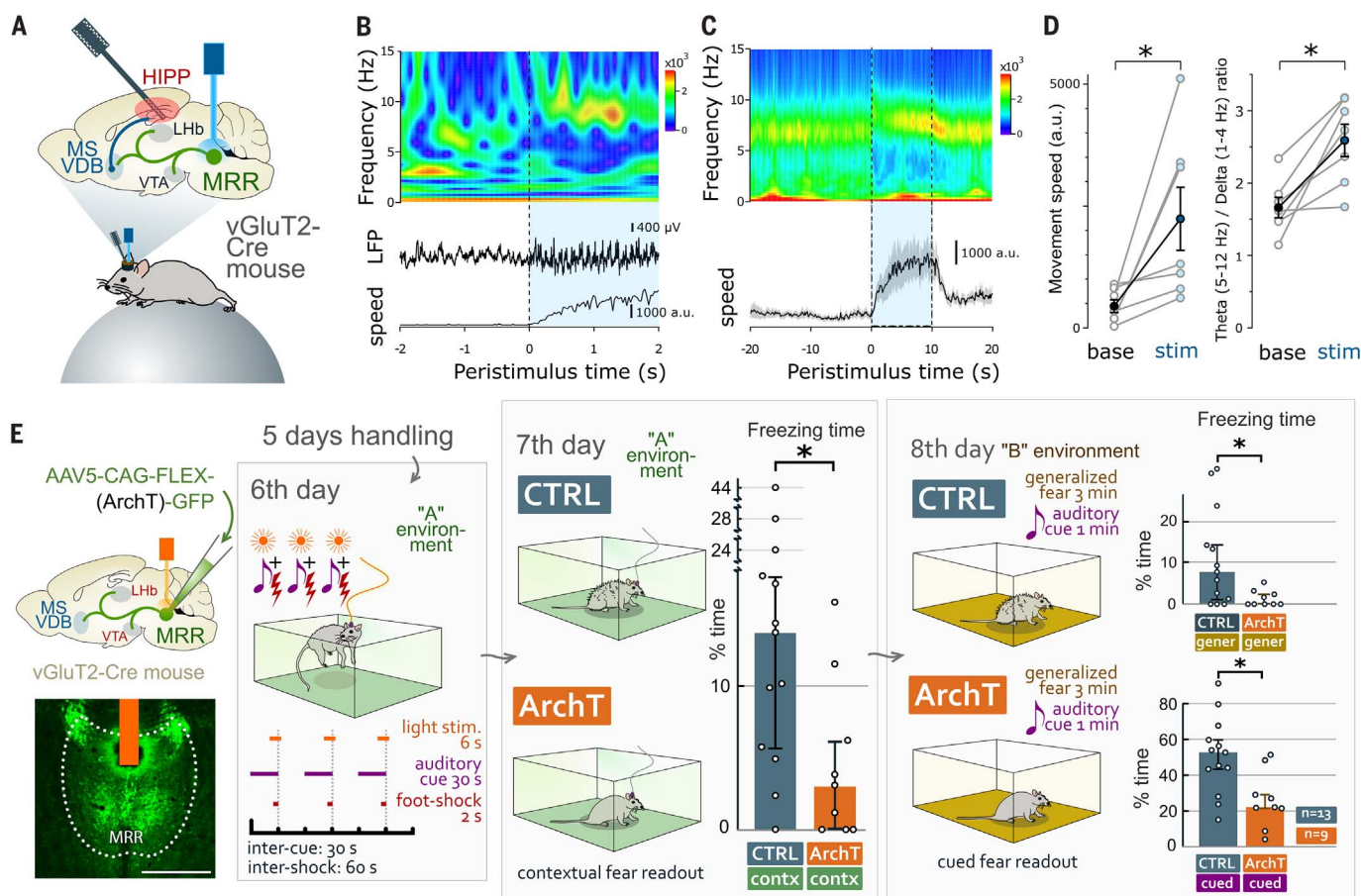


Fig. 8. MRR vGluT2 neurons promote hippocampal theta rhythm and are necessary for fear memory formation. (A) AAV2/5-EF1 α -DIO-ChR2-eYFP was injected into the MRR of vGluT2-Cre mice. An optical fiber was inserted into the MRR and a silicone probe was implanted into the hippocampal CA1 region to measure concurrent hippocampal network activity ($n = 7$ mice). Mice were head-fixed on top of an air-supported spherical treadmill and their behavior was monitored. (B) Running speed (bottom), local field potential (LFP) recordings from the pyramidal layer of the dorsal hippocampal CA1 region (middle), and corresponding wavelet spectrogram (top). Vertical dashed line indicates the onset of laser stimulation, which rapidly induced a switch to theta oscillation. (C) Average running speed (mean $\pm$ SEM) (bottom) and corresponding averaged wavelet spectrogram (seven mice) (top). Vertical dashed lines highlight the MRR laser illumination period. (D) Paired plots indicating movement speed (left) and

theta/delta ratios (right) during baseline (10-s window before laser onset) versus stimulation (10-s window after laser onset) periods (gray, individual animals; black, mean $\pm$ SEM). (E) Design of cued fear conditioning experiments with optogenetic inhibition of MRR vGluT2 neurons. Fluorescent image represents one of the injection sites to label MRR vGluT2 neurons, and the orange area represents the position of the optic fiber. Scale bar: 500 μ m. After handling, light illumination of MRR was switched on precisely during foot shocks that mice received at the end of the auditory cue presentation on day 6. On days 7 and 8, mice received no more light. On day 7, contextual freezing behavior in ArchT mice in the same environment was almost completely diminished (below the 5% threshold) compared with CTRL mice, which received virus without ArchT. On day 8, ArchT mice showed significantly less generalized fear and cued fear in a novel environment compared with CTRL mice. Medians and interquartile ranges are shown on the graphs.

Meanwhile, the MS/VDB-hippocampal system must also be switched into the state optimal for memory acquisition to record the context of such events (8, 14). These processes are also necessary for the prediction and prevention of negative experience in the future. Yet it was not clear which neuronal pathway orchestrates the coordinated activation of these networks. Although the MRR was known to play a central role in processing negative experience (13, 15), its known cell types did not project to the LHB, and the transmitter phenotypes and targets of almost 25% of the MRR neurons were not even known (22).

We discovered that these previously unrecognized vGluT2-positive MRR neurons project heavily into the LHB, mVTA, and MS/VDB (fig. S7). They are the largest population of projection neurons from the MRR, are glutamatergic, and do not express 5-HT, vGluT3, or vGAT. We detected an extensive convergence of monosynaptic inputs to MRR vGluT2 neurons from several environmental experience-related brain centers (fig. S7) (7, 40, 54). MRR vGluT2 neurons predominantly innervate the medial (limbic) division of the LHB that projects to the MRR and mVTA, but they mostly avoid lateral (pallidal) division of the LHB that receives a different set of inputs

(25, 51, 55, 56). MRR vGluT2 neurons directly innervate MRR- or mVTA-projecting LHB cells, creating a direct feedback in the MRR-LHB-mVTA axis.

Our *in vivo* physiological measurements confirmed the central and specific role of MRR vGluT2 neurons in the formation of negative experience. MRR vGluT2 neurons were strongly and specifically activated by strong aversive stimuli, mildly activated by weak aversive stimuli, and unaffected by rewarding stimuli. This suggests that these neurons are primarily responsible and necessary for relaying negative experience in the brainstem. Indeed, specific optogenetic inhibition

of these neurons precisely during the presentation of adverse stimuli eliminated or significantly decreased hippocampus-dependent contextual or hippocampus-independent cued fear memories, respectively. These data showed that this neural hub is essential for processing negative experience.

The elevation of excitatory transmission in the LHB and the stimulation of the mVTA have similar effects (23, 46, 57, 58). MRR vGluT2 neurons heavily project to the LHB and also selectively innervate mVTA DA neurons (but do not innervate neurons in lateral VTA that are mostly reward-related), suggesting that they can effectively activate negative prediction centers. Indeed, their optogenetic activation triggered immediate behavioral changes by inducing acute place aversion. The adverse event was effectively memorized because it induced conditioned place aversion. Furthermore, the selective activation of MRR vGluT2 neurons could negate an otherwise strongly rewarding behavior in an operant conditioning task. The activation of MRR vGluT2 neurons did not induce passive freezing behavior directly; instead, MRR vGluT2 neurons seem to be involved in active responses to negative experience, and therefore they induce avoidance or flight-or-fight behavior.

Depression is thought to be a result of a learning mechanism based on chronically sustained negative experiences (59, 60). Glutamatergic synapses on LHB neurons show long-term plasticity mechanisms, and LHB neurons can learn to respond to cues preceding aversive events (28, 29, 50, 67). The chronic elevation of glutamatergic transmission in the LHB causes depressive disorder through the promotion of burst-firing of LHB neurons, which is also regulated by adjacent glial cell coverage (28–30, 50, 62, 63). MRR vGluT2 neurons massively innervate LHB neurons with different types of NMDA receptor-containing excitatory contacts that are mostly covered by glial processes on LHB neurons. They can also evoke burst-firing of LHB neurons. We revealed an abundant reciprocal excitatory connection between vGluT2-positive glutamatergic neurons of the LHB and MRR that may result in a reverberation and may support pathological learning and excessive activation of LHB neurons if the loop is not controlled effectively. To mimic a chronically maladapted circuitry, we chemogenetically induced chronic overactivation of vGluT2-positive MRR neurons and it caused anhedonia in the sucrose-preference test, which is a typical sign of depressive disorder in mice.

Aggression is another well-known symptom of depression in mice and humans (32–34), and both the LHB and MS/VDB play a role in the emotional processing of aggressive behavior (31). The activation of MRR vGluT2

neurons also promoted aggressive behavior. Aggression is commonly associated with agitation, the most distinguishing features of which include restlessness, pacing, and motor activities. We observed elevated locomotor activity and exploration in chemogenetically stimulated mice, which together suggest that activation of vGluT2-positive MRR neurons promotes agitation and active aversive behaviors (flight-or-fight responses) in neutral or social situations.

MRR has a complex effect on MS/VDB-hippocampal activity and the formation of contextual fear memories, although its mechanism is not well understood (9, 11). In vivo optogenetic activation of MRR promoted memory acquisition-related theta rhythm through a mechanism conveyed by a non-serotonergic and non-GABAergic MRR cell population (13). Activation of pacemaker PV-positive MS/VDB neurons is essential for the generation of hippocampal theta rhythm and proper episodic memory formation (38, 39, 64). PV-positive MS/VDB neurons are innervated by glutamatergic cells of the MRR (18, 65, 66), but the identity of those neurons was unknown. We discovered that MRR vGluT2 neurons not only project to the LHB-mVTA aversion axis, but the axons of most of these MRR vGluT2-positive excitatory neurons bifurcate and simultaneously innervate PV-positive neurons in the MS/VDB and can instantly and reliably promote hippocampal theta rhythm generation necessary for memory acquisition. MRR vGluT2 neurons increased theta rhythm-related exploratory behavior, while animals exhibited conditioned place aversion on the day after stimulation, suggesting the facilitation of strong memory formation. MRR vGluT2 neurons may also facilitate the phase-locking of theta rhythm in the LHB and the hippocampus.

Our data revealed that the MRR vGluT2-positive neuronal population is a previously unrecognized neural hub of the brain that is both necessary and sufficient for the acquisition of negative experience, but which is not activated by positive rewarding experience. MRR vGluT2 neurons receive extensive convergence of inputs from sensory experience-related brain areas, their inhibition disrupts aversive memory formation, and their activation promotes agitated and aversive behavior and flight, which quickly negates motivated behavior. If flight is not an option during social interaction, MRR vGluT2 neurons provoke fight and aggression. MRR vGluT2 neurons also facilitate long-term memory formation related to negative experience, and their chronic activation produces a depression-like phenotype, likely through inducing long-term bursting activity in LHB neurons (28, 30), which we showed in our *in vitro* experiments. Therefore, vGluT2-positive

MRR neurons control the acquisition of negative experience by simultaneously triggering the activity of brain aversion centers and hippocampal episodic memory encoding. Maladaptations in processing negative experience are the basis of several types of mood disorders, which have a huge social and economic impact on individuals and society. Selective targeting of this neural hub may form the basis of new therapies.

Materials and methods summary

Ethical considerations and mouse strains

All experiments were performed in accordance with regulations. For details, see the supplementary methods section of the supplementary materials. We used male and female vGluT2-iRES-Cre, vGAT-iRES-Cre, BAC-vGluT3/iCre, and TpH2/iCre-ERT2 mice and males C57Bl/6 wild-type mice. We used adult (at least 6-week-old) mice.

Viral gene transfer, retrograde and mono-transsynaptic rabies tracing, and optic fiber implantations

Mice were anesthetized and mounted in an animal stereotaxic frame. A microinjector pump was used for injections. For anterograde tracing, optogenetic and chemogenetic experiments, we injected different amounts of viruses into the target brain areas. For retrograde tracing experiments, we injected FluoroGold or cholera toxin B subunit into the target areas. For behavioral experiments, 4 to 6 weeks after virus injections, optic fibers were implanted over the MRR. Positions of the optic fibers are illustrated in fig. S5. See (26) for a detailed description of the monosynaptic rabies tracing technique used.

Perfusions

Mice were anesthetized and were perfused transcardially with different solutions, depending on the experiments. After perfusions, brains were cut into 30- μ m-thick sections using a sliding microtome or into 50-, 60-, or 100- μ m-thick sections using a vibratome.

Immunohistochemistry and antibodies

Generally, perfusion-fixed sections were cryoprotected, and after antigen retrieval they were incubated in a blocking medium, and sections were incubated in a mixture of primary antibodies. This was followed by extensive washes and incubation in the mixture of appropriate secondary antibodies. Then, sections were placed on slides and covered with mounting medium. Fluorescent immunohistochemistry for laser-scanning confocal microscopy, immunoperoxidase labeling for localization of virus injections, immunogold-immunoperoxidase double labeling for electron microscopy, silver-gold intensified and nickel-intensified immunoperoxidase double

labeling, and sample preparation for 3D block-face scanning electron microscopy required different procedures, which are described in detail in the supplementary methods.

The list and specifications of the primary and secondary antibodies used can be found in tables S1 and S2. Combinations of the used primary and secondary antibodies in the different experiments are listed in tables S3 and S4.

Block-face scanning electron microscopy and image analysis

We used an FEI Apreo VolumeScope. Images in the z-stacks measured 16,384 pixels $\times$ 16,383 pixels with 70 nm thickness, and the final z-stacks contained 300 slices. Voxel size was 4 nm $\times$ 4 nm $\times$ 70 nm. All image post-processing was done in FIJI ImageJ. Segmentation was performed manually. Data were analyzed using Tibco Statistica 13.4. After segmentation, the models were exported in .obj format and imported into Blender (Blender Foundation, Amsterdam, the Netherlands) for further investigation and visualization.

Stereology measurements

Unbiased design-based stereological measurements were carried out using the optical fractionator method. Cell counting was carried out in Stereo Investigator 10.0 stereology software, while cells were identified parallel using NIS Elements AR 4.3 software.

In vitro electrophysiological experiments

In all slice experiments, mice were decapitated under deep isoflurane anesthesia. The brain was removed, and coronal slices of 300 μ m thickness were cut using a vibratome. Slices were placed into an interface-type holding chamber, and cells were recorded. Drugs were administered from stock solutions via pipettes into the ACSF-containing superfusion system. All data were processed and analyzed off-line using standard built-in functions of Python 2.7.0. Latency was defined as a time between light stimulus onset and time of reaching 10% of maximal EPSC amplitude. Throughout this article, for in vitro electrophysiology data, we used median, first, and third quartiles for the description of data groups because they did not show Gaussian distribution.

In vivo electrophysiological recordings in head-fixed mice

Animals were anesthetized and mounted in a stereotaxic frame. After surgery, mice were continuously monitored. After a recovery period of 3 to 7 days, mice were water-restricted and habituated for 10 to 14 days to the spherical treadmill setup with their head fixed. Ani-

mal weight was monitored, and when a mouse reached 85% of the initially measured baseline weight, the daily volume of water supplement was individually adjusted in order to maintain this 85% target weight. Habituation is described in the supplementary methods. On the day of the experiment, the animal was head-fixed above the air-supported spherical treadmill. Hippocampal and median raphe silicone probes were lowered through the cranial window. Ground electrode was placed above the cerebellum. Electrophysiological recordings were performed by a signal multiplexing head-stage. At the end of each recording session, brief laser pulses were applied for offline identification of responsive units. Units (i.e., neurons) were categorized as “tagged” if their firing followed the laser pulse with short latency (within 4 ms) and >50% success rate.

The mean baseline firing rate values and standard deviations were calculated from a 4-s time window preceding stimulus onset. A change in firing rate was declared significant if it deviated more than 2 standard deviations from the mean baseline value (z -score larger than 2 or smaller than -2) in a 200-ms time window after stimulus onset. Both responsive neurons (which increased their firing frequency) and nonresponsive neurons distributed across animals. All statistical analyses were performed with standard Igor Pro 8 functions.

Optogenetic behavioral experiments

After optic fiber implantations, mice were transferred to the animal room of the behavioral unit of the institute to rest and then received 5 days of handling. We carried out real-time place-aversion tests (RTPA), conditioned place-aversion tests (CPA), operant conditioning tests, optogenetic contextual fear conditioning tests (Opto-CFC), and delayed cued-fear conditioning tests (CuedFC). The experimenters evaluating behavioral experiments were blind to the conditions and treatment of the mice. Experimental data were collected and analyzed using the Noldus EthoVision 13.0 and Tibco Statistica 13.4 software.

Behavioral test battery for chemogenetic experiments

We carried out a series of behavioral tests with mice that were injected either with the hM3Dq-containing virus [that expressed a clozapine-*N*-oxide (CNO)-sensitive, excitatory G protein-coupled receptor plus a fluorescent protein] or with a control virus (that expressed only a fluorescent protein). Mice were allowed 48 to 72 hours of rest between tests. To activate the hM3Dq virus, CNO (1 mg/kg in 10 ml saline, Tocris) was injected intraperitoneally 30 min before each behavioral test. Tests were video recorded and analyzed later by an observer blind to the treatment protocols. After this battery of behavioral tests, mice were

sacrificed, and their adrenal glands were removed and weighed.

REFERENCES AND NOTES

1. E. S. Bromberg-Martin, M. Matsumoto, H. Nakahara, O. Hikosaka, Multiple timescales of memory in lateral habenula and dopamine neurons. *Neuron* **67**, 499–510 (2010). doi: [10.1016/j.neuron.2010.06.031](https://doi.org/10.1016/j.neuron.2010.06.031); pmid: [20696385](https://pubmed.ncbi.nlm.nih.gov/20696385/)
2. E. S. Bromberg-Martin, O. Hikosaka, Lateral habenula neurons signal errors in the prediction of reward information. *Nat. Neurosci.* **14**, 1209–1216 (2011). doi: [10.1038/nn.2902](https://doi.org/10.1038/nn.2902); pmid: [21857659](https://pubmed.ncbi.nlm.nih.gov/21857659/)
3. E. S. Bromberg-Martin, M. Matsumoto, O. Hikosaka, Distinct tonic and phasic anticipatory activity in lateral habenula and dopamine neurons. *Neuron* **67**, 144–155 (2010). doi: [10.1016/j.neuron.2010.06.016](https://doi.org/10.1016/j.neuron.2010.06.016); pmid: [20624598](https://pubmed.ncbi.nlm.nih.gov/20624598/)
4. C. A. Orsini, D. E. Moorman, J. W. Young, B. Setlow, S. B. Floresco, Neural mechanisms regulating different forms of risk-related decision-making: Insights from animal models. *Neurosci. Biobehav. Rev.* **58**, 147–167 (2015). doi: [10.1016/j.neubiorev.2015.04.009](https://doi.org/10.1016/j.neubiorev.2015.04.009); pmid: [26072028](https://pubmed.ncbi.nlm.nih.gov/26072028/)
5. C. M. Stopper, S. B. Floresco, What's better for me? Fundamental role for lateral habenula in promoting subjective decision biases. *Nat. Neurosci.* **17**, 33–35 (2014). doi: [10.1038/nn.3587](https://doi.org/10.1038/nn.3587); pmid: [24270185](https://pubmed.ncbi.nlm.nih.gov/24270185/)
6. C. M. Stopper, M. T. L. Tse, D. R. Montes, C. R. Wiedman, S. B. Floresco, Overriding phasic dopamine signals redirects action selection during risk/reward decision making. *Neuron* **84**, 177–189 (2014). doi: [10.1016/j.neuron.2014.08.033](https://doi.org/10.1016/j.neuron.2014.08.033); pmid: [25220811](https://pubmed.ncbi.nlm.nih.gov/25220811/)
7. P. M. Baker, S. E. Oh, K. S. Kidder, S. J. Y. Mizumori, Ongoing behavioral state information signaled in the lateral habenula guides choice flexibility in freely moving rats. *Front. Behav. Neurosci.* **9**, 295 (2015). doi: [10.3389/fnbeh.2015.00295](https://doi.org/10.3389/fnbeh.2015.00295); pmid: [26582981](https://pubmed.ncbi.nlm.nih.gov/26582981/)
8. R. Goutagny *et al.*, Interactions between the lateral habenula and the hippocampus: Implication for spatial memory processes. *Neuropsychopharmacology* **38**, 2418–2426 (2013). doi: [10.1038/npp.2013.142](https://doi.org/10.1038/npp.2013.142); pmid: [23736315](https://pubmed.ncbi.nlm.nih.gov/23736315/)
9. R. Crooks, J. Jackson, B. H. Bland, Dissociable pathways facilitate theta and non-theta states in the median raphe–Septohippocampal circuit. *Hippocampus* **22**, 1567–1576 (2012). doi: [10.1002/hipo.20999](https://doi.org/10.1002/hipo.20999); pmid: [22180148](https://pubmed.ncbi.nlm.nih.gov/22180148/)
10. A. Domonkos *et al.*, Divergent in vivo activity of non-serotonergic and serotonergic VGLUT3-neurons in the median raphe region. *J. Physiol.* **594**, 3775–3790 (2016). doi: [10.1113/JP272036](https://doi.org/10.1113/JP272036); pmid: [27028801](https://pubmed.ncbi.nlm.nih.gov/27028801/)
11. J. Jackson, C. T. Dickson, B. H. Bland, Median raphe stimulation disrupts hippocampal theta via rapid inhibition and state-dependent phase reset of theta-related neural circuitry. *J. Neurophysiol.* **99**, 3009–3026 (2008). doi: [10.1152/jn.00065.2008](https://doi.org/10.1152/jn.00065.2008); pmid: [18436639](https://pubmed.ncbi.nlm.nih.gov/18436639/)
12. G. Viana Di Prisco, Z. Albo, R. P. Vertes, B. Kocsis, Discharge properties of neurons of the median raphe nucleus during hippocampal theta rhythm in the rat. *Exp. Brain Res.* **145**, 383–394 (2002). doi: [10.1007/s00221-002-1123-8](https://doi.org/10.1007/s00221-002-1123-8); pmid: [12136388](https://pubmed.ncbi.nlm.nih.gov/12136388/)
13. D. V. Wang *et al.*, Mesopontine median raphe regulates hippocampal ripple oscillation and memory consolidation. *Nat. Neurosci.* **18**, 728–735 (2015). doi: [10.1038/nn.3998](https://doi.org/10.1038/nn.3998); pmid: [25867120](https://pubmed.ncbi.nlm.nih.gov/25867120/)
14. H. Aizawa *et al.*, The synchronous activity of lateral habenula neurons is essential for regulating hippocampal theta oscillation. *J. Neurosci.* **33**, 8909–8921 (2013). doi: [10.1523/JNEUROSCI.4369-12.2013](https://doi.org/10.1523/JNEUROSCI.4369-12.2013); pmid: [23678132](https://pubmed.ncbi.nlm.nih.gov/23678132/)
15. T. G. C. S. Andrade, H. Zangrossi Jr., F. G. Graeff, The median raphe nucleus in anxiety revisited. *J. Psychopharmacol.* **27**, 1107–1115 (2013). doi: [10.1177/0269881113499208](https://doi.org/10.1177/0269881113499208); pmid: [23999409](https://pubmed.ncbi.nlm.nih.gov/23999409/)
16. D. G. Balázsfi *et al.*, Median raphe region stimulation alone generates remote, but not recent fear memory traces. *PLOS ONE* **12**, e0181264 (2017). doi: [10.1371/journal.pone.0181264](https://doi.org/10.1371/journal.pone.0181264); pmid: [28708877](https://pubmed.ncbi.nlm.nih.gov/28708877/)
17. T. F. Freund, A. I. Gulyás, L. Acsády, T. Görös, K. Tóth, Serotonergic control of the hippocampus via local inhibitory interneurons. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8501–8505 (1990). doi: [10.1073/pnas.87.21.8501](https://doi.org/10.1073/pnas.87.21.8501); pmid: [1700433](https://pubmed.ncbi.nlm.nih.gov/1700433/)
18. J. Jackson, B. H. Bland, M. C. Antle, Nonserotonergic projection neurons in the midbrain raphe nuclei contain the vesicular glutamate transporter VGLUT3. *Synapse* **63**, 31–41 (2009). doi: [10.1002/syn.20581](https://doi.org/10.1002/syn.20581); pmid: [18925658](https://pubmed.ncbi.nlm.nih.gov/18925658/)

19. V. Varga *et al.*, Fast synaptic subcortical control of hippocampal circuits. *Science* **326**, 449–453 (2009). doi: [10.1126/science.1178307](https://doi.org/10.1126/science.1178307); pmid: [19833972](https://pubmed.ncbi.nlm.nih.gov/19833972/)
20. S. J. Bang, P. Jensen, S. M. Dymecki, K. G. Commons, Projections and interconnections of genetically defined serotonin neurons in mice. *Eur. J. Neurosci.* **35**, 85–96 (2012). doi: [10.1111/j.1460-9568.2011.07936.x](https://doi.org/10.1111/j.1460-9568.2011.07936.x); pmid: [22151329](https://pubmed.ncbi.nlm.nih.gov/22151329/)
21. C. Leranth, R. P. Vertes, Median raphe serotonergic innervation of medial septum/diagonal band of broca (MSDB) parvalbumin-containing neurons: Possible involvement of the MSDB in the desynchronization of the hippocampal EEG. *J. Comp. Neurol.* **410**, 586–598 (1999). doi: [10.1002/\(SICI\)1096-9861\(19990809\)410:4<586::AID-CNE6>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1096-9861(19990809)410:4<586::AID-CNE6>3.0.CO;2-H); pmid: [10398050](https://pubmed.ncbi.nlm.nih.gov/10398050/)
22. K. E. Sos *et al.*, Cellular architecture and transmitter phenotypes of neurons of the mouse median raphe region. *Brain Struct. Funct.* **222**, 287–299 (2017). doi: [10.1007/s00429-016-1217-x](https://doi.org/10.1007/s00429-016-1217-x); pmid: [27044051](https://pubmed.ncbi.nlm.nih.gov/27044051/)
23. S. Lammel *et al.*, Input-specific control of reward and aversion in the ventral tegmental area. *Nature* **491**, 212–217 (2012). doi: [10.1038/nature11527](https://doi.org/10.1038/nature11527); pmid: [23064228](https://pubmed.ncbi.nlm.nih.gov/23064228/)
24. S. Lammel, B. K. Lim, R. C. Malenka, Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology* **76**, 351–359 (2014). doi: [10.1016/j.neuropharm.2013.03.019](https://doi.org/10.1016/j.neuropharm.2013.03.019); pmid: [23578393](https://pubmed.ncbi.nlm.nih.gov/23578393/)
25. L. A. Quina *et al.*, Efferent pathways of the mouse lateral habenula. *J. Comp. Neurol.* **523**, 32–60 (2015). doi: [10.1002/cne.23662](https://doi.org/10.1002/cne.23662); pmid: [25099741](https://pubmed.ncbi.nlm.nih.gov/25099741/)
26. I. R. Wickersham *et al.*, Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* **53**, 639–647 (2007). doi: [10.1016/j.neuron.2007.01.033](https://doi.org/10.1016/j.neuron.2007.01.033); pmid: [17329205](https://pubmed.ncbi.nlm.nih.gov/17329205/)
27. A. Szőnyi *et al.*, Brainstem nucleus incertus controls contextual memory formation. *Science* **364**, eaaw0445 (2019). doi: [10.1126/science.aaw0445](https://doi.org/10.1126/science.aaw0445); pmid: [31123108](https://pubmed.ncbi.nlm.nih.gov/31123108/)
28. Y. Cui *et al.*, Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression. *Nature* **554**, 323–327 (2018). doi: [10.1038/nature25752](https://doi.org/10.1038/nature25752); pmid: [29446379](https://pubmed.ncbi.nlm.nih.gov/29446379/)
29. K. Li *et al.*, β CaMKII in lateral habenula mediates core symptoms of depression. *Science* **341**, 1016–1020 (2013). doi: [10.1126/science.1240729](https://doi.org/10.1126/science.1240729); pmid: [23990563](https://pubmed.ncbi.nlm.nih.gov/23990563/)
30. Y. Yang *et al.*, Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature* **554**, 317–322 (2018). doi: [10.1038/nature25509](https://doi.org/10.1038/nature25509); pmid: [29446381](https://pubmed.ncbi.nlm.nih.gov/29446381/)
31. S. A. Golden *et al.*, Basal forebrain projections to the lateral habenula modulate aggression reward. *Nature* **534**, 688–692 (2016). doi: [10.1038/nature18601](https://doi.org/10.1038/nature18601); pmid: [27357796](https://pubmed.ncbi.nlm.nih.gov/27357796/)
32. L. A. Martin, H. W. Neighbors, D. M. Griffith, The experience of symptoms of depression in men vs women: Analysis of the National Comorbidity Survey Replication. *JAMA Psychiatry* **70**, 1100–1106 (2013). doi: [10.1001/jamapsychiatry.2013.1985](https://doi.org/10.1001/jamapsychiatry.2013.1985); pmid: [23986338](https://pubmed.ncbi.nlm.nih.gov/23986338/)
33. N. Verdolini *et al.*, Aggressiveness in depression: A neglected symptom possibly associated with bipolarity and mixed features. *Acta Psychiatr. Scand.* **136**, 362–372 (2017). doi: [10.1111/acps.12777](https://doi.org/10.1111/acps.12777); pmid: [28741646](https://pubmed.ncbi.nlm.nih.gov/28741646/)
34. C. R. Yang *et al.*, Enhanced aggressive behaviour in a mouse model of depression. *Neurotox. Res.* **27**, 129–142 (2015). doi: [10.1007/s12640-014-9498-4](https://doi.org/10.1007/s12640-014-9498-4); pmid: [25367807](https://pubmed.ncbi.nlm.nih.gov/25367807/)
35. B. Li *et al.*, Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* **470**, 535–539 (2011). doi: [10.1038/nature09742](https://doi.org/10.1038/nature09742); pmid: [21350486](https://pubmed.ncbi.nlm.nih.gov/21350486/)
36. S. J. Russo, E. J. Nestler, The brain reward circuitry in mood disorders. *Nat. Rev. Neurosci.* **14**, 609–625 (2013). doi: [10.1038/nrn3381](https://doi.org/10.1038/nrn3381); pmid: [23942470](https://pubmed.ncbi.nlm.nih.gov/23942470/)
37. G. Buzsáki, E. I. Moser, Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nat. Neurosci.* **16**, 130–138 (2013). doi: [10.1038/nrn3304](https://doi.org/10.1038/nrn3304); pmid: [23354386](https://pubmed.ncbi.nlm.nih.gov/23354386/)
38. B. Hangya, Z. Borhegyi, N. Szilágyi, T. F. Freund, V. Varga, GABAergic neurons of the medial septum lead the hippocampal network during theta activity. *J. Neurosci.* **29**, 8094–8102 (2009). doi: [10.1523/JNEUROSCI.5665-08.2009](https://doi.org/10.1523/JNEUROSCI.5665-08.2009); pmid: [19553449](https://pubmed.ncbi.nlm.nih.gov/19553449/)
39. Z. Borhegyi, V. Varga, N. Szilágyi, D. Fábó, T. F. Freund, Phase segregation of medial septal GABAergic neurons during hippocampal theta activity. *J. Neurosci.* **24**, 8470–8479 (2004). doi: [10.1523/JNEUROSCI.1413-04.2004](https://doi.org/10.1523/JNEUROSCI.1413-04.2004); pmid: [15456820](https://pubmed.ncbi.nlm.nih.gov/15456820/)
40. D. J. Barker *et al.*, Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission. *Cell Rep.* **21**, 1757–1769 (2017). doi: [10.1016/j.celrep.2017.10.066](https://doi.org/10.1016/j.celrep.2017.10.066); pmid: [29141211](https://pubmed.ncbi.nlm.nih.gov/29141211/)
41. M. Stephenson-Jones *et al.*, A basal ganglia circuit for evaluating action outcomes. *Nature* **539**, 289–293 (2016). doi: [10.1038/nature19845](https://doi.org/10.1038/nature19845); pmid: [27652894](https://pubmed.ncbi.nlm.nih.gov/27652894/)
42. J. W. de Jong *et al.*, A neural circuit mechanism for encoding aversive stimuli in the mesolimbic dopamine system. *Neuron* **101**, 133–151.e7 (2019). doi: [10.1016/j.neuron.2018.11.005](https://doi.org/10.1016/j.neuron.2018.11.005); pmid: [30503173](https://pubmed.ncbi.nlm.nih.gov/30503173/)
43. K. Brinschwitz *et al.*, Glutamatergic axons from the lateral habenula mainly terminate on GABAergic neurons of the ventral midbrain. *Neuroscience* **168**, 463–476 (2010). doi: [10.1016/j.neuroscience.2010.03.050](https://doi.org/10.1016/j.neuroscience.2010.03.050); pmid: [20353812](https://pubmed.ncbi.nlm.nih.gov/20353812/)
44. T. C. Jhou, S. Geisler, M. Marinelli, B. A. Degarmo, D. S. Zahm, 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.* **513**, 566–596 (2009). doi: [10.1002/cne.21891](https://doi.org/10.1002/cne.21891); pmid: [19235216](https://pubmed.ncbi.nlm.nih.gov/19235216/)
45. S. Hong, T. C. Jhou, M. Smith, K. S. Saleem, O. Hikosaka, Negative reward signals from the lateral habenula to dopamine neurons are mediated by rostromedial tegmental nucleus in primates. *J. Neurosci.* **31**, 11457–11471 (2011). doi: [10.1523/JNEUROSCI.1384-11.2011](https://doi.org/10.1523/JNEUROSCI.1384-11.2011); pmid: [21832176](https://pubmed.ncbi.nlm.nih.gov/21832176/)
46. A. M. Stamatakis, G. D. Stuber, Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance. *Nat. Neurosci.* **15**, 1105–1107 (2012). doi: [10.1038/nn.3145](https://doi.org/10.1038/nn.3145); pmid: [22729176](https://pubmed.ncbi.nlm.nih.gov/22729176/)
47. J. Tian, N. Uchida, Habenula lesions reveal that multiple mechanisms underlie dopamine prediction errors. *Neuron* **87**, 1304–1316 (2015). doi: [10.1016/j.neuron.2015.08.028](https://doi.org/10.1016/j.neuron.2015.08.028); pmid: [26365765](https://pubmed.ncbi.nlm.nih.gov/26365765/)
48. M. Matsumoto, O. Hikosaka, Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* **447**, 1111–1115 (2007). doi: [10.1038/nature05860](https://doi.org/10.1038/nature05860); pmid: [17522629](https://pubmed.ncbi.nlm.nih.gov/17522629/)
49. H. Li, D. Pullmann, T. C. Jhou, Valence-encoding in the lateral habenula arises from the entopeduncular region. *eLife* **8**, e41223 (2019). doi: [10.7554/eLife.41223](https://doi.org/10.7554/eLife.41223); pmid: [30855228](https://pubmed.ncbi.nlm.nih.gov/30855228/)
50. F. J. Meye *et al.*, Cocaine-evoked negative symptoms require AMPA receptor trafficking in the lateral habenula. *Nat. Neurosci.* **18**, 376–378 (2015). doi: [10.1038/nn.3923](https://doi.org/10.1038/nn.3923); pmid: [25643299](https://pubmed.ncbi.nlm.nih.gov/25643299/)
51. C. D. Proulx, O. Hikosaka, R. Malinow, Reward processing by the lateral habenula in normal and depressive behaviors. *Nat. Neurosci.* **17**, 1146–1152 (2014). doi: [10.1038/nn.3779](https://doi.org/10.1038/nn.3779); pmid: [25157511](https://pubmed.ncbi.nlm.nih.gov/25157511/)
52. M. Trusel *et al.*, Punishment-predictive cues guide avoidance through potentiation of hypothalamus-to-habenula synapses. *Neuron* **102**, 120–127.e4 (2019). doi: [10.1016/j.neuron.2019.01.025](https://doi.org/10.1016/j.neuron.2019.01.025); pmid: [30765165](https://pubmed.ncbi.nlm.nih.gov/30765165/)
53. D. Wang *et al.*, Learning shapes the aversion and reward responses of lateral habenula neurons. *eLife* **6**, e23045 (2017). doi: [10.7554/eLife.23045](https://doi.org/10.7554/eLife.23045); pmid: [28561735](https://pubmed.ncbi.nlm.nih.gov/28561735/)
54. D. H. Root, C. A. Mejias-Aponte, J. Qi, M. Morales, Role of glutamatergic projections from ventral tegmental area to lateral habenula in aversive conditioning. *J. Neurosci.* **34**, 13906–13910 (2014). doi: [10.1523/JNEUROSCI.2029-14.2014](https://doi.org/10.1523/JNEUROSCI.2029-14.2014); pmid: [25319687](https://pubmed.ncbi.nlm.nih.gov/25319687/)
55. D. S. Zahm, D. H. Root, Review of the cytology and connections of the lateral habenula, an avatar of adaptive behaving. *Pharmacol. Biochem. Behav.* **162**, 3–21 (2017). doi: [10.1016/j.pbb.2017.06.004](https://doi.org/10.1016/j.pbb.2017.06.004); pmid: [28647565](https://pubmed.ncbi.nlm.nih.gov/28647565/)
56. U. Kim, Topographic midbrain and descending projections of the habenula in the rat. *J. Comp. Neurol.* **513**, 173–187 (2009). doi: [10.1002/cne.21951](https://doi.org/10.1002/cne.21951); pmid: [19123238](https://pubmed.ncbi.nlm.nih.gov/19123238/)
57. J. Tooley *et al.*, Glutamatergic ventral pallidum neurons modulate activity of the habenula-ventral tegmental circuitry and constrain reward seeking. *Biol. Psychiatry* **83**, 1012–1023 (2018). doi: [10.1016/j.biopsych.2018.01.003](https://doi.org/10.1016/j.biopsych.2018.01.003); pmid: [29452828](https://pubmed.ncbi.nlm.nih.gov/29452828/)
58. A. M. Stamatakis *et al.*, Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward. *J. Neurosci.* **36**, 302–311 (2016). doi: [10.1523/JNEUROSCI.1202-15.2016](https://doi.org/10.1523/JNEUROSCI.1202-15.2016); pmid: [26758824](https://pubmed.ncbi.nlm.nih.gov/26758824/)
59. J. Kauffling, Alterations and adaptation of ventral tegmental area dopaminergic neurons in animal models of depression. *Cell Tissue Res.* **377**, 59–71 (2019). doi: [10.1007/s00441-019-03007-9](https://doi.org/10.1007/s00441-019-03007-9); pmid: [30848354](https://pubmed.ncbi.nlm.nih.gov/30848354/)
60. S. F. Maier, M. E. P. Seligman, Learned helplessness at fifty: Insights from neuroscience. *Psychol. Rev.* **123**, 349–367 (2016). doi: [10.1037/rev0000033](https://doi.org/10.1037/rev0000033); pmid: [27337390](https://pubmed.ncbi.nlm.nih.gov/27337390/)
61. I. Lazaridis *et al.*, A hypothalamus-habenula circuit controls aversion. *Mol. Psychiatry* **24**, 1351–1368 (2019). doi: [10.1038/s41380-019-0369-5](https://doi.org/10.1038/s41380-019-0369-5); pmid: [30755721](https://pubmed.ncbi.nlm.nih.gov/30755721/)
62. D. Knowland *et al.*, Distinct ventral pallidal neural populations mediate separate symptoms of depression. *Cell* **170**, 284–297.e18 (2017). doi: [10.1016/j.cell.2017.06.015](https://doi.org/10.1016/j.cell.2017.06.015); pmid: [28689640](https://pubmed.ncbi.nlm.nih.gov/28689640/)
63. A. Friedman *et al.*, Electrical stimulation of the lateral habenula produces an inhibitory effect on sucrose self-administration. *Neuropharmacology* **60**, 381–387 (2011). doi: [10.1016/j.neuropharm.2010.10.006](https://doi.org/10.1016/j.neuropharm.2010.10.006); pmid: [20955718](https://pubmed.ncbi.nlm.nih.gov/20955718/)
64. G. Gangadharan *et al.*, Medial septal GABAergic projection neurons promote object exploration behavior and type 2 theta rhythm. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 6550–6555 (2016). doi: [10.1073/pnas.1605019113](https://doi.org/10.1073/pnas.1605019113); pmid: [27208094](https://pubmed.ncbi.nlm.nih.gov/27208094/)
65. S. Aznar, Z. X. Qian, G. M. Knudsen, Non-serotonergic dorsal and median raphe projection onto parvalbumin- and calbindin-containing neurons in hippocampus and septum. *Neuroscience* **124**, 573–581 (2004). doi: [10.1016/j.neuroscience.2003.12.020](https://doi.org/10.1016/j.neuroscience.2003.12.020); pmid: [14980728](https://pubmed.ncbi.nlm.nih.gov/14980728/)
66. A. Szőnyi *et al.*, The ascending median raphe projections are mainly glutamatergic in the mouse forebrain. *Brain Struct. Funct.* **221**, 735–751 (2016). doi: [10.1007/s00429-014-0935-1](https://doi.org/10.1007/s00429-014-0935-1); pmid: [25381463](https://pubmed.ncbi.nlm.nih.gov/25381463/)
67. A. M. Barth, LFP_analysis, software, Figshare (2019); <https://doi.org/10.6084/m9.figshare.8924771.v1>

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Competing interests: The authors have no competing interests. **Data and materials availability:** Viruses used in this study were obtained under material transfer agreements, as follows: AAV2/5-EFlα-DIO-eYFP, AAV2/5-EFlα-DIO-mCherry, and AAV2/5-CAG-FLEX-ArchT-GFP from the UNC Vector Core; AAV2/5-EFlα-DIO-hChR2 (H134R)-eYFP from the Penn Vector Core; AAV2/8-hSyn-DIO-mCherry and AAV2/8-hSyn-DIO-hm3D(Gq)-mCherry from Addgene; and AAV2/8-hSyn-FLEX-TVA-p2A-eGFP-p2A-oG and Rabies(ΔG)-EnvA-mCherry from the Salk GT3 Vector Core. Data generated and analyzed during this study are presented in the manuscript or in the supplementary materials, and additional custom written codes for in vivo electrophysiological recordings are available online (67).

SUPPLEMENTARY MATERIALS

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Median raphe controls acquisition of negative experience in the mouse

András Szonyi, Krisztián Zichó, Albert M. Barth, Roland T. Gönczi, Dániel Schlingloff, Bibiána Török, Eszter Sipos, Abel Major, Zsuzsanna Bardóci, Katalin E. Sos, Attila I. Gulyás, Viktor Varga, Dóra Zelena, Tamás F. Freund and Gábor Nyiri

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Keeping tabs on bad experiences

Identifying the neural basis underlying how we acquire, process, and store negative experiences could help the search for effective treatments for mood disorders. Szonyi *et al.* used a range of neuroscientific tools to elucidate the role of a specific neural circuit that originates in the median raphe region of the murine brainstem (see the Perspective by Ikemoto). A subpopulation of excitatory neurons projected to aversive brain areas and received recurrent feedback from the lateral habenula and convergent feedback from a range of fear-related circuitry. These neurons were activated by aversive stimuli, and artificial stimulation promoted aversion or anxiety-related behavior. This group of cells thus plays a pivotal role in a network that helps to mediate aversive motivation.

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