NEUROSCIENCE

Fear balance is maintained by bodily feedback to the insular cortex in mice

Alexandra S. Klein[1,2], Nate Dolensek[1,3], Caroline Weiand[1,4]†, Nadine Gogolla[1]*†

How does the brain maintain fear within an adaptive range? We found that the insular cortex acts as a state-dependent regulator of fear that is necessary to establish an equilibrium between the extinction and maintenance of fear memories in mice. Whereas insular cortex responsiveness to fear-evoking cues increased with their certainty to predict harm, this activity was attenuated through negative bodily feedback that arose from heart rate decelerations during freezing. Perturbation of body-brain communication by vagus nerve stimulation disrupted the balance between fear extinction and maintenance similar to insular cortex inhibition. Our data reveal that the insular cortex integrates predictive sensory and interoceptive signals to provide graded and bidirectional teaching signals that gate fear extinction and illustrate how bodily feedback signals are used to maintain fear within a functional equilibrium.

Fear is essential for survival but has to be maintained within a functional balance that minimizes risk-taking while allowing for the pursuit of important needs (*1*). Next to defensive behaviors, fear evokes strong bodily reactions, such as changes in heart and breathing rates (*2*–*5*). Although bodily feedback signals to the brain are thought to play a crucial role in emotion regulation (*6*–*9*), the neural mechanisms that mediate fear regulation through bodily feedback are incompletely understood. The insular cortex (InsCtx) is a core region involved in the processing of bodily signals, also referred to as interoception (*10*). It receives strong inputs from distinct thalamic and brainstem nuclei that transmit visceral and cardiovascular signals from the periphery to the brain (*11*–*13*). Data from human and other animals have implicated the InsCtx in fear and extinction learning, as well as the learning of safety (*14*–*21*). In addition, human neuroimaging studies have identified structural and functional alterations of the InsCtx as a hallmark of anxiety disorders (*6*, *22*). We therefore sought to address how the interoceptive InsCtx may contribute to fear regulation.

To investigate the influence of InsCtx activity on fear regulation, we performed classical auditory fear conditioning (FC) and used optogenetic inhibition during fear extinction learning in mice (Fig. 1A). Viral vectors leading to excitatory-neuron specific expression of an inhibitory opsin [halorhodopsin (NpHR)] or a control protein [enhanced yellow fluorescent protein (eYFP)] were bilaterally injected into the visceral InsCtx. Optical fibers were placed above the injection sites (Fig. 1B and fig. S1). Efficacy of NpHR-mediated neuronal inhibition in behaving animals was confirmed using extracellular recordings of single units (fig. S2). We first used a relatively weak FC protocol with mildly aversive footshocks as unconditioned stimuli (US), resulting in moderate fear levels [measured as freezing in response to the conditioned stimulus (CS+) during recall; see supplementary materials for details on experimental design and a glossary of key terms]. InsCtx inhibition during the presentation of the CS+ throughout the extinction session led to acute facilitation of extinction and improved extinction performance (measured as the normalized freezing decrease between recall and late extinction; Fig. 1C and fig. S3, A and B). Human studies suggest that InsCtx engagement as well as the intensity of bodily reactions increase proportional to fear levels (*3*, *6*, *23*–*26*). We therefore next assessed the dependence of InsCtx activity manipulations on the level of fear. We used a strong FC protocol by enhancing the US, which produced higher fear levels in reaction to the CS+. Paradoxically, inhibiting InsCtx during extinction after strong FC led to the opposite results as observed upon weak FC, namely an impairment in extinction learning (Fig. 1D and fig. S3, A and C).

These results raised the question of whether the observed opposite effects upon the same activity manipulation were caused by different fear levels resulting from differently strong FC protocols, individual differences in fear reactivity to an aversive experience, or a combination of both. We therefore designed a consecutive FC paradigm, where the same mice subsequently underwent first weak and then strong FC (Fig. 1, E to H; fig. S4; and materials and methods). This experiment confirmed the initial observations (Fig. 1E, left) that InsCtx inhibition upon different FC protocols produced opposite effects. Whereas control mice exhibited stable fear extinction performances after both FC sessions, upon InsCtx inhibition, extinction performances were enhanced after weak FC but reduced after strong FC (Fig. 1E, right). To assess the influence of individual differences in fear reactivity, we assessed each animal's fear level during recall after the first FC session (measured as freezing in response to the CS+) and correlated it to their extinction performance after both weak and strong FC. Whereas in control animals individual fear levels did not affect extinction performances, upon InsCtx inhibition, fear extinction performances were dependent on individual fear levels. Animals that exhibited high fear levels during the recall session extinguished less efficiently, and animals that exhibited low fear levels during the recall session extinguished more rapidly (Fig. 1F and fig. S4C).

To address how individual differences in reactivity to an aversive experience and conditioning strength interacted, we divided animals into low- and high-fear groups based on their individual fear level and assessed their extinction performance separately for strong and weak FC. Again, control animals exhibited stable extinction performances across different combinations of individual fear levels and strengths of aversive experiences (Fig. 1G). However, InsCtx inhibition affected extinction performances only in situations where fear level and experience were on extreme ends: high-fear animals experiencing strong FC or low-fear animals experiencing weak FC (Fig. 1, G and H; and fig. S4D). Intermediate combinations of aversive experience and fear level (weak FC in high-fear animals or strong FC in low-fear animals) were unaffected by InsCtx inhibition (Fig. 1G).

Our results so far revealed that the InsCtx gates extinction learning acutely. We next tested whether InsCtx inhibition also had persistent effects. To avoid complete extinction of fear as observed upon prolonged extinction training (see figs. S3 and S4), mice underwent a shortened extinction session under InsCtx inhibition and were tested on a subsequent day for extinction memories during retrieval in absence of InsCtx inhibition. Like the acute effects during extinction, extinction memories during retrieval were largely stable across different fear levels in control animals, whereas InsCtx inhibition rendered extinction retrieval depending on initial fear levels (Fig. 1, I and J).

To assess how the InsCtx may gate fear extinction, we recorded the responsiveness of the InsCtx to fear-associated cues across fear conditioning and extinction by fiber photometry (*27*) (Fig. 2A). We first quantified InsCtx responses averaged across all animals. Whereas during habituation the presentation of the initially innocuous auditory stimuli did not activate the InsCtx, the InsCtx was increasingly activated by successive CS+ and US pairings during FC. CS+ evoked InsCtx responses

[1]Circuits for Emotion Research Group, Max Planck Institute of Neurobiology, Martinsried, Germany. [2]International Max-Planck Research School for Molecular Life Sciences, Munich, Germany. [3]Graduate School of Systemic Neurosciences, Ludwig-Maximilians University, Munich, Germany. [4]International Max-Planck Research School for Translational Psychiatry, Munich, Germany.
*Corresponding author. Email: ngogolla@neuro.mpg.de
†Present address: Emotion Research Department, Max Planck Institute of Psychiatry, Munich, Germany.

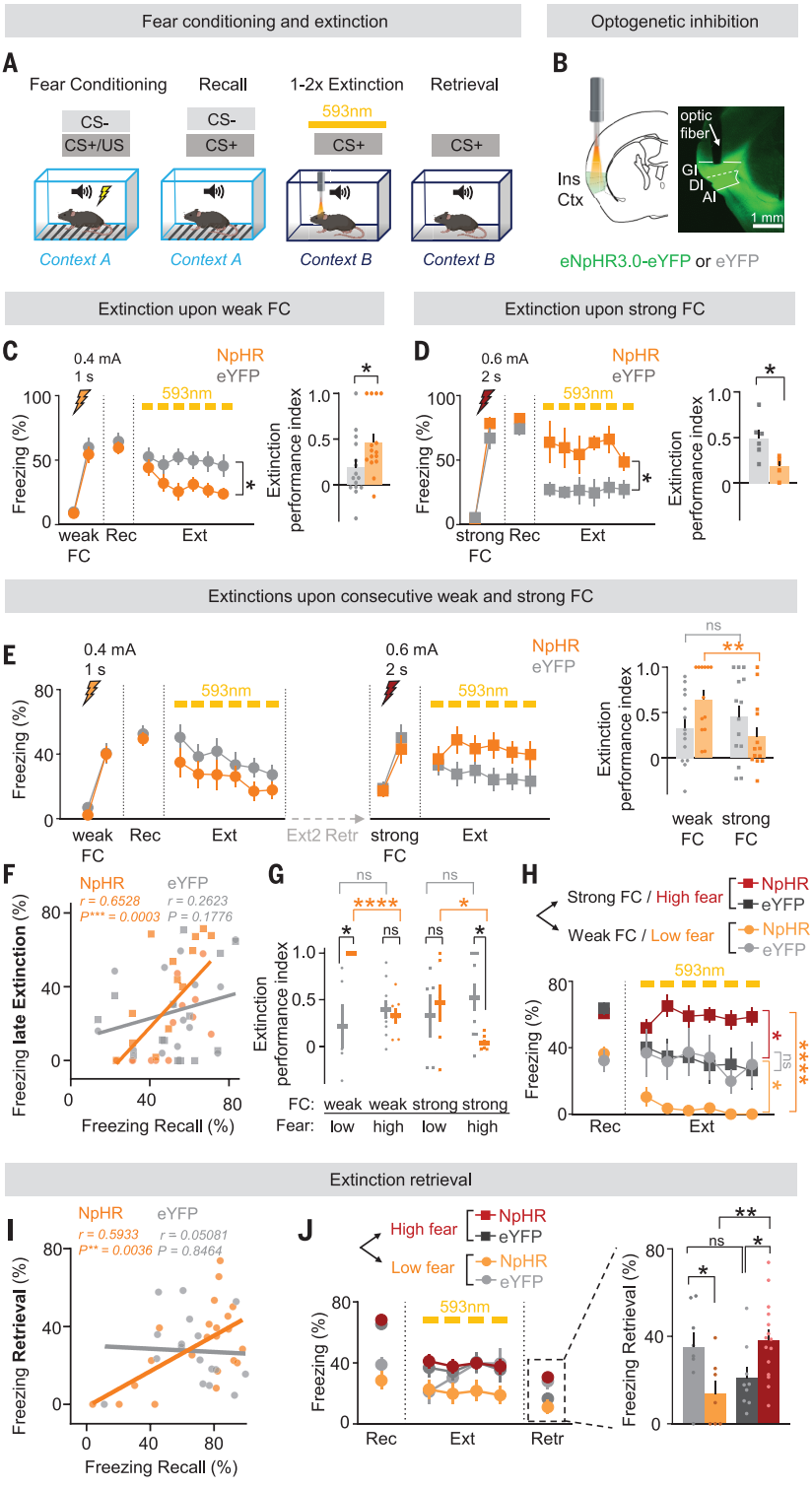

Fig. 1. The InsCtx balances fear extinction and maintenance. (**A**) The FC and extinction paradigm. (**B**) Strategy to inhibit the InsCtx. GI, granular InsCtx; DI, dysgranular InsCtx; AI, agranular InsCtx. (**C**) Extinction upon weak FC (left) and extinction performance indices (right) in N = 15 NpHR and N = 15 eYFP mice. Rec, recall; Ext, extinction. (**D**) Extinction upon strong FC (left) and extinction performance indices (right) in N = 5 NpHR and N = 6 eYFP mice. (**E** to **H**) Extinction upon consecutive weak and strong FC. Retr, retrieval; r, Pearson's correlation coefficient. Shown in (E) are the freezing behavior across all sessions (left) and extinction performance indices (right) in N = 13 NpHR and N = 14 eYFP mice. In (F), the correlation between fear level (freezing during recall) and freezing during late extinction upon weak (circles) or strong FC (squares) is shown. Shown in (G) are extinction performance indices of individual animals upon weak or strong FC depending on their fear level (high-fear mice: N = 7 NpHR, N = 9 eYFP; low-fear mice: N = 6 NpHR, N = 5 eYFP). Shown in (H) are the separation into the most affected groups (top; strong FC, high-fear mice: N = 7 NpHR, N = 9 eYFP; weak FC, low-fear mice: N = 6 NpHR, N = 5 eYFP) and freezing behavior of these groups (bottom). (**I** and **J**) The effect of InsCtx inhibition on extinction retrieval. In (I), the correlation between fear levels and freezing during extinction retrieval (N = 22 NpHR and N = 17 eYFP mice) is shown. Shown in (J) are the separation of the same mice into two groups according to their fear levels (top left; high fear: N = 15 NpHR, N = 9 eYFP; low fear: N = 7 NpHR, N = 8 eYFP), freezing behavior of high- and low-fear animals (bottom left), and quantification of freezing during retrieval (right). For (C) to (E), (H), and (J), filled symbols are average freezing values pooled from two CS+ presentations and four CS+ for recall. For behavior and protocol of the entire experiments, see figs. S3 and S4. The statistical tests used were as follows: for the left panels of (C) to (E), (H), and (J), two-way repeated measures analysis of variance (ANOVA); for the right panels of (C), (D), (G), and (J), two-tailed unpaired t tests; and for the right panel of (E), two-tailed paired t test. Detailed statistical results are shown in table S1. For all panels in which they appear, *$P \leq 0.05$, **$P < 0.01$, ****$P < 0.0001$, ns is not significant, and error bars represent mean ± SEM.

persisted during recall and eventually decreased back to baseline levels with progressing extinction learning (Fig. 2, B and C). InsCtx responsiveness to the CS+ and the amount of CS+ evoked freezing averaged across all animals were positively correlated throughout the different sessions of the paradigm (Fig. 2C). However, InsCtx responses did not increase to the same degree in response to a control tone, which was never paired with an aversive outcome (CS–; fig. S5, A and B).

Although these data revealed that InsCtx activity scales with the certainty to which the CS+ predicted the aversive outcome, they did not explain the observed paradoxical effects of InsCtx inhibition on extinction learning. We thus assessed InsCtx CS+ responsiveness in relation to individual fear levels. Contrary to the observation that CS+ responses in the InsCtx correlate to overall freezing across the fear and extinction paradigm (Fig. 2C), CS+ responses throughout extinction were negatively correlated to individual fear levels: Animals with higher recall fear exhibited lower CS+ evoked

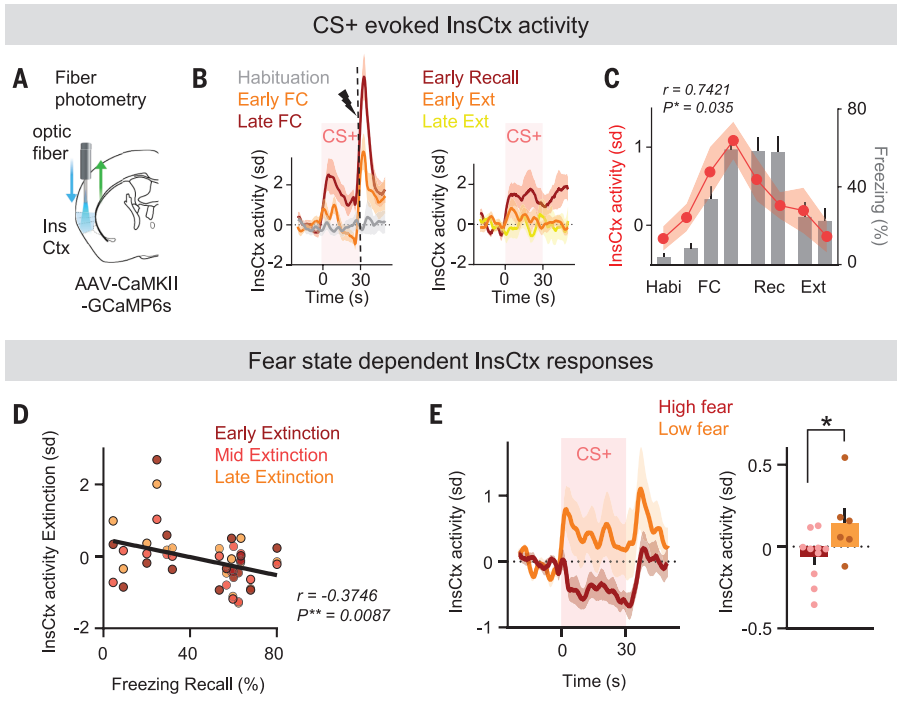

Fig. 2. InsCtx CS+ responses are modulated by fear state. (**A**) Fiber photometry strategy. (**B**) CS+ evoked InsCtx activity during the fear conditioning and extinction paradigm (N = 16 mice). Lines represent average z-scored fluorescence traces ($\Delta F/F_0$) normalized to 30 s preceding each CS+. Habituation (Habi): first CS+; early-late FC: first-last CS+; early recall: first CS+; early-late extinction: first-last CS+. The dashed line in the left panel indicates the timing of the footshock (only delivered during FC). sd, standard deviation. (**C**) Average CS+ evoked InsCtx activity (red) and freezing (gray) during 30 s of CS+ presentations across the experiment [average of two CS+ presentations, except for mid-FC (one CS+)]. (**D**) Correlation between fear level and InsCtx activity during early, mid, and late extinction for each animal. (**E**) Mean CS+ evoked InsCtx activity during all CS+ presentations throughout extinction in high-fear (N = 10 mice) versus low-fear (N = 6 mice) animals (left); lines represent average z-scored fluorescence traces (ΔF/F0) normalized to 30 s preceding each CS+. The quantification of the same data using two-tailed unpaired t tests is shown on the right. Detailed statistical results are shown in table S1. For all panels in which they appear, *$P \leq 0.05$, ns is not significant, and bars and shaded areas represent SEM.

InsCtx responses, and animals with lower recall fear responded with higher CS+ evoked InsCtx responses (Fig. 2D). Given the bidirectional effects of InsCtx inhibition on extinction learning, we again divided animals into high- and low-fear groups (fig. S5C). Consistent with the opposite behavioral changes upon InsCtx inhibition in high- versus low-fear animals, InsCtx activity increased upon CS+ presentations in low-fear animals, but exhibited an inverted response, namely an activity decrease, in high-fear animals (Fig. 2E).

Which process results in lowered InsCtx responses to the CS+ in high-fear compared with low-fear animals? High-fear animals exhibited substantially more freezing. Data from humans and other species suggest that freezing slows heart rate (*28*–*31*). The InsCtx receives rich interoceptive information from the body, including from the heart (*11*, *13*, *32*). We therefore tested how heart rate varies throughout fear conditioning and extinction. We used pulse oximetry in freely moving mice and analyzed heart rate deviations from baseline throughout all phases of the paradigm (Fig. 3, A to D; and fig. S6A). FC resulted in strong heart rate deviations that increased with successive CS+ and US pairings. Although painful footshocks resulted in heart rate increases, heart rates subsequently decelerated and were lowest during and after the CS+ presentations. During recall and extinction, strong fluctuations were triggered by the onset of the CS+ presentations but progressively diminished with ongoing extinction learning (Fig. 3, A to D). Overall, heart rate deviations were greater during FC and recall and decreased during extinction learning back to baseline levels (Fig. 3B). Notably, heart rates varied more strongly in high-fear than in low-fear animals (Fig. 3, A, C, and D).

To determine whether deviations in heart rate were reflected in InsCtx activity changes, we combined pulse oximetry and fiber photometry (Fig. 3, E to K; and fig. S6B). Heart rate deviation and InsCtx activity were not correlated during the initial phases of each session. We observed strong correlations upon the presentation of the first CS+ in FC, recall, and extinction (Fig. 3, E and F). Because the presentation of the CS+ marks the onset of a shift into a higher fear state, we hypothesized that the onset of the correlation between InsCtx activity and heart rate could be explained by changes in bodily feedback during fear expression. Indeed, heart rate decelerated with freezing onset, as reported previously (*28*–*31*) (Fig. 3G). Similarly, InsCtx activity decreased with the onset of freezing but was not correlated to speed of movement (fig. S6C), supporting the role of the InsCtx in interoception (Fig. 3H). Freezing-induced heart rate and InsCtx activity decreases were equally pronounced in high- and low-fear animals (Fig. 3, G and H) and thus could not explain the observed differences in InsCtx activities between these two groups (Fig. 2F). However, during extinction, high-fear animals exhibited overall more frequent and longer freezing bouts in response to CS+ presentations than low-fear animals (Fig. 3I). CS+ evoked InsCtx responses were negatively correlated with acute freezing during extinction (Fig. 3J). Analyzing high- and low-fear animals separately highlighted that InsCtx activity decreased during CS+ presentations in high-fear animals but increased in low-fear animals (Fig. 3K).

Our data suggest that InsCtx reactivity to a fear-associated cue results from the integration of the predictive value of the CS+ modulated by negative bodily feedback signals during acute freezing. Further, the different CS+ responsiveness in high- and low-fear states could be a product of stronger negative bodily feedback signals received in the InsCtx during frequent freezing, which is characteristic of high-fear animals. To test this hypothesis, we directly interfered with the communication between body and brain by means of vagus nerve stimulation (VNS). The vagus nerve is the main pathway carrying interoceptive information from the body to the brain (*33*–*35*) and is thought to relay bodily signals through a multisynaptic pathway to the visceral InsCtx (*11*, *13*, *36*) (Fig. 4A). We thus combined VNS (fig. S7B) with fiber photometry in the InsCtx (Fig. 4B). VNS increased the activity of the InsCtx when applied in a neutral context, confirming our approach (Fig. 4C). Analogous to our optogenetic inhibition experiments, we applied VNS during the CS+ presentations throughout extinction learning (Fig. 4D and fig. S7A). First, we tested whether we could override bodily feedback signals that modulate InsCtx responses during freezing. Indeed, during VNS application, InsCtx activity increased with the onset of a freezing episode,

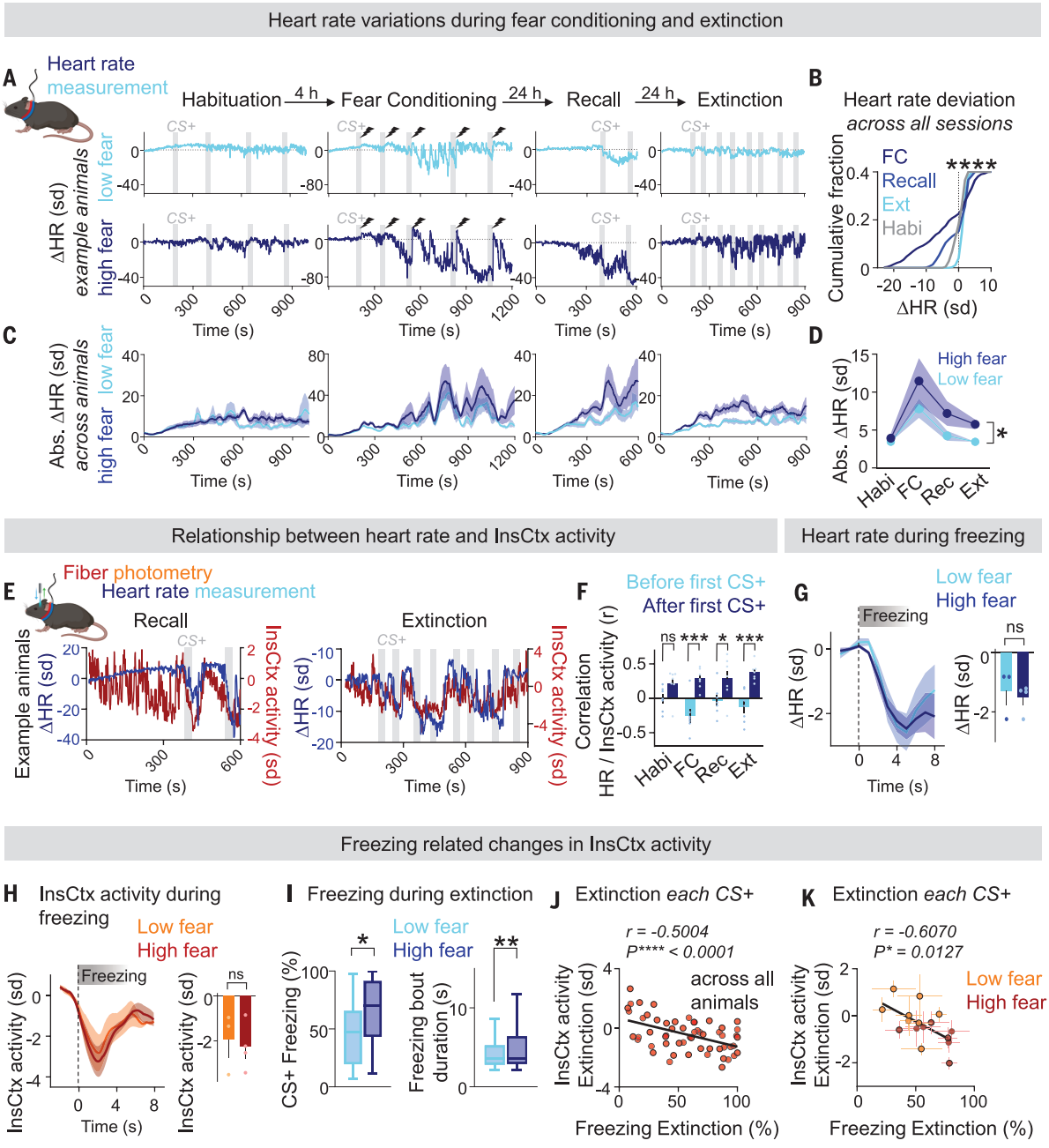

Fig. 3. Fear expression modulates heart rate and InsCtx activity. (**A** to **D**) Heart rate (HR) measurements using pulse oximetry during the FC and extinction paradigm. Shown in (A) are heart rate deviations (normalized to the first 100 s of each session) during each session of the paradigm in exemplary low-fear (top, light blue) and high-fear animals (bottom, dark blue). Shown in (B) are cumulative fractions of normalized heart rate deviations for each session of the paradigm, averaged across all animals (N = 15 mice). In (C), absolute heart rate deviations of low-fear (N = 7 mice) and high-fear (N = 8 mice) animals across all four sessions of the experiment are shown. Quantification of the data in (C) is shown in (D). (**E** to **K**) Combination of fiber photometry recordings in the InsCtx with heart rate measurements. In (E), heart rate deviation (dark blue) and InsCtx activity (dark red) during recall (left) and extinction (right) in individual exemplary animals are shown. In (F), correlations between heart rate deviation and InsCtx activity before and after the first CS+ presentation in each session (N = 7 mice) are shown. Shown in (G) are heart rate during freezing normalized to 2 s preceding freezing onset and averaged across high-fear (N = 4 mice) and low-fear (N = 3 mice) animals (left) and quantification of the same data (right). In (H), InsCtx activity during freezing normalized to 2 s preceding freezing onset and averaged across high- and low-fear animals (left) and quantification of the same data (right) are shown. Shown in (I) are the average freezing to each CS+ presentation during extinction [left; n = 24 CS+ for low-fear animals; n = 32 CS+ for high-fear animals (total of eight per animal)] and average freezing bout duration in low-fear (n = 136 freezing episodes) and high-fear (n = 132 freezing episodes) animals (right). In (J), the correlation between freezing and InsCtx activity for each CS+ presentation during extinction for all animals (N = 7 mice) is shown. In (K), the correlation between freezing and InsCtx activity to each CS+ presentation during extinction averaged across high-fear (N = 4 mice) and low-fear (N = 3 mice) animals is shown. The statistical tests used were as follows: for (B), Kolmogorov-Smirnoff tests; for (D), two-way repeated measures ANOVA; for (F), two-way repeated measures ANOVA with Bonferroni correction; and for (G) to (I), two-tailed unpaired t tests, with box-and-whisker plots in the style of Tukey. Detailed statistical results are shown in table S1. For all panels in which they appear, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P < 0.0001$, ns is not significant, and error bars and shaded areas represent SEM.

whereas, consistent with our previous findings, freezing resulted in InsCtx activity decreases in periods when no VNS was applied (Fig. 4E). Notably, VNS resulted in an inversion of the previously observed correlation between CS+ evoked InsCtx activity and freezing, such that in the presence of VNS, InsCtx activity was positively correlated to CS+-evoked freezing levels (Fig. 4F). Separating the VNS cohort into high- and low-fear animals (fig. S7C) revealed that inversely to the observations in nonmanipulated animals (Fig. 2F), upon VNS, high-fear animals responded with an InsCtx activity increase, whereas low-fear animals exhibited an activity decrease, in response to the CS+ presentations (Fig. 4G).

Finally, we addressed how VNS affects extinction learning. Consistent with the idea that bodily feedback transmitted by the vagus nerve provides the necessary bodily feedback signals to gate fear extinction learning, we found that VNS had fear state–dependent effects on extinction performance. Similar to InsCtx inhibition, under VNS, high-fear animals exhibited impaired extinction performance, whereas low-fear animals exhibited an extinction facilitation, both acutely during extinction and at retrieval of extinction memories (Fig. 4, H to J, and fig. S7D).

Our findings reveal that fear is actively maintained within balance by the InsCtx, which can either boost or weaken extinction learning depending on the fear state of the subject. Freezing, a shared means of fear expression across species (*28*, *37*), occurred together with heart rate decelerations, which are transmitted by the vagus nerve to the InsCtx and attenuate its responses to aversive cues. These findings highlight a neural mechanism by which freezing gains a coping function: It dampens aversive signaling in the InsCtx through bodily feedback. Our data suggest that disrupting this coping function may result in exaggerated fear memories and identify bodily changes that occur during freezing as an essential part of emotion regulation. Interestingly, stronger heartbeat perception in humans has been linked to pathological fear

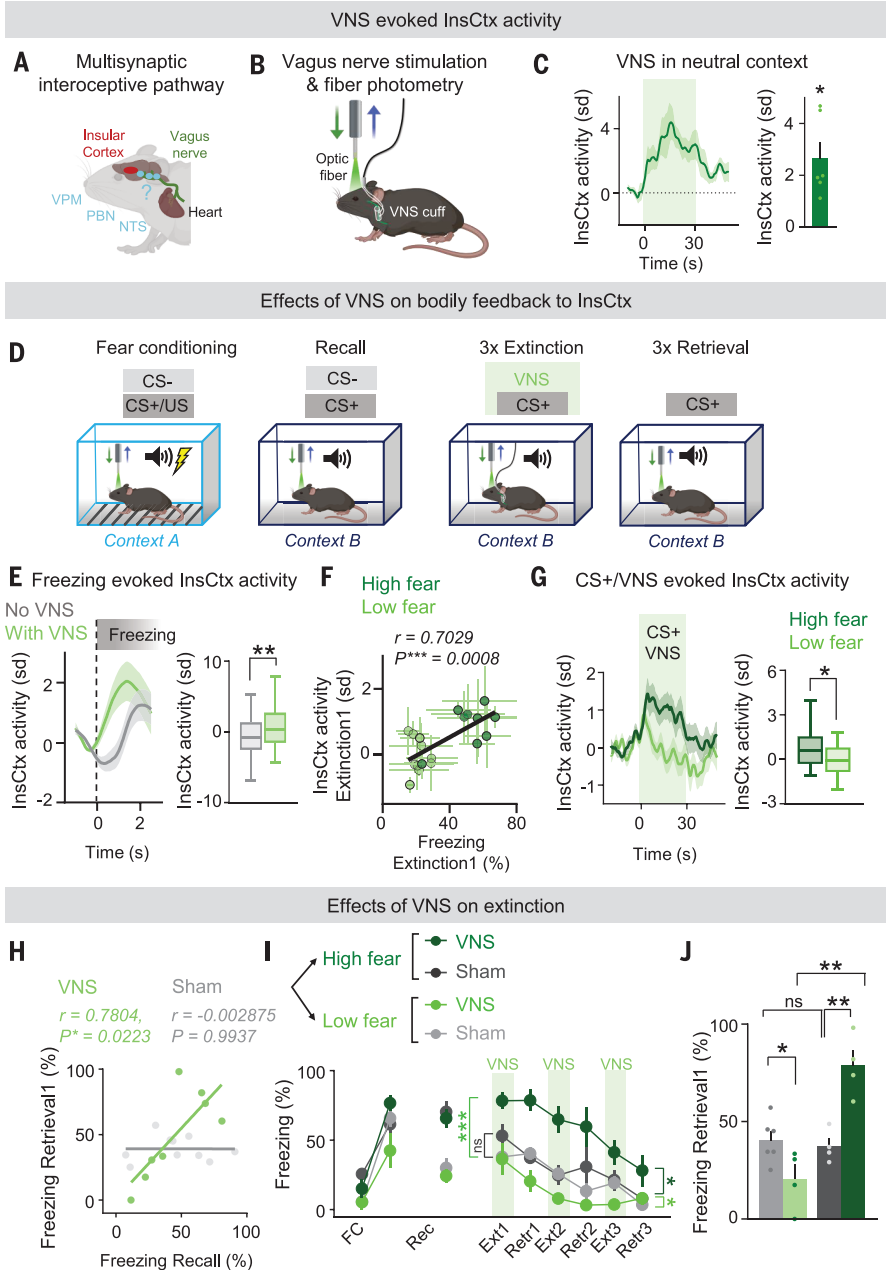

Fig. 4. VNS disrupts fear balance. (**A**) Proposed multisynaptic interoceptive pathway connecting cardiac activity to InsCtx activity. VPM, ventral posteromedial nucleus of the thalamus; PBN, parabrachial nucleus; NTS, nucleus of the solitary tract. (**B** to **G**) Fiber photometry in the InsCtx combined with VNS. Shown in (C) are VNS-evoked InsCtx activity (average trace of two stimulations) under neutral conditions (left) and quantification of the same data (right; *N* = 6 mice). In (D), VNS was applied during CS+ presentations throughout three consecutive extinction sessions. Shown in (E) are the average InsCtx activity during freezing events occurring within (*n* = 64 events) or outside (*n* = 100 events) VNS (left) and quantification of the same data (right). In (F), the correlation between freezing and InsCtx activity to each CS+ presentation during extinction (session 1) averaged across high-fear (*N* = 3 mice) and low-fear (*N* = 3 mice) animals is shown. Shown in (G) are InsCtx activity during all 10 CS+ presentations combined with VNS during extinction in the same high- and low-fear animals as in (F) (left) and quantification of the same data [right; *n* = 30 CS+ for high-fear mice; *n* = 30 CS+ presentations for low-fear mice (total of 10 CS+ per mouse)]. (**H** to **J**) Effects of VNS on fear extinction. In (H), the correlation between fear level and freezing during first retrieval of VNS (*N* = 8 mice) and sham control (*N* = 10 mice) animals is shown. Shown in (I) are the separation of the same mice as in (H) into groups according to their fear levels (top; *N* = 4 high-fear VNS, *N* = 4 high-fear sham, *N* = 4 low-fear VNS, *N* = 6 low-fear sham) and freezing behavior in both groups during FC and extinction (bottom). One data point represents the average of two CS+ for FC, recall, and retrievals and 10 CS+ for extinctions. For the protocol of the entire experiment, see fig. S7. Shown in (J) is the quantification of freezing during retrieval. The statistical tests used were as follows: for (C), one-sample *t* test with hypothetical mean of zero; for (E) right, (G) right, and (J), two-tailed unpaired *t* tests; and for (I), two-way repeated measures ANOVA. Detailed statistical results are shown in table S1. For all panels in which they appear, box-and-whisker plots are in the style of Tukey, $*P \leq 0.05$, $**P \leq 0.01$, ns is not significant, and error bars and shaded areas represent SEM.

and anxiety (*7*) and is known to engage the insular and opercular regions as key structures of the interoceptive brain (*9*, *32*).

Our data further suggest the integration of two opposite signals within the InsCtx: prediction of threat by fear-associated cues and negative feedback signals from the body. Future work is needed to address how interoception may modulate other parts of the fear circuitry, such as the amygdala or prefrontal cortex, which have also been reported to respond to bodily signals (*38*–*41*).

Although the role of the InsCtx as a regulator of bodily homeostasis has been suggested for a long time (*10*, *42*–*44*), we show in this study that the InsCtx also serves as homeostatic regulator of emotion. The InsCtx may detect deviations from established set points of adaptive functioning, for example, responding to fear as a deviation from an affective and bodily set point, which has already been suggested previously (*22*, *42*–*45*). Additionally, to reestablish affective homeostasis, the InsCtx may provide teaching signals to downstream effector systems, such as the known extinction circuitry (*46*–*48*) to which it is heavily connected (*11*, *12*). Future work should address how other types of bodily feedback to the InsCtx—such as hunger, craving, and gut- or breathing-related signals (*13*, *44*, *45*, *49*, *50*)—may influence InsCtx function in regulating affective states.

Although our results indicate that the InsCtx may be dispensable for extinction in subjects with moderate fear levels, its gating function becomes important in high- or low-fear subjects that are experiencing extreme situations. These findings may relate to the dual role of the InsCtx in anxiety and addiction disorders (*22*, *49*, *51*–*53*), which are characterized by opposing ways to deal with threat and harm (*49*). Furthermore, people suffering from anxiety disorders exhibit hyperactivity in the InsCtx when compared with healthy controls (*22*), possibly owing to the lack of negative feedback from the body.

Although VNS facilitates fear extinction in rats (*54*) and is tested in clinical studies to treat anxiety disorders in humans (*55*), our results reveal that VNS may worsen extinction outcomes under certain conditions. This is in agreement with a recent study (*54*) and emphasizes the need to individually adjust VNS therapy. Despite this caveat, our study highlights the potency of engaging a bodily route in emotion regulation. Given the overall poor accessibility of the human brain for targeted therapeutic interventions, the detailed description of this bodily feedback mechanism may bear enormous therapeutic potential for human anxiety disorders.

REFERENCES AND NOTES

1. D. Mobbs, J. J. Kim, *Curr. Opin. Behav. Sci.* **5**, 8–15 (2015).
2. O. Stiedl, J. Spiess, *Behav. Neurosci.* **111**, 703–711 (1997).
3. I. Van Diest, M. M. Bradley, P. Guerra, O. Van den Bergh, P. J. Lang, *Biol. Psychol.* **80**, 212–217 (2009).
4. S. Bagur *et al.*, *Nat. Commun.* **12**, 2605 (2021).
5. M. Maschke *et al.*, *J. Neurol. Neurosurg. Psychiatry* **72**, 116–118 (2002).
6. M. P. Paulus, M. B. Stein, *Biol. Psychiatry* **60**, 383–387 (2006).
7. A. J. Willem Van der Does, M. M. Antony, A. Ehlers, A. J. Barsky, *Behav. Res. Ther.* **38**, 47–62 (2000).
8. L. Quadt, H. D. Critchley, S. N. Garfinkel, *Ann. N. Y. Acad. Sci.* **1428**, 112–128 (2018).
9. S. N. Garfinkel, H. D. Critchley, *Trends Cogn. Sci.* **20**, 34–46 (2016).
10. A. D. Craig, *Nat. Rev. Neurosci.* **3**, 655–666 (2002).
11. G. V. Allen, C. B. Saper, K. M. Hurley, D. F. Cechetto, *J. Comp. Neurol.* **311**, 1–16 (1991).
12. D. A. Gehrlach *et al.*, *eLife* **9**, e55585 (2020).
13. D. F. Cechetto, C. B. Saper, *J. Comp. Neurol.* **262**, 27–45 (1987).
14. J. P. Casanova *et al.*, *Behav. Brain Res.* **296**, 70–77 (2016).
15. C. Sehlmeyer *et al.*, *PLOS ONE* **4**, e5865 (2009).
16. A. R. Foilb, J. G. Flyer-Adams, S. F. Maier, J. P. Christianson, *Neurobiol. Learn. Mem.* **134**, 317–327 (2016).
17. J. P. Casanova, M. Aguilar-Rivera, M. L. Á. Rodríguez, T. P. Coleman, F. Torrealba, *J. Neurophysiol.* **120**, 1906–1913 (2018).
18. N. M. Brydges *et al.*, *PLOS ONE* **8**, e54197 (2013).
19. M. A. Fullana *et al.*, *Mol. Psychiatry* **21**, 500–508 (2016).
20. J. A. Greco, I. Liberzon, *Neuropsychopharmacology* **41**, 320–334 (2016).
21. D. Kargl *et al.*, *eLife* **9**, e60336 (2020).
22. A. Etkin, T. D. Wager, *Am. J. Psychiatry* **164**, 1476–1488 (2007).
23. S. S. Khalsa *et al.*, *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **3**, 501–513 (2018).
24. T. Klucken, O. Kruse, J. Schweckendiek, R. Stark, *Front. Behav. Neurosci.* **9**, 132 (2015).
25. D. W. Grupe, J. B. Nitschke, *Nat. Rev. Neurosci.* **14**, 488–501 (2013).
26. K. Preuschoff, S. R. Quartz, P. Bossaerts, *J. Neurosci.* **28**, 2745–2752 (2008).
27. L. A. A. Gunaydin *et al.*, *Cell* **157**, 1535–1551 (2014).
28. K. Roelofs, *Philos. Trans. R. Soc. London Ser. B* **372**, 20160206 (2017).
29. M. A. Hagenaars, M. Oitzl, K. Roelofs, *Neurosci. Biobehav. Rev.* **47**, 165–176 (2014).
30. L. C. Schenberg, E. C. Vasquez, M. B. da Costa, *Brain Res.* **621**, 50–58 (1993).
31. J. Iwata, J. E. LeDoux, *Behav. Neurosci.* **102**, 66–76 (1988).
32. H. D. Critchley, S. Wiens, P. Rotshtein, A. Öhman, R. J. Dolan, *Nat. Neurosci.* **7**, 189–195 (2004).
33. H. R. Berthoud, W. L. Neuhuber, *Auton. Neurosci.* **85**, 1–17 (2000).
34. R. Câmara, C. J. Griessenauer, in *Nerves and Nerve Injuries*, R. S. Tubbs, E. Rizk, M. M. Shoja, M. Loukas, N. Barbaro, R. J. Spinner, Eds. (Academic Press, 2015), pp. 385–397.
35. S. Ito, *Neurosci. Lett.* **148**, 151–154 (1992).
36. M. Kalia, J. M. Sullivan, *J. Comp. Neurol.* **211**, 248–265 (1982).
37. A. D. Zych, N. Gogolla, *Curr. Opin. Neurobiol.* **68**, 57–66 (2021).
38. R. C. Frysinger, R. M. Harper, *Electroencephalogr. Clin. Neurophysiol.* **72**, 463–470 (1989).
39. J. Liu, L. Lin, D. V. Wang, *J. Neurosci.* **41**, 1080–1091 (2021).
40. A. Adhikari *et al.*, *Nature* **527**, 179–185 (2015).
41. L. B. M. Resstel, F. M. A. Corrêa, *Auton. Neurosci.* **126-127**, 130–138 (2006).
42. G. Egan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 15241–15246 (2003).
43. Y. Livneh *et al.*, *Nature* **546**, 611–616 (2017).
44. Y. Livneh *et al.*, *Neuron* **105**, 1094–1111.e10 (2020).
45. D. A. Gehrlach *et al.*, *Nat. Neurosci.* **22**, 1424–1437 (2019).
46. P. Tovote, J. P. Fadok, A. Lüthi, *Nat. Rev. Neurosci.* **16**, 317–331 (2015).
47. C. Herry *et al.*, *Eur. J. Neurosci.* **31**, 599–612 (2010).
48. G. G. Calhoon, K. M. Tye, *Nat. Neurosci.* **18**, 1394–1404 (2015).
49. N. H. Naqvi, N. Gaznick, D. Tranel, A. Bechara, *Ann. N. Y. Acad. Sci.* **1316**, 53–70 (2014).
50. M. Aguilar-Rivera, S. Kim, T. P. Coleman, P. E. Maldonado, F. Torrealba, *Sci. Rep.* **10**, 21642 (2020).
51. J. Downar, D. M. Blumberger, Z. J. Daskalakis, *Trends Cogn. Sci.* **20**, 107–120 (2016).
52. M. Goodkind *et al.*, *JAMA Psychiatry* **72**, 305–315 (2015).
53. H. Namkung, S.-H. Kim, A. Sawa, *Trends Neurosci.* **40**, 200–207 (2017).
54. R. R. Souza *et al.*, *Exp. Neurol.* **341**, 113718 (2021).
55. M. S. George *et al.*, *Brain Stimul.* **1**, 112–121 (2008).
56. A. S. Klein, N. Dolensek, C. Weiand, *N*. Gogolla, Source data for "Fear balance is maintained by bodily feedback to insular cortex in mice." Zenodo (2021);

ACKNOWLEDGMENTS

We thank members of the Gogolla laboratory, C. Herry, J. J. Letzkus, and R. Portugues for technical guidance, discussions, and feedback; F. Lyonnaz and E. Cho for technical assistance; and M. Malezieux for feedback on the manuscript. **Funding:** This work was supported by the Max-Planck Society, the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (ERC-2017-STG, grant agreement n° 758448 to N.G.), and the ANR-DFG project "SAFENET" (ANR-17-CE37-0021). **Author contributions:** N.G. and A.S.K. conceived the project and designed the experiments. A.S.K. performed all experiments and analyses. N.D. provided technical assistance and helped with analyses and programming. C.W. helped with experiments, controls, and histology. N.G. wrote the manuscript with the assistance of all authors. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data are available in the manuscript and the supplementary materials and at Zenodo (*56*). Viruses were packaged at the University of North Carolina (UNC) Vector Core or at Addgene and are made available under material transfer agreements. Illustrations containing drawings were created with BioRender.com.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abj8817
Materials and Methods
Supplementary Text
Figs. S1 to S7
Table S1
References (*57*–*60*)

View/request a protocol for this paper from *Bio-protocol*.

8 June 2021; accepted 21 September 2021
10.1126/science.abj8817

Fear balance is maintained by bodily feedback to the insular cortex in mice

Alexandra S. KleinNate DolensekCaroline WeiandNadine Gogolla

Science, 374 (6570), • DOI: 10.1126/science.abj8817

How the body regulates fear

Although fear is important for survival, it is maladaptive if it is either too strong, as in anxiety disorders, or too weak, as in exaggerated risk taking. Working in mice, Klein *et al*. observed that the insular cortex has an unparalleled dual role in either enhancing or weakening the extinction of fear, depending on the internal fear state of the animal (see the Perspective by Christianson). This insula function helps to maintain fear within a homeostatic range and depends on bodily feedback signals: Fear-induced freezing behavior is associated with a slowed heart rate, which in turn dampens fear-evoked activity of the insular cortex. Two opposite signals, prediction of threat by fear-associated cues and negative feedback signals from the body, are thus integrated within the insular cortex. —PRS

Science (ISSN) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.