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Supplementary Information for

Stress transforms lateral habenula reward responses into punishment signals

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Figs. S1 to S8
References

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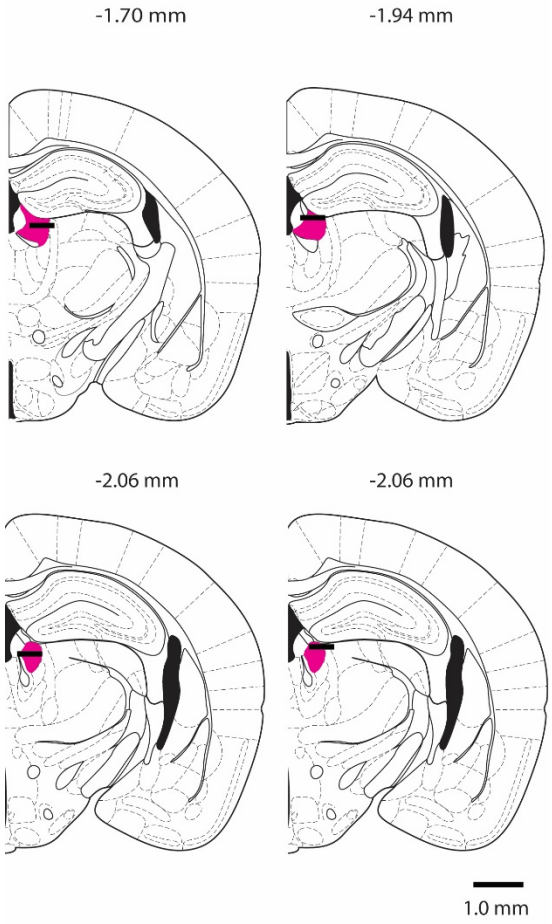

Fig. S1. Location of GRIN lenses. Approximate locations of bottoms of each GRIN lens are indicated by black bar. Pink, lateral habenula. Diagrams adapted from *The Mouse Brain in Stereotaxic Coordinates*, Paxinos and Franklin (2001). Measurements above diagrams indicate distance from bregma.

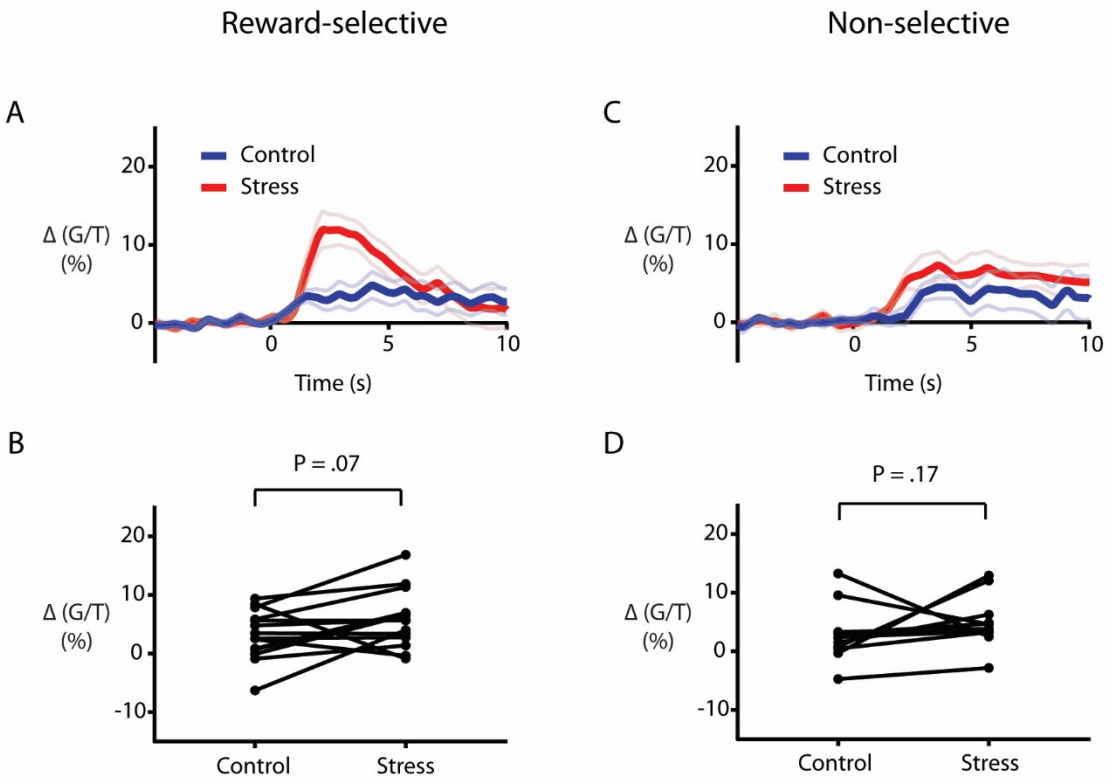

Fig. S2. Neuronal responses to CS- before and after pairing with shock stress. Control CS responses in Reward-selective neurons (**A**, **B;** $N = 14$) or Non-selective neurons (**C, D**; $N = 11$) during control trials (Control; before shock stress; $N = 33$-108 trials) and trials during pairing with shock stress (Stress; $N = 72$-108 trials). (**A, C**) Mean change in activity during control CS, 0-10 s, compared to 5 seconds before CS. (**B, D**) Mean change in activity during control CS, 0-10 s, for each neuron compared to 5 seconds before CS. Wilcoxon signed rank test used for all statistical tests.

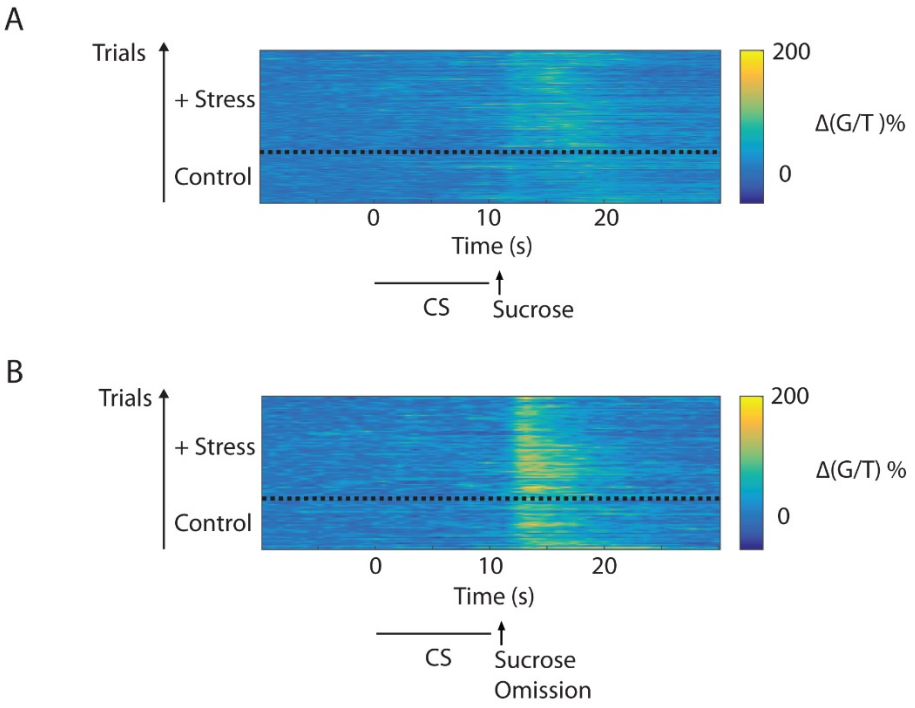

Fig. S3. Trial-by-trial activity of example reward-selective neuron. (**A**) Sucrose trials. (**B**) Sucrose omission trials. Dotted line, first trial after start of tail shock stress. Activity measurements for each trial normalized to the ten second epoch before the start of the CS. There were twenty-four sucrose trials per session/day and 12 sucrose omission trials per session/day.

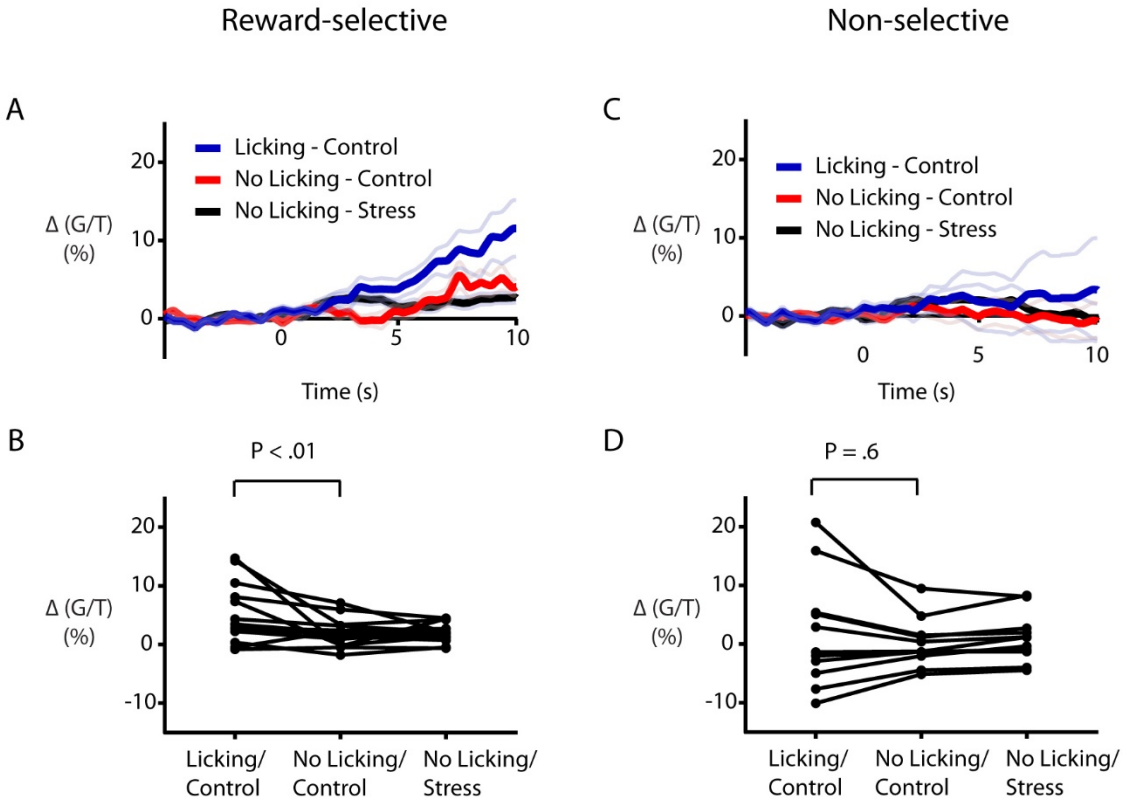

Fig. S4. Neuronal CS+ responses with and without licking and stress. CS+ responses in Reward-selective neurons (**A**, **B;** $N = 14$) or Non-selective neurons (**C, D**; $N = 11$) during control trials with anticipatory licking during the CS+ (Licking/Control), control trials without anticipatory licking during the CS+ (No Licking/Control), and trials during stress without anticipatory licking during the CS+ (No Licking/Stress). There were not enough trials with anticipatory licking during stress for a meaningful analysis. (**A, C**) Mean change in activity during CS+, 0-10 s, compared to 5 seconds before CS+. (**B, D**) Mean change in activity during CS+, 0-10 s, for each neuron compared to 5 seconds before CS+. (**B**) Greater increase in activity to CS+ during trials with anticipatory licking in Reward-selective neurons. (**D**) Although there was no consistent directional change in activity in Non-selective neurons during licking (P = .6), there was a statistically significant increase in the magnitude of CS+ activity in Non-selective neurons during control trials with licking compared to control trials without licking (computed using absolute values of changes in activity; $P < .01$). All other unmarked comparisons were not statistically significant (P > .05). Wilcoxon signed rank test used for all statistical tests.

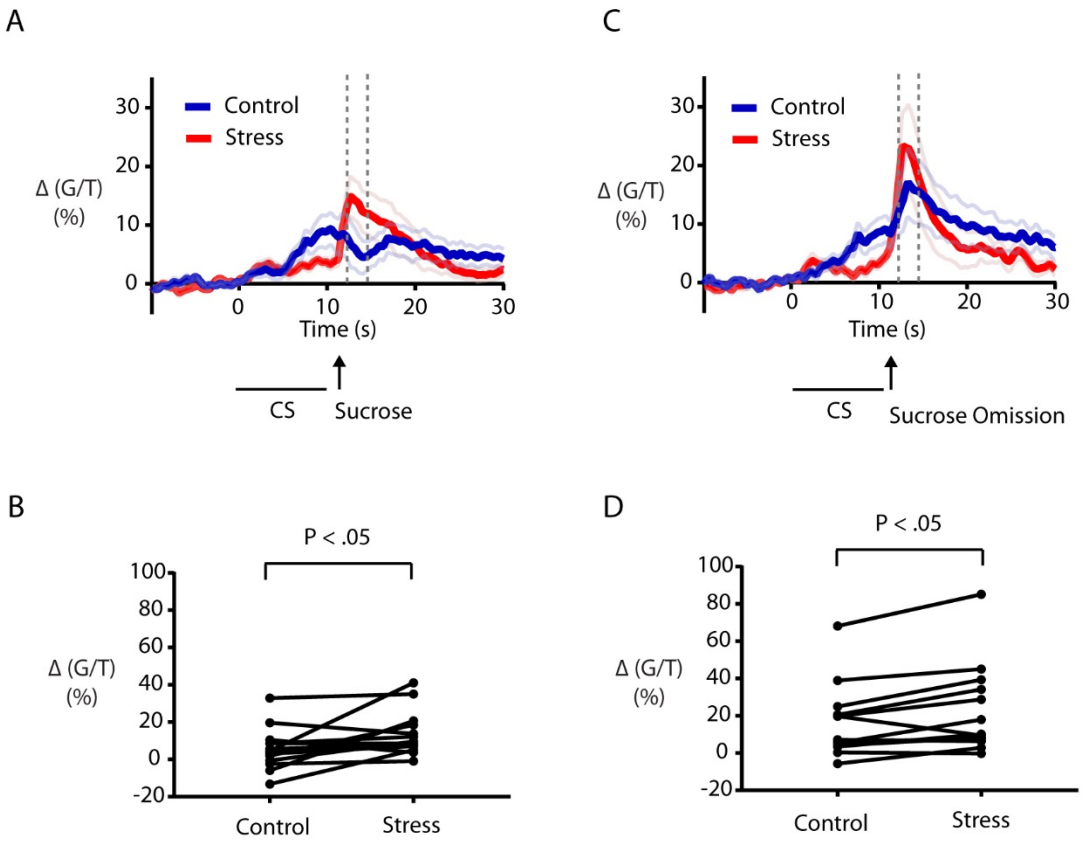

Fig. S5. Responses of Reward-selective neurons during sucrose consumption and sucrose omission compared to pre-CS. Mean change in activity of Reward-selective neurons ($N = 14$) during sucrose consumption (**A**, **B**) or sucrose omission (**C**, **D**) before stress (Control) and trials during stress (Stress). (**A**, **C**) Mean change in activity compared to 5 seconds before CS. Dashed lines indicate time interval used to compute individual neuron responses for (**B**, **D**). (**B**, **D**) Mean change in activity during sucrose consumption or sucrose omission (time intervals indicated by dashed lines in (**A**, **C**)) for each neuron compared to 5 seconds before CS. Only trials with licking within 1 second of sucrose delivery or omission were included in these analyses. Wilcoxon signed rank test used for all statistical tests.

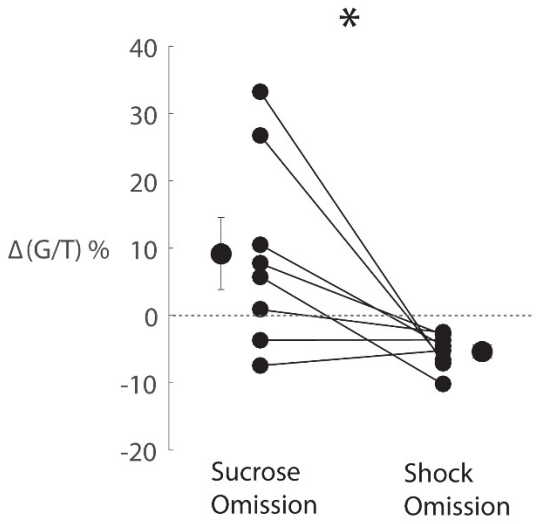

Fig. S6. Decrease in activity during shock omission but not sucrose omission. Comparison of changes in activity during sucrose omission and shock omission from the same Reward-selective neurons during the same imaging sessions. All Reward-selective (N = 8; 3 mice) neurons decreased activity in a 2 second window starting 2.5 seconds after the termination of the CS paired with shock (N = 24 trials). Changes in activity during sucrose omission are shown for the 2 second window starting 2.5 seconds after termination of the CS paired with sucrose (N = 24 trials). *, $P < .05$, Wilcoxon signed rank test.

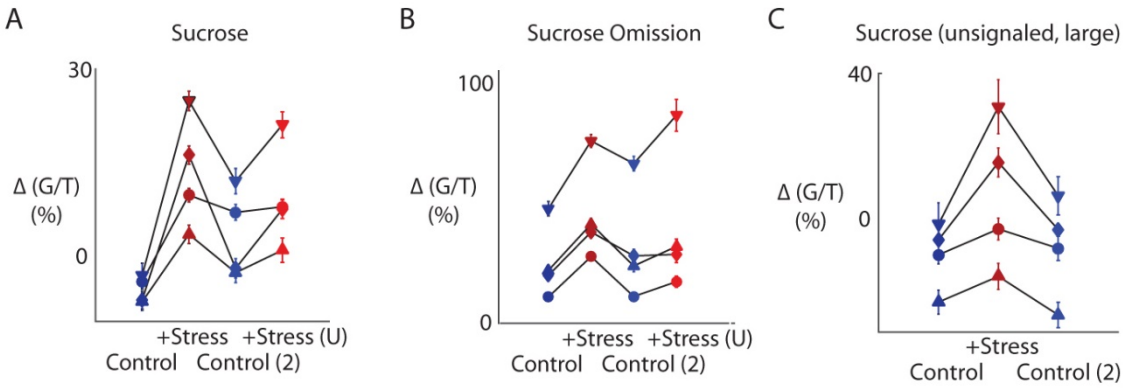

Fig. S7. **Effects of stress on LHb neuronal reward signaling are robust.** (**A**) Responses of Reward-selective neurons to sucrose during control sessions or stress sessions. +Stress, signaled stress. +Stress (U), unsignaled stress. Each data point is the average response of one Reward-selective neuron (*N* = 216, 216, 216, 195 trials). (**B**) Responses of Reward-selective neurons to sucrose omission, conditions as (**A**) (*N* = 104, 101, 98, 57 trials). (**C**) Responses of Reward-selective neurons to unsignaled, long duration (2 s) reward in the presence (+Stress) or absence (Control) of signaled stress (*N* = 52, 50, 53 trials). Only trials in which the mouse licked within 1 second of sucrose delivery or omission were included in all panels.

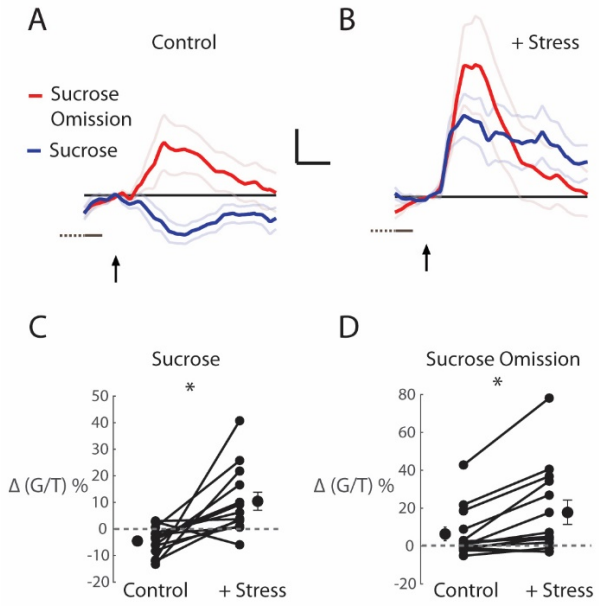

Fig. S8. **Effects of stress are present during trials with reward expectation.** Activity of Reward-selective neurons on trials with anticipatory licking prior to sucrose or sucrose omission. Anticipatory licking is defined here as licking of spout during the 0.5 s epoch between the end of the CS and sucrose delivery or omission. $N = 14$ Reward-selective neurons. (**A, B**) Mean post-CS neural responses (for 3 or 6 days before or after stress introduction, respectively) of all responses for indicated condition. Scale bars: 2 s, 5% ΔG/T. Arrows: sucrose or sucrose omission; bars: CS+ (sucrose-conditioned). Black lines indicate zero. (**C, D**) Mean response of indicated trial type, for individual neurons (connected with line) before and during stress. *, $P < .01$, Wilcoxon signed rank test.

References

1. Paxinos, G. and Franklin, K.B (2001).The Mouse Brain in Stereotaxic Coordinates. (Academic Press, London) 2nd Ed.