Supplementary figures

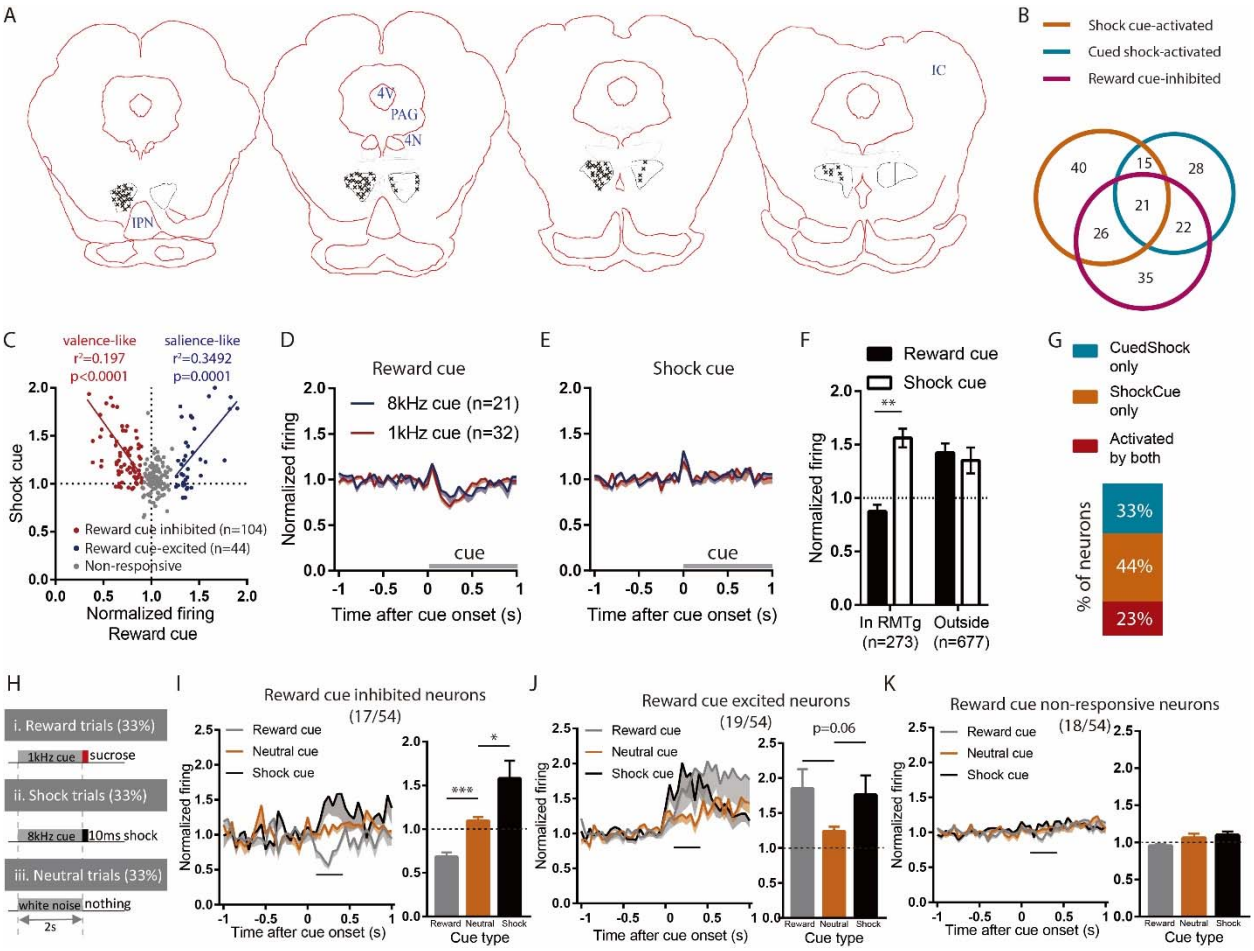

Figure S1. Histology and supplemental electrophysiological data from RMTg recordings, related to Figure 1. (**A**) RMTg electrophysiological recording sites in all experiments. (**B**) Venn diagram of individual neuron's response to reward-predictive cues, shock-predictive cues, and shocks. (**C**) Responses of individual neurons to reward- and shock-predictive cues. Responses to reward and shock cues correlated inversely with each other in reward cue-inhibited neurons, consistent with valence encoding pattern, and positively correlated in reward cue-excited neurons, consistent with salience encoding pattern (r^2=0.197, p<0.0001, and r^2=0.3492, p=0.0001). (**D, E**) Reward and shock cues consisted of distinct auditory 1kHz or 8kHz tones, counterbalanced between animals. RMTg responses to these cues depended on the predicted outcome, and were indifferent to specific cue used. (**F**) Averaged responses of neurons outside the RMTg revealed excitations to both reward and shock-predictive cues that did not discriminate between cues, unlike RMTg neurons that showed strong discrimination (p=0.0053 and p=0.668 for reward cue vs shock cue responses in neurons inside the RMTg and outside the RMTg respectively, two-way ANOVA, with Holm-Sidak multiple comparisons test). (**G**) Responses to shock cues and shocks were usually encoded in distinct RMTg subpopulations. (**H**) Recording paradigm in which reward trials, neutral trials, and shock trials were randomly presented. (**I-K**) Responses of reward cue-inhibited, reward cue-excited, and reward cue-non responsive neurons, respectively. (reward cue-inhibited neurons: F=11.07, p<0.0001 and p=0.04, for reward cues and shock cues compared to neutral

cues; reward cue-excited neurons: F=3.733, p=0.068, for both reward and shock cues compared to neutral cues; reward cue-non responsive neurons: F=2.574, p=0.274 and p=0.681, for reward cues and shock cues compared to neutral cues, repeated measures one-way ANOVA, with Holm-Sidak multiple comparisons.

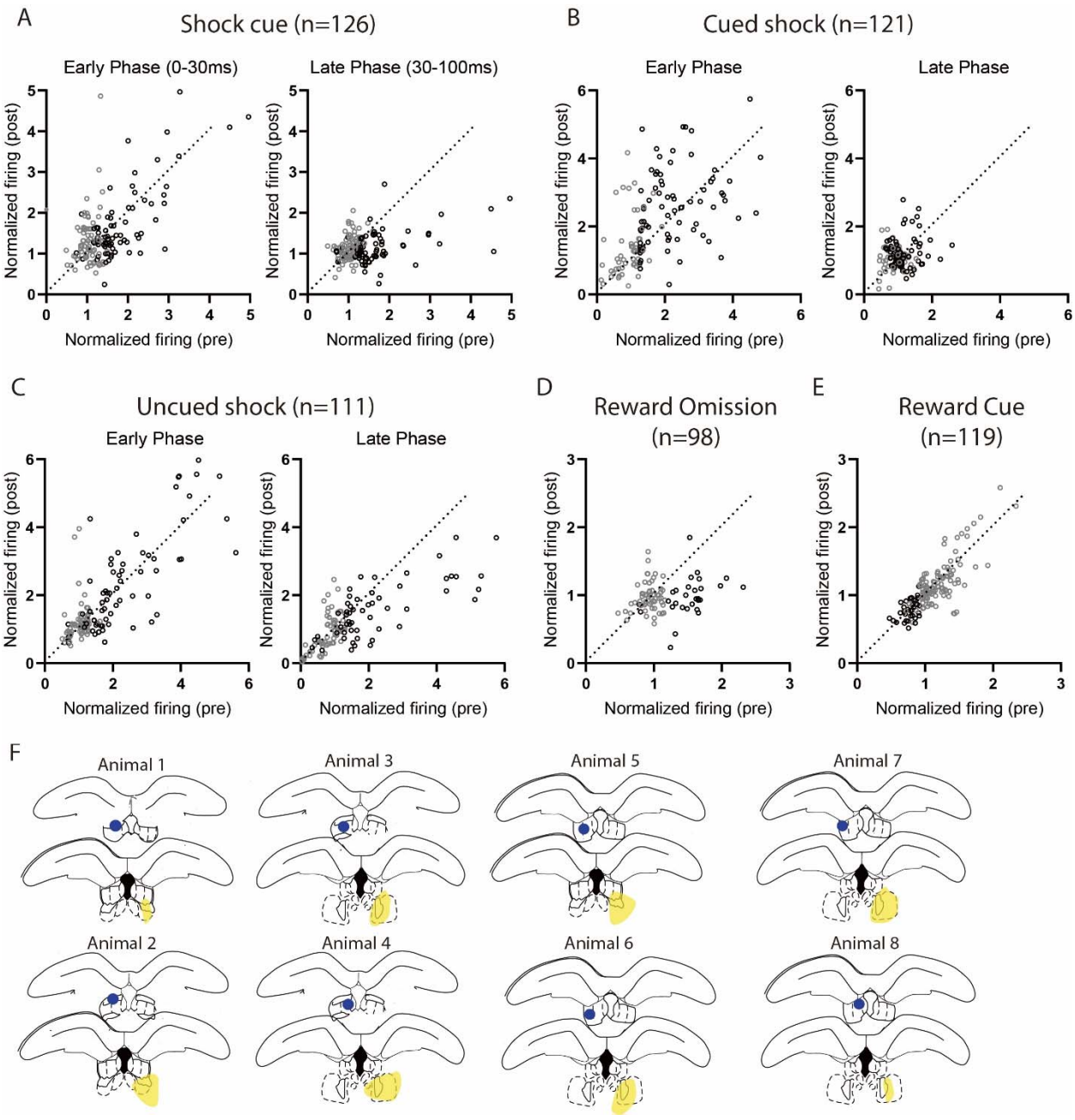

Figure S2. Histology and individual animal data for RMTg recordings during LHb inactivation, related to Figure 2. (**A-D**) Individual responses to shock cues, cued shocks, uncued shocks, reward omissions, and reward cues before and after LHb inactivation. Black dots indicate neurons that showed significant inhibition to reward cues or significant excitations to shocks, shock cues, or reward omissions. Grey dots indicate all other neurons (including non-responsive). (**F**) LHb cannula placements (blue dots), and the size of electrolytic lesions on the contralateral fasciculus retroflexus, the major output from the LHb carrying axons to the RMTg region (yellow areas).

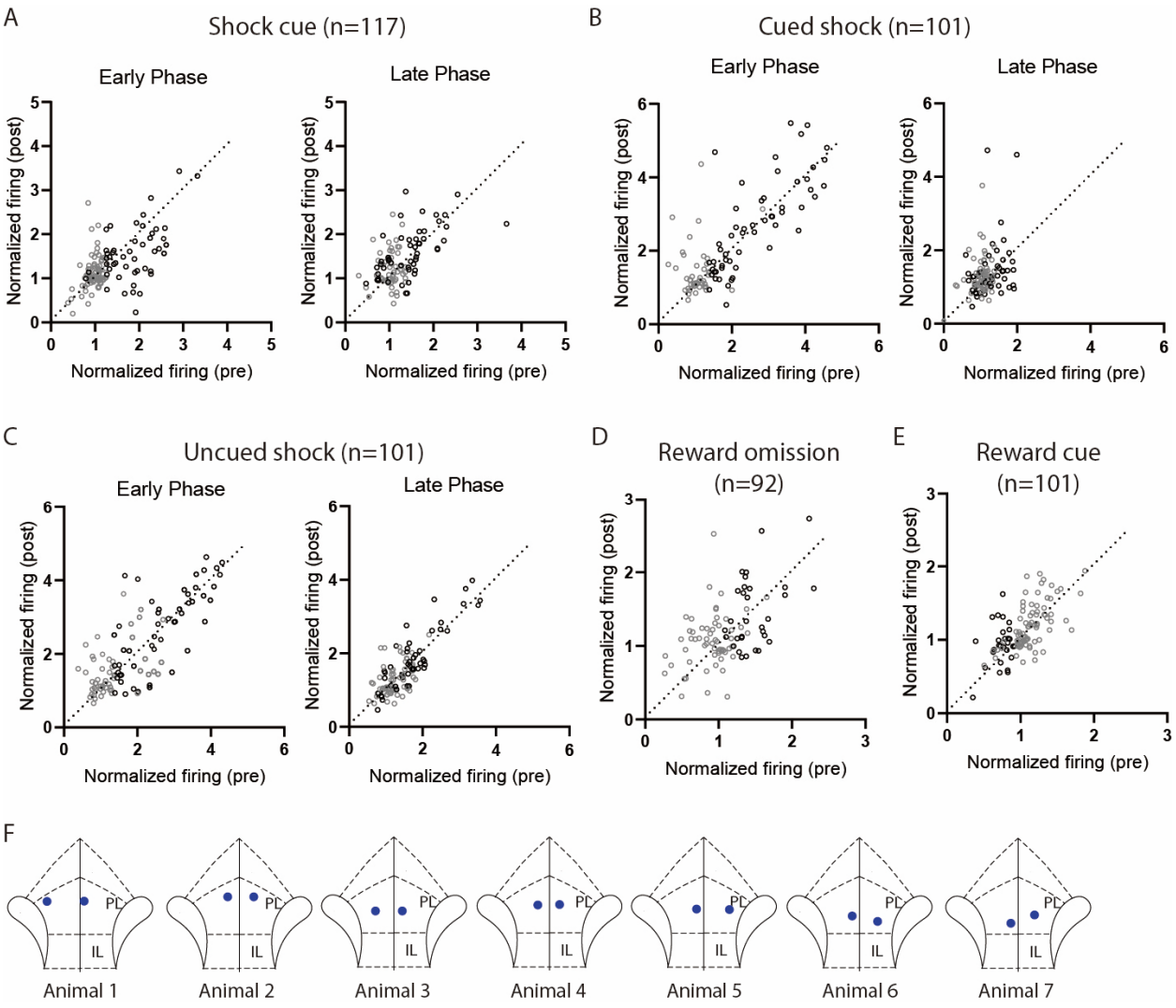

Figure S3. Histology and individual animal data for RMTg recordings after PL inactivation, related to Figure 3. (**A-D**) Individual responses to shock cues, cued shocks, uncued shocks, reward omissions, and reward cues before and after PL inactivation. Black dots indicate neurons that showed significant inhibition to reward cues or significant excitations to shocks, shock cues, or reward omissions. Grey dots indicate all other neurons (including non-responsive). (**F**) PL cannula placements (blue dots).

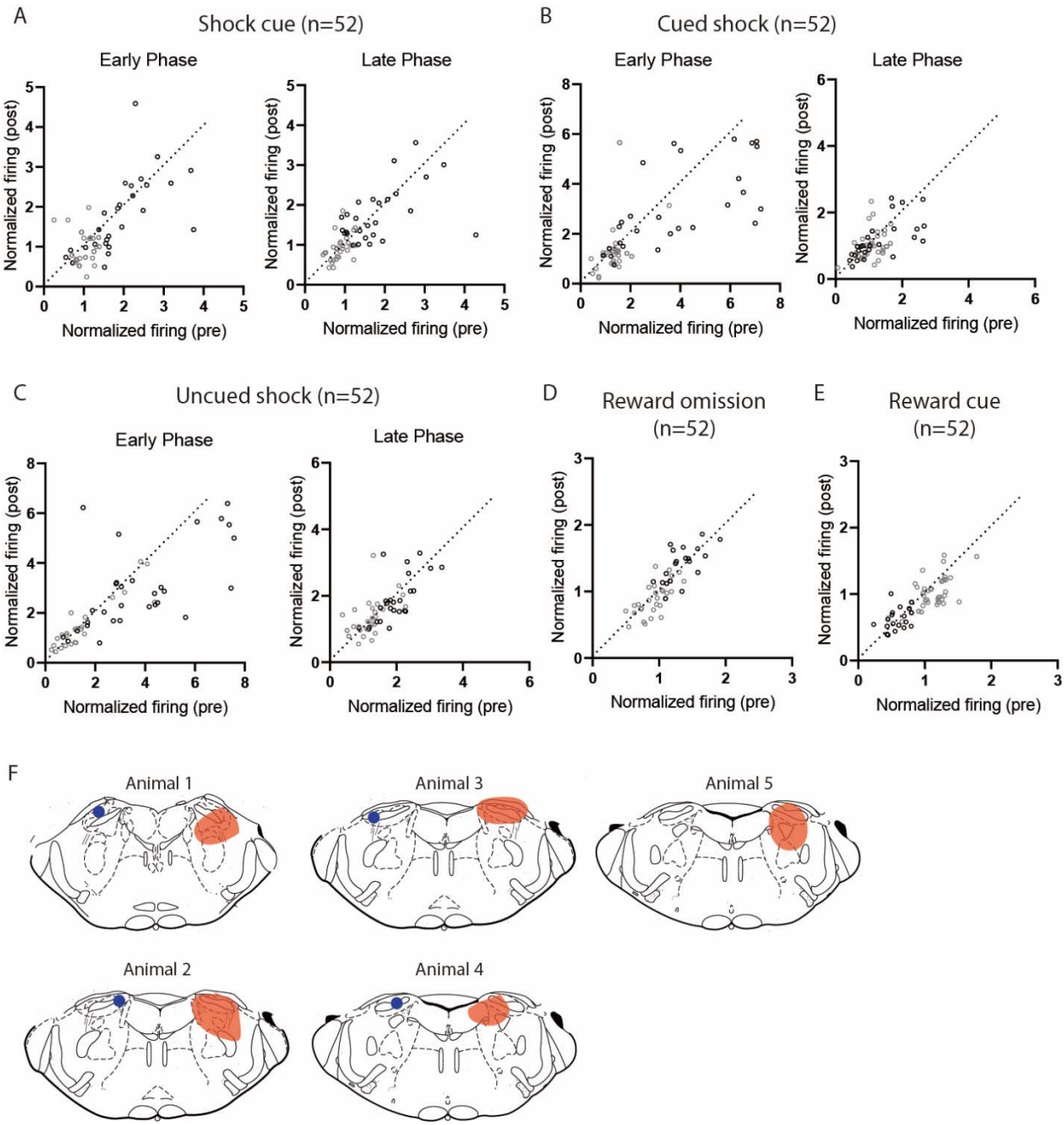

Figure S4. Histology and individual animal data for RMTg recordings after PBN inactivation, related to Figure 3. (**A-D**) Individual responses to shock cues, cued shocks, uncued shocks, reward omissions, and reward cues before and after PBN inactivation. Black dots indicate neurons that showed significant inhibition to reward cues or significant excitations to shocks, shock cues, or reward omissions. Grey dots indicate all other neurons (including non-responsive). (**F**) PBN cannula placements (blue dots), and the size of excitotoxic lesions on the contralateral PBN (red areas).

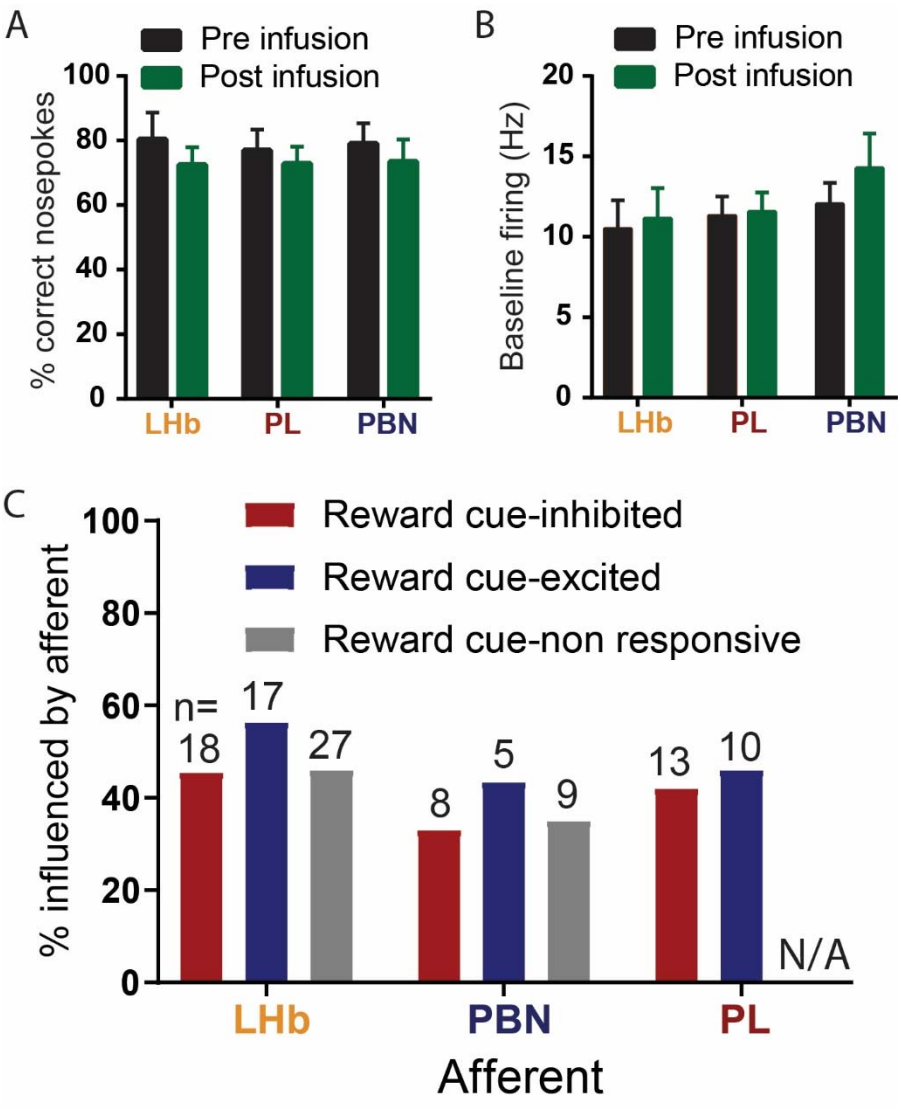

Figure S5. Supplementary data for pharmacological inactivation, related to Figures 2-3. (**A, B**) Microinfusion of drug did not alter nosepoke behavior (p=0.33, p=0.148, and p=0.164 for PL, PBN, and LHb inactivation, respectively) nor baseline RMTg firing rate (p=0.7669, p=0.2413 and p=0.2175). (**C**) Inactivation of PL, PBN, or LHb reduced the proportion of RMTg neurons activated by shock cues, shocks, or surprise, respectively. These proportions did not differ between reward cue-inhibited, reward cue excited, and reward cue-non responsive RMTg neurons (p=0.578, p=0.893, and p=0.462 for inhibited vs. excited, inhibited vs. non-responsive, and excited vs. non-responsive in LHb group, p=0.645, p=0.946, and p=0.681 in PNB group, and p=0.865 for inhibited vs. excited in PL groups, Chi-square). Reward cue-non responsive neurons in PL group were excluded from the analysis, as they tend not to respond to shock cues. Numbers of affected neurons are shown above each individual bar.

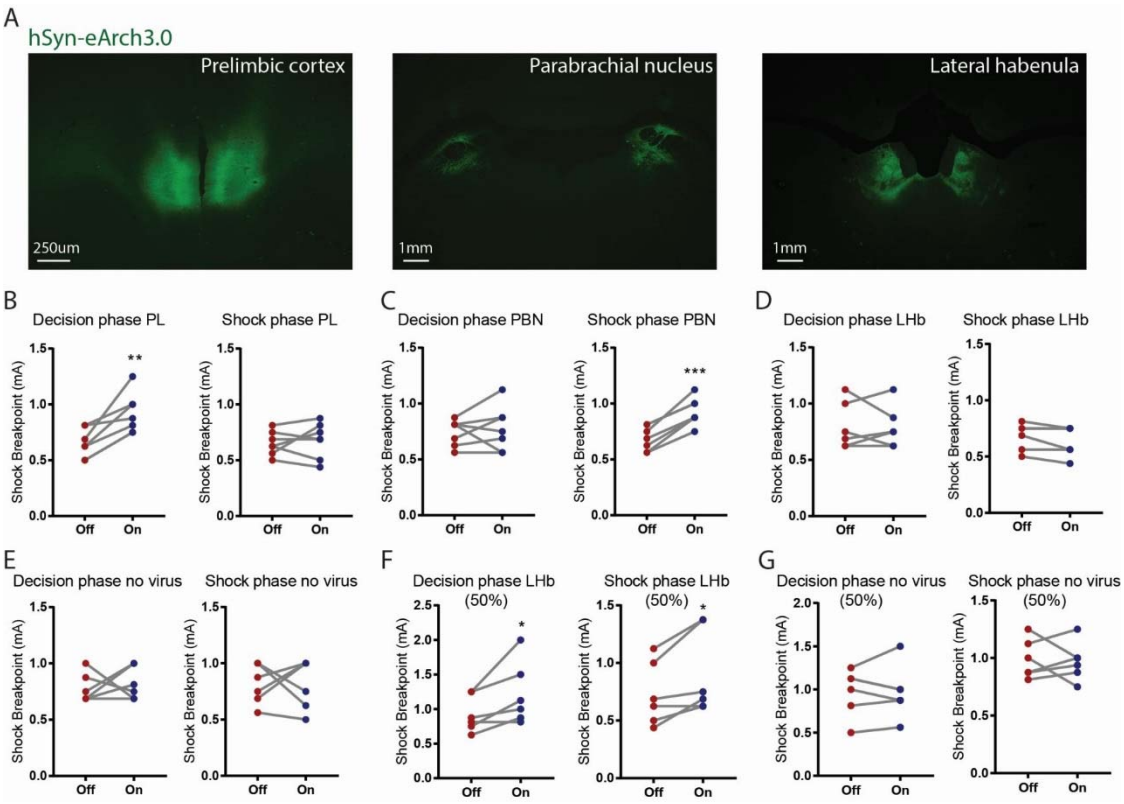

Figure S6. Histology and individual animal behavioral data after optogenetic inhibition of RMTg afferents, related to Figure 4. (**A**) Representative photos of virus injection sites in the PL, PBN, and LHb. (**B-E**) Individual performance in the optogenetic experiments using 100% shock probability (**B**: p=0.004 and p=0.587; **C**: p=0.603 and p=0.0002; **D**: p=0.871 and p=0.102; **E**: p=0.685 and p>0.999, paired t-test). (**F, G**) Individual performance after optogenetic inhibition of LHb projections to the RMTg using 50% probability of shock delivery (**F**: p=0.039 and p=0.026; **G**: p>0.999 and p=0.787, paired t-test).

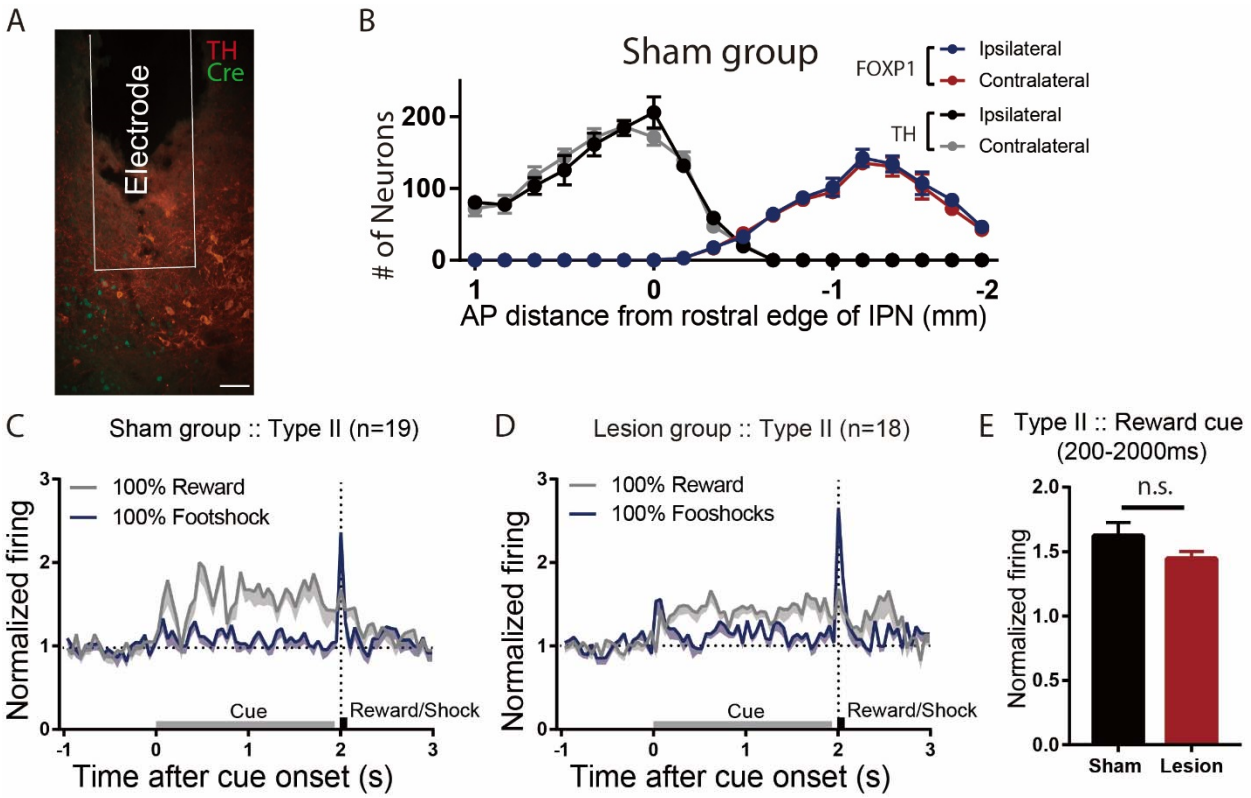

Figure S7. Histology and supplemental electrophysiological data from VTA recordings, related to Figure 5. (**A**) Electrode (white rectangles) and Cre-recombinase (green fluorescence) placements in VTA, along with red TH immunofluorescence. Scale bar: 100um. (**B**) Quantitation of RMTg FOXP1-positive cells and VTA TH-positive cells ipsilateral to the site of Cre injection compared with the contralateral side in sham-lesioned rats. (**B**) Time course of non-pDA neuron responses to reward and reward cues in lesioned rats. (**C**) Non-pDA neurons lacked phasic activation to reward-predictive cue and had greatly reduced excitation to footshock, relative to DA-like neurons. (**D, E**) Responses of type II VTA neurons to reward cues were not affected by RMTg lesion (p=0.1508, unpaired t-test).