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Propranolol decreases fear expression by modulating fear memory traces

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Abstract

BACKGROUND: Post-traumatic stress disorder (PTSD) can develop following a traumatic event and results in heightened, inappropriate fear and anxiety. Although approximately 8% of the United States population suffers from PTSD, only two drugs have been approved by the FDA to treat it, both with limited efficacy. Propranolol, a non-selective β-adrenergic antagonist, has shown efficacy in decreasing exaggerated fear, and there has been renewed interest in using it to treat fear disorders.

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FINANCIAL DISCLOSURES

BKC and CAD are named on provisional and non-provisional patent applications for the prophylactic use of (*R,S*)-ketamine and related compounds against stress-related psychiatric disorders. SLS, MS, AMZ, AM, ADL, NV, and ML report no biomedical financial interests or potential conflicts of interest.

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METHODS: Here, we sought to determine the mechanisms by which propranolol attenuates fear by utilizing an activity-dependent tagging system, the ArcCreER^{T2} x enhanced yellow fluorescent protein (eYFP) mice. 129S6/SvEv mice were administered a 4-shock contextual fear conditioning (CFC) paradigm followed by immediate or delayed context re-exposures. Saline or propranolol was administered either prior to or following the first context re-exposure. To quantify hippocampal, prefrontal and amygdalar memory traces, ArcCreER^{T2} x eYFP mice were administered a delayed context re-exposure with either a saline or propranolol injection prior to context re-exposure.

RESULTS: Propranolol decreased fear expression only when administered prior to a delayed context re-exposure. Fear memory traces were affected in the dorsal dentate gyrus and basolateral amygdala following propranolol administration in the ArcCreER^{T2} x eYFP mice. Propranolol acutely altered functional connectivity between hippocampal, cortical, and amygdalar regions.

CONCLUSIONS: These data indicate that propranolol may decrease fear expression by altering network correlated activity and by weakening the reactivation of the initial traumatic memory trace. This work contributes to the understanding of noradrenergic drugs as therapeutic aids for PTSD patients.

Keywords

propranolol; engram; memory trace; c-Fos; Arc; contextual fear conditioning

INTRODUCTION

Post-traumatic stress disorder (PTSD) is triggered by a traumatic event and results in heightened, inappropriate fear and anxiety, flashbacks, and intrusive thoughts (1). Although approximately 8% of the United States general population is affected by PTSD, only two drugs, sertraline (Zoloft) and paroxetine (Paxil), have been approved by the FDA for the treatment of PTSD, both with limited efficacy (2, 3). Other drugs that target noradrenergic (NA) transmission have been shown to have some efficacy against fear expression associated with PTSD (4, 5, 6).

Propranolol, a non-selective β-adrenergic antagonist which inhibits both β1- and β2-adrenoreceptors (AR), has shown efficacy in decreasing exaggerated fear in PTSD patients (5) and rodents (7, 8, 9). In healthy humans, a meta-analysis revealed that a single session of memory reactivation paired with propranolol administration reduced the strength of negatively valanced emotional memories (10). Additionally, propranolol can successfully reduce performance anxiety in musicians (11), in dental phobic patients (12), and in patients with arachnophobia (13). However, results of propranolol's efficacy in humans have been mixed, with some studies showing no efficacy in phobic behavior following administration (14, 15). It remains to be determined the extent to which propranolol could be used for fear disorders, as well as the appropriate timing, dosage, and number of administrations.

Understanding how propranolol alters brain activity and affects the retrieval and the reconsolidation of fear memories is crucial to utilizing this drug in an efficacious manner. Notably, several regions involved in fear learning have been shown to encode fear memory

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traces or engrams. Engrams are defined as a neural ensemble activated during learning and whose reactivation by the original stimuli results in memory retrieval, a concept first proposed by Richard Semon in the early 20th century (16). Recent genetic and optogenetic techniques have allowed scientists to manipulate engrams and to alter their content, suggesting that deleterious memories, such as fear memories (17–22) in PTSD, or lost memories, such as in Alzheimer's disease (AD) (23, 24), may be modified to improve mood and cognition (16, 25).

To study engrams, numerous technologies utilize immediate early genes (IEGs), which are activated upon learning (26). Our lab previously created an indelible, activity-dependent tagging murine line, based on the IEG activity-regulated cytoskeleton-associated protein *Arc/Arg3.1* (27–29), which has been widely implicated in synaptic plasticity. This line of ArcCreER^{T2} mice allows for the permanent labeling of activated *Arc*$^{+}$ neurons to study long-term engrams (17, 22, 23, 30–33). Using this transgenic murine line, it has been shown that, in the hippocampus (HPC), the subregions dentate gyrus (DG) and *cornu ammonis* 3 (CA3) encode memory traces of contextual fear memories (17), and that the neuronal ensembles active upon encoding of contextual fear conditioning (CFC) memories are necessary for memory retrieval (17, 22).

No study to date has investigated whether and how systemic administration of propranolol affects fear memory trace activity during fear retrieval. Brain regions that have been implicated in mediating the effects of propranolol on fear memory retrieval and reconsolidation in humans (34–36) and in rodents (8, 9, 37) constitute the optimal targets for research, in particular, subregions of the HPC (8, 34, 38–40), of the prefrontal cortex (PFC) and of the amygdala (AMG) (8, 9, 35, 37, 41, 42).

Here, we investigated the mechanisms of a propranolol-induced decrease in fear expression at the level of PFC, HPC, and AMG fear memory traces. We show that following CFC, propranolol decreased fear expression only when administered prior to a delayed, but not immediate, context re-exposure. Propranolol's effects on fear expression did not extend past the first context re-exposure. Behavioral controls indicated that propranolol's effects were centrally mediated and were due to impaired fear memory retrieval that was context specific, as opposed to an anxiolytic effect or to changes in generalized fear. Utilizing the ArcCreER^{T2} x eYFP mice we show that during fear retrieval propranolol modulates activity in HPC, PFC and AMG regions, changing their activity levels, functional connectivity, and the reactivation rates of fear memory traces.

METHODS AND MATERIALS

Mice

Male 129S6/SvEvTac mice were purchased from Taconic (Hudson, NY) at 7-8 weeks of age. For memory trace tagging experiments, ArcCreER^{T2}(+) (26) x R26R-STOP-floxed-eYFP (43) homozygous female mice were bred with R26R-STOP-floxed-eYFP homozygous male mice (43). All experimental mice were ArcCreER^{T2}(+) and homozygous for the eYFP reporter. ArcCreER^{T2}(+) x eYFP mice are on a 129S6/SvEv background, as they have been backcrossed for more than 10 generations onto a 129S6/SvEv line.

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Mice were housed 4-5 per cage in a 12-h (06:00-18:00) light-dark colony room at 22°C. Food and water were provided *ad libitum*. Behavioral testing was performed during the light phase. All mice utilized for behavioral experiments were approximately 9-12 weeks of age. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the New York State Psychiatric Institute (NYSPI). Numbers of animals in each experimental cohort are included in Table S1.

Drugs

Propranolol (P)—A single injection of saline (Sal) (0.9% NaCl) or propranolol hydrochloride (P) ((±)-Propranolol hydrochloride, Sigma Aldrich, #PHR1308) (10 mg/kg) was administered once or twice during each experiment. Propranolol was prepared in physiological saline. All injections were administered intraperitoneally (i.p.) in volumes of 0.1 cc per 10 mg body weight. Dosing was based on previous studies, indicating that 10 mg/kg of propranolol was effective at decreasing fear expression either acutely or in subsequent re-exposures in mice (7, 8, 9, 44).

Sotalol (S)—A single injection of saline (Sal) (0.9% NaCl) or Sotalol (Sot) ((±)-Sotalol hydrochloride, Sigma Aldrich, #S0278) (10 mg/kg), a peripheral β-adrenergic blocker, was administered once during each experiment. Sotalol was prepared in physiological saline and all injections were administered i.p. in volumes of 0.1 cc per 10 mg body weight.

4-hydroxytamoxifen (4-OHT)—Recombination was induced using 4-OHT (Sigma, St. Louis, MO, H7904) as previously described (39). 4-OHT was dissolved by sonication in 10% EtOH / 90% corn oil at a concentration of 10 mg/ml. One injection of 200 μl (2 mg) was administered i.p. into adult mice.

Statistical Analysis

Data analysis is described in the Supplemental Materials. All statistical results are listed in Tables S2–S4.

RESULTS

Delayed, but not immediate administration of propranolol decreases fear expression

We first sought to determine the effect of propranolol on fear memory retrieval in 129S6/SvEv mice as to our knowledge no prior studies have tested propranolol in 129S6/SvEv mice. Mice were administered a 4-shock CFC paradigm and tested for memory retrieval 5 days later (delayed context re-exposure 1 (RE1)). Injecting propranolol immediately following RE1 did not impact fear expression during the second context re-exposure (RE2) (Figure 1A–1B). We next tested whether two administrations of propranolol following RE1 could impact fear expression. Using a separate cohort of mice, we administered an additional re-exposure (RE3) followed by a second injection of propranolol, which did not impact fear expression in a fourth re-exposure (RE4) (Figure S1A–S1C). Administering propranolol prior to RE1 significantly decreased fear expression during RE1, but 24 h later propranolol-injected mice did not exhibit decreased fear expression during RE2 relative to saline-injected mice (Figure 1C–1D).

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Next, we administered the 4-shock CFC paradigm and tested for retrieval 30 min following CFC training (immediate RE1). Injecting propranolol immediately following RE1 did not impact fear expression during RE2 (Figure 1E–1F). Injecting propranolol prior to RE1 did not impact fear expression during RE1 or RE2 (Figure 1G–1H). These data indicate that propranolol is only effective at decreasing contextual fear expression when injected following a delayed interval and before RE1 in the 129S6/SvEv mice.

Delayed administration of propranolol decreases cued fear expression

Here, we tested the effect of delayed administration of propranolol on retrieval of cued fear conditioning (FC) (Figure 2A). The two groups showed no differences during cued FC (Figure 2B). During the first tone test in context B, the propranolol-injected mice froze significantly less to the tones than the saline-injected mice (Figure 2C). The mice were then tested again 24 h later, and the propranolol-injected mice froze significantly more to the tones than the saline-injected mice (Figure 2D). The following day, mice were tested in context A, without any tone presentations, and the propranolol-injected mice froze significantly more than saline-injected mice (Figure 2E). These data indicate that administration of propranolol prior to a delayed cue RE acutely lowers freezing behavior, similarly to what was observed with CFC, but also alters subsequent RE tests, unlike with CFC.

Propranolol does not affect anxiety-like behavior, fear generalization, or recall of a non-fearful memory

Next, we sought to determine if the decrease in fear expression following propranolol administration was due to effects on anxiety-like behavior. Mice were administered a 4-shock CFC paradigm and 5 days later tested in either the elevated plus maze (EPM) (Figure S2A) or the open field (OF) (Figure S2J). Injection of propranolol prior to the EPM did not impact any of the behavioral measures assessed in the EPM (Figure S2B–S2I) nor in the OF (Figure S2K–S2Q). These data indicate that the propranolol-induced decrease in fear expression is not due to effects on anxiety-like behavior.

To investigate the context specificity of propranolol’s effects, we replicated the delayed CFC paradigm, but we exposed mice to an unconditioned, yet similar context B after context A re-exposure (Figure 3A). We replicated the acute effect of propranolol in reducing contextual fear expression but found no effect on fear generalization (Figure 3B–3C). Additionally, we re-exposed the mice to context A 35 days following CFC to assess long-term memory (LTM) and found no differences between the groups (Figure 3B–3C). These data suggest the effect of propranolol on fear retrieval is context specific and does not affect LTM retrieval.

To understand if the effects of propranolol are specific to fear memories or generalized to other types of memories, we tested the impact of propranolol in a social memory task (Figure 3D). During the social memory retrieval test, saline- and propranolol-injected mice spent significantly more time exploring the cup with the novel mouse compared to the cup with the familiar mouse (Figure 3E). There were no differences between the groups in time spent exploring the familiar mouse or the novel mouse over the course of the test (Figure

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3F–3G). These data suggest the effect of propranolol on learned fear retrieval may not be replicated with retrieval of other types of consolidated memories.

Sotalol, a peripheral β-adrenergic blocker, does not decrease fear expression

To determine if the observed propranolol-induced decrease in fear expression was due to central or peripheral β-adrenergic blockade, we administered sotalol, a β1 and β2-adrenergic blocker which does not cross the blood-brain barrier (45). Mice were administered a delayed CFC paradigm (Figure S3A) and received sotalol or saline prior to RE1. No differences in freezing behavior were observed during either RE1 or RE2 (Figure S3B–S3C), indicating that the propranolol-induced decrease in fear expression is not peripherally mediated.

Propranolol decreases fear expression in the ArcCreER^{T2} x eYFP mice

To determine how propranolol alters the memory traces of the fear-inducing stimuli, we utilized the ArcCreER^{T2} x eYFP mice for indelible, whole-brain activity dependent tagging (Figure S4). Mice were injected with 4-OHT 5 h prior to 4-shock CFC (Figure 4A). Five days later, mice were administered saline or propranolol prior to RE1 and euthanized 1 h following RE1. Propranolol significantly decreased fear expression during RE1 when compared with saline (Figure 4B).

Propranolol alters memory traces in the dDG of ArcCreER^{T2} x eYFP mice

We next quantified the activated neural ensembles in HPC, PFC and AMG of ArcCreER^{T2} x eYFP mice utilizing the data analysis pipeline described in the Supplement Materials (Figure S4–S10) (46–49). In the HPC, the numbers of eYFP^{+} and c-Fos^{+} cells in the DG, CA3, and CA1 did not differ between groups (Figure 4D–4E, 4I–4J). The memory trace reactivation percentages of co-labeled/c-Fos^{+} cells (Figure 4F) and co-labeled/eYFP^{+} cells (Figure 4G) cells were significantly lower in the dDG following propranolol administration when compared to saline administration. Propranolol did not alter the average dCA3 or dCA1 (Figure 4F–4G), nor vDG, vCA3, or vCA1 memory traces (Figure 4K–4L). These data indicate that propranolol specifically alters dDG memory traces. These data were corroborated by manual quantification (Figure S11).

Propranolol decreases c-Fos^{+} cells in the ILA of the PFC

In the PFC (Figure 5A), there were no differences in eYFP^{+} cells between saline- or propranolol-administered mice (Figure 5B). There were significantly less c-Fos^{+} cells in the ILA of propranolol-administered mice (Figure 5C). No other differences were observed in number of c-Fos^{+} cells (Figure 5C) nor in memory traces (Figure 5D–5E).

Propranolol decreases c-Fos^{+} cells in the LA and alters memory traces in the BLA

In the AMG, no differences were observed in number of eYFP+ cells active between saline- and propranolol-administered mice (Figure 5F–5G). Propranolol resulted in significantly less c-Fos^{+} cells in the LA, but not in any other amygdalar nuclei (Figure 5H). In the BLA, the percentage of co-labeled/c-Fos^{+} cells was higher in the propranolol group relative to controls (Figure 5I). No other differences in reactivation were observed in AMG (Figure 5I–5J).

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Propranolol alters functional connectivity within and between the HPC, PFC, and AMG

To understand how the functional connectivity between brain regions implicated in fear memory retrieval is altered by propranolol, we computed the correlations of cell counts between brain regions, as done before (50, 51). First, we characterized the correlation of activity during fear encoding for all mice (Figure S12). This analysis showed significant positive correlations within the dHPC, but not within the vHPC. Within the AMG, all nuclei were positively correlated with the BMA, except for the LA, which correlated only with the BLA. The dHPC and vHPC were positively correlated through CA1, and both were positively correlated with PFC and with different nuclei of the AMG.

To assess the effect of propranolol on the functional connectivity during RE1, we performed correlation analyses of c-Fos levels across brain regions for both groups. Relative to saline-administered mice (Figure 6A, 6C), propranolol-administered mice maintained positive correlations within the dHPC but showed fewer positive correlations and more negative correlations within the vHPC, and within the AMG (Figure 6B, 6D). Across regions, compared to controls, in the propranolol group there were fewer positive correlations between dHPC and vHPC, and a negative correlation between dCA3 and vCA3. Between dHPC and AMG regions, in the controls there were positive correlations between dCA3 and the AMG nuclei LA, BMA and CEA, and between vCA1 and LA, but these were absent in the propranolol-administered group. Between dHPC and PFC, only in propranolol-administered mice we observed a negative correlation between dDG and PL. Analyses of correlations between number of cells active during memory retrieval and freezing levels showed no significant correlation for any region for either group (Figure 6A–6B). These data suggest that propranolol alters the correlated activity between several HPC, PFC and AMG regions, predominantly decreasing positive correlations and increasing negative correlations; and that no individual region's activity correlated with freezing behavior in either condition.

Propranolol alters the correlations of memory trace reactivation rates across regions

We analyzed how fear memory trace reactivation upon RE1 correlated across regions (Figure 7A–7D). Regarding the percentage of co-labeled/eYFP$^+$ cells, we found that in control mice (Figure 7A), but not in propranolol administered mice (Figure 7B), dCA1 and BLA reactivation levels were positively correlated; all vHPC subregions were positively correlated with each other, and both dDG and BLA reactivation levels were negatively correlated with IA. Conversely, only in propranolol-administered mice, the percentage of co-labeled/eYFP$^+$ cells in dDG and LA were negatively correlated (Figure 7B). When analyzing the correlations of reactivation levels and freezing behavior, in the control condition, the ACA (Figure 7E) was the one region in which percentage of co-labeled/eYFP$^+$ cells was positively correlated with freezing, and this relation was absent in propranolol-injected mice. The LA percentage of co-labeled/eYFP$^+$ cells (Figure 7F) was positively correlated with freezing levels only in propranolol-administered mice. Regarding the percentage of co-labeled/c-Fos$^+$ cells (Figure 7C–7D), in control mice only, we observed significant negative correlations between dDG and ACA, and between BLA and vCA3. In contrast, exclusively in propranolol-administered mice we found positive correlations between the reactivation levels of dCA3 and LA, of vCA1 and ILA, and of BLA, ILA, and ACA with each other. In dCA3, the percentage of co-labeled/c-Fos$^+$ cells was negatively

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correlated with freezing behavior, only in controls (Figure 7G). These data suggest that memory trace reactivation across regions may be synchronized, and that ACA, LA and dCA3 memory reactivation levels correlate with freezing levels depending on the treatment. Non-significant correlations between memory trace reactivation and freezing levels are shown in Figure S13.

DISCUSSION

Here, we investigated whether propranolol affected learned fear retrieval and whether this was accompanied by changes in memory trace reactivation. We show that propranolol acutely impairs contextual fear expression. We quantified neurons active during memory encoding and during retrieval across brain regions by utilizing an activity-dependent tagging system. We show that propranolol's acute effect is associated with: 1) decreased functional connectivity within and between HPC, PFC and AMG regions, 2) decreased activity in the LA and ILA, 3) changes in memory trace reactivation in the dDG and BLA, and 4) changes in the correlation between memory trace reactivation in ACA, LA and dCA3 and freezing.

Propranolol has been used to manipulate the reconsolidation of the fearful memory that is elicited by re-exposure/retrieval, and previous animal studies have elucidated to an extent conditions that allow for propranolol's efficacy and the neural mechanisms that mediate its action (52–54). However, there has been difficulty establishing the boundary conditions in terms of timing, dosing, and exposure/stimulation that lead to a successful and reproducible therapeutic outcome (55), warranting further research to understand how to best utilize the potential of propranolol for treating fear disorders.

Here, we show that propranolol had an acute effect on fear expression during CFC memory retrieval in a delayed RE, with no effect on RE trials after drug washout, as had been shown in (7). The lack of an effect during immediate RE could be due to higher levels of NA following CFC, rendering propranolol insufficient to alter behavior. On a cued fear retrieval test, we observed a similar acute effect of propranolol during the first tone test. However, we observed a long-lasting effect of heightened fear in the following exposures to the tone, and to the original FC context, absent in the CFC paradigm. Other studies have shown an effect of propranolol in impairing extinction/reconsolidation of auditory cued fear (9, 56, 57), although the literature is not unanimous (44). These differences may be due to one task being HPC-dependent and the other HPC-independent, and different components of the memory trace responding differently after drug washout.

We further probed into how specific the acute effect of propranolol was to fear memory retrieval. Our results indicate that in certain circumstances propranolol affects fear retrieval but does not affect innate anxiety or generalized fear, nor retrieval of a non-fearful social memory. Instead, propranolol's effect on fear behavior can be specific to learned fear and to the original conditioned stimuli. These findings add to the debate on the use of propranolol for performance anxiety (58–60) and suggest that its apparent anxiolytic effect may be impaired retrieval of learned fear.

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To understand how propranolol affects brain activity during the reactivation of fear memories, we used the ArcCreER^{T2} x eYFP mouse model. In the HPC, we found that propranolol affected fear memory traces in the dDG, but not in other hippocampal subregions. In rodents, β1- and β2-AR, which have been extensively implicated in the modulation of memory formation (reviewed in (38)), are expressed throughout the HPC, with higher levels of expression of both receptor subtypes in the dDG compared to dCA1 and dCA3 (61–64). Here, we provide evidence of NA modulation of dDG fear memory traces upon retrieval of a contextual fear memory. A more subtle effect was observed in dCA3 in the correlation between reactivation rates and freezing levels. The dHPC is necessary for learning and memory associated with spatial navigation and exploration (65, 66), whereas the vHPC is associated with innate fear and anxiety and with emotional and motivational aspects of learning (67–71). Our data suggest that the acute effect of propranolol on fear behavior may be due to a lower reactivation of the contextual components of the fear memory in the dHPC. The lack of an effect in activity or memory reactivation in vHPC is consistent with the lack of an observed effect on anxiety.

In the PFC, we found that propranolol decreased global activity only in the ILA. When assessing correlated activity, interestingly we found that PL negatively correlated with dDG, where we found differences in memory trace reactivation, suggesting either mutual inhibition or a disconnection due to upstream signaling. Average memory trace reactivation was unaffected in the PFC, yet ACA fear memory reactivation rate of encoding cells was positively correlated with freezing levels in controls, but not with propranolol, indicating a role for ACA in fear memory encoding and retrieval and in mediating propranolol's effects.

In the AMG, the BLA was the only region where average reactivation rate was altered, with higher reactivation rate of retrieval cells in propranolol-administered mice, which tended to be negatively correlated with freezing levels. The LA was the region that showed changes in more measures: lower activity in propranolol-administered mice relative to controls, correlations with dHPC and vHPC present in controls but absent in propranolol-administered mice, and reactivation rates of encoding cells positively correlated with freezing levels, only with propranolol.

From a circuit perspective, if propranolol disrupts the coordination between regions involved in fear retrieval and/or fear expression, it can interfere with fear retrieval by altering the upstream input into an area with a critical memory trace (for example the PL-DG projection). Additionally, the ILA, which has fear-dampening/pro-extinction effects, becomes positively correlated with vCA3 under propranolol. The ventral HPC is thought to harbor a memory component of emotional valence, and so a greater inhibitory influence of ILA on vCA3 could influence fear retrieval. We also observe that propranolol disrupts the coordinated activity between amygdalar nuclei, and between the vHPC subdivisions. Disruption to the communication between these regions that harbor different components of the memory trace could contribute to the behavioral effect. Further studies characterizing the necessity or sufficiency of particular pathways in modulating the effects of propranolol will be needed.

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Overall, we found that regions that did not have global differences in activity could have differences in correlated activity between regions, a dichotomy shown before (50). Notably, we also found that regions could have no differences in total activity but show differences in average reactivation levels between groups (e.g., dDG, BLA) or in the correlation between reactivation and freezing (e.g., ACA, LA, dCA3). If β-adrenergic signaling is altered in the memory trace cells following encoding, this could explain why specifically that subset of neurons would have altered activity in response to propranolol, but not the whole region. Simultaneously, the correlations between reactivation rates raise another question. Notably, in the dDG, reactivation rates of encoding cells were lower in propranolol-administered mice, but there was no significant correlation between memory reactivation and freezing levels. Yet, memory trace reactivation rates were negatively correlated specifically between the dDG and the two regions where reactivation rates of encoding cells did correlate with freezing levels, the LA and the ACA. This suggests either common modulation by upstream regions, or an interplay between components of a memory trace across regions, so that subsets of neurons are preferentially connected, and modulation of one component elicits changes in the ones it connects to. These effects may be discreet enough that they are not reflected in global activity changes by region.

This work contributes to the understanding of NA signaling during memory retrieval and how NA drugs may exert their therapeutic effects in fear disorders. Following this proof of concept, future work must explore how different parameters of timing and stimulus presentation can lead to different neuronal activity patterns, with different responses to propranolol, in both males and females. Considering our previous work (31), we believe that utilizing activity-dependent tagging strategies to understand how drugs affect fear ensembles can improve our understanding of stress-induced disorders such as PTSD and pave the way for novel therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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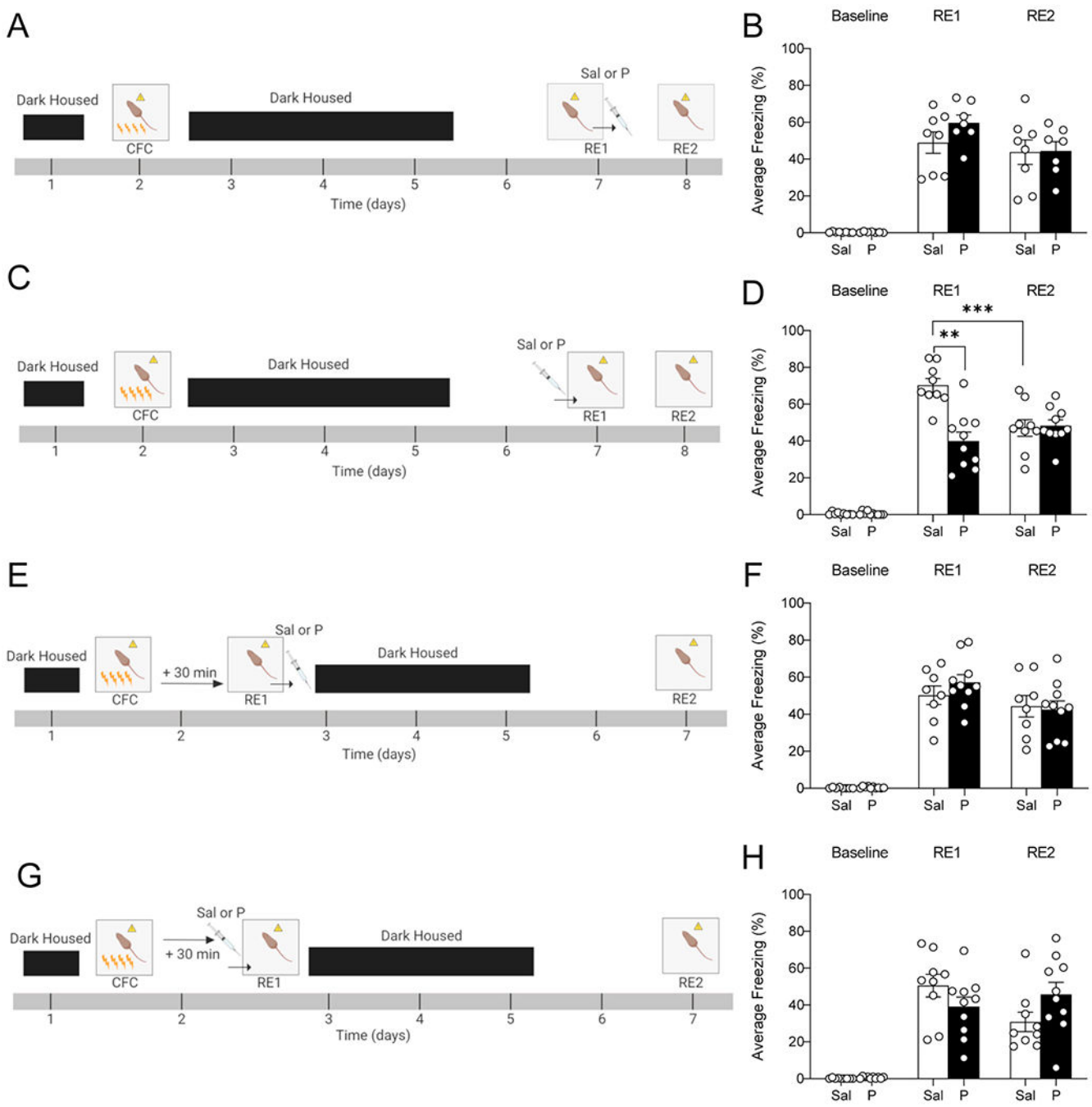

Figure 1. Administration of propranolol prior to delayed, but not immediate, context re-exposure decreases fear expression in 129S6/SvEv mice.

(A) Experimental design. **(B)** Injection of propranolol following RE1 does not impact fear expression during RE2. **(C)** Experimental design. **(D)** Injection of propranolol prior to RE1 decreases fear expression during RE1 but not during RE2. **(E)** Experimental design. **(F)** Injection of propranolol following RE1 does not impact fear expression during RE2. **(G)** Experimental design. **(H)** Injection of propranolol prior to RE1 does not impact fear expression during RE1 or RE2. (n = 7-10 male mice per group). **p < 0.01, ***p < 0.001.

Sal, saline; P, propranolol; CFC, contextual fear conditioning; RE, context re-exposure; min, minutes.

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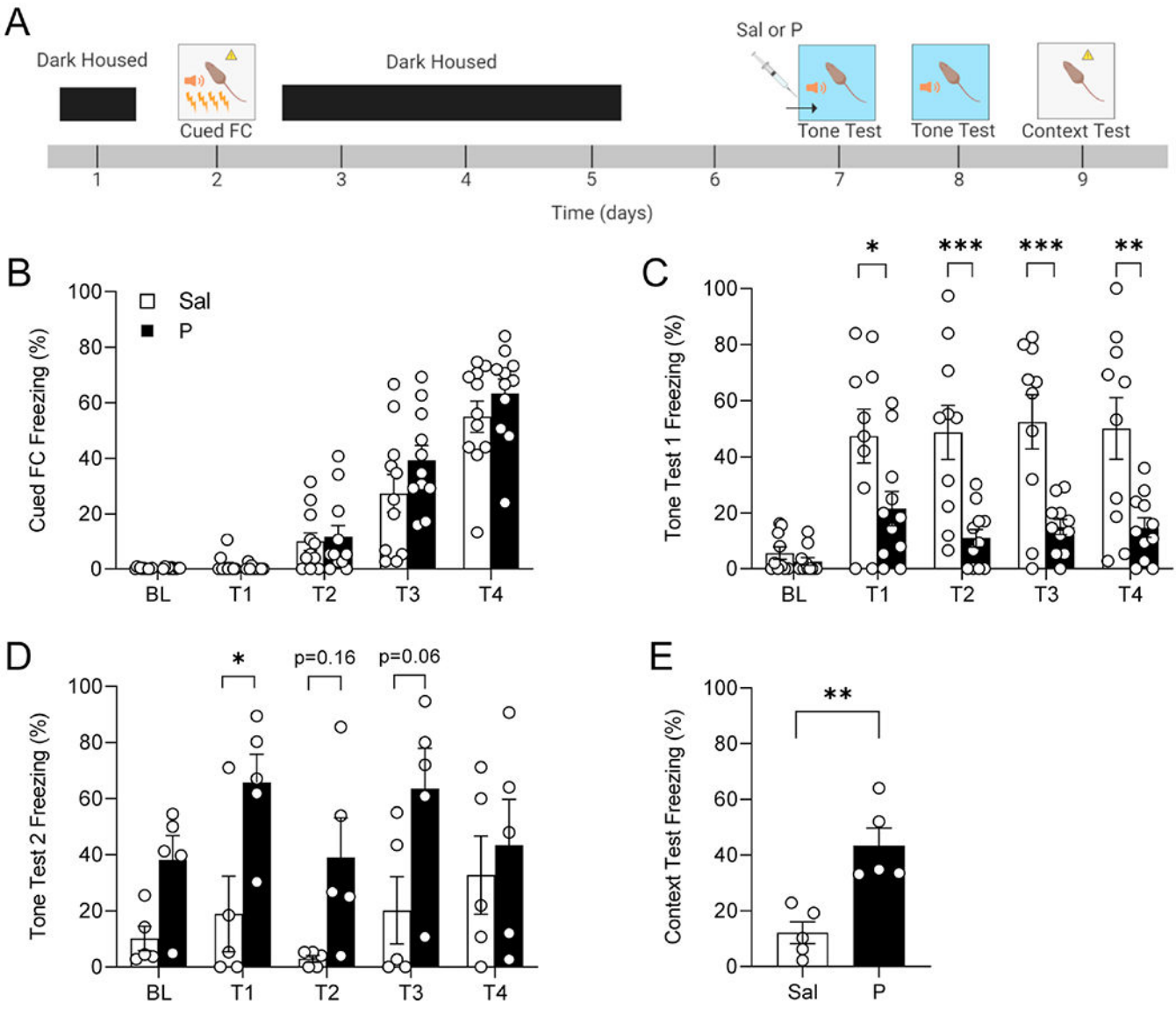

Figure 2. Administration of propranolol in a delayed re-exposure alters freezing behavior in a cued fear conditioning paradigm in 129S6/SvEv mice.

(A) Experimental design. **(B)** There are no differences between groups in freezing behavior during cued fear conditioning in context A. **(C)** Injection of propranolol before tone test in context B reduced freezing behavior during tone presentation in propranolol-administered mice. Freezing presented in bouts of 15s, and 20s for the tones. **(D)** Mice that were administered propranolol the previous day showed significantly higher freezing behavior than mice that had received saline during exposure to the tone in context B. **(E)** The mice were tested again in the original FC context and the propranolol group showed higher freezing levels compared to the saline group. (n = 9-10 male mice per group). *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent ± SEM. Sal, saline; P, propranolol; FC, fear conditioning; min, minutes.

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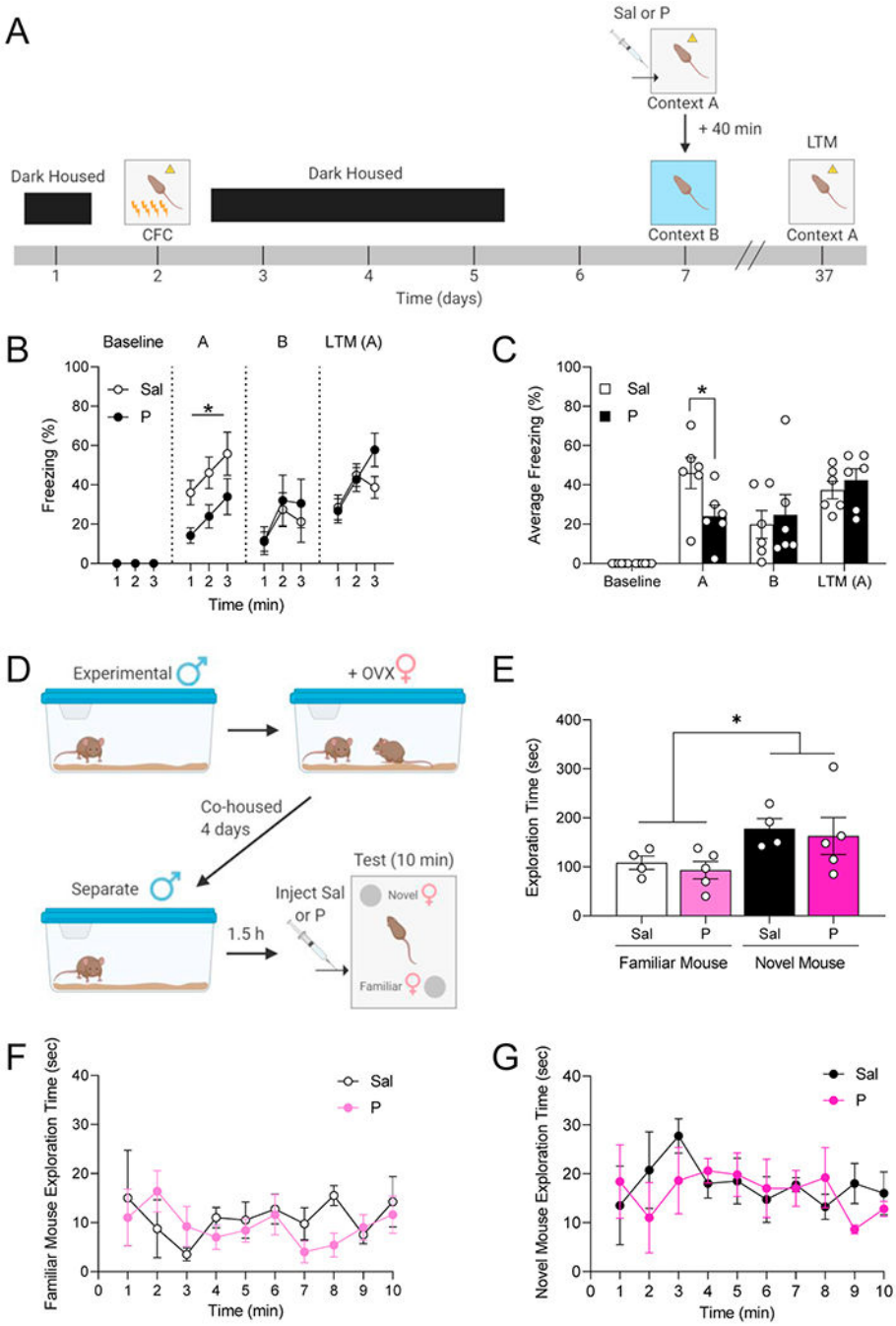

Figure 3. Administration of propranolol does not affect fear generalization, long-term memory retrieval, or social recognition in 129S6/SvEv mice.

(A) Experimental design. **(B)** Injection of propranolol prior to the re-exposure decreases freezing behavior in the original CFC context (context A) but not in a different context (context B), and no differences are observed in freezing in long term memory retrieval 30 days later. **(C)** Injection of propranolol prior to the re-exposure decreases average freezing behavior in the original CFC context (context A) but not in a different context (context B), and no differences are observed in average freezing in long term memory retrieval 30 days

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later. (n = 9-10 male mice per group). **(D)** Experimental design. **(E)** The mice spent significantly more time exploring the cup with the novel mouse compared to the cup with the familiar mouse. No differences between mice administered propranolol or saline. **(F)** Time spent exploring the familiar mouse. **(G)** Time spent exploring the novel mouse. (n = 5 male mice per group). *$p < 0.05$. Error bars represent ± SEM. Sal, saline; P, propranolol; CFC, contextual fear conditioning; LTM, long-term memory; min, minutes.

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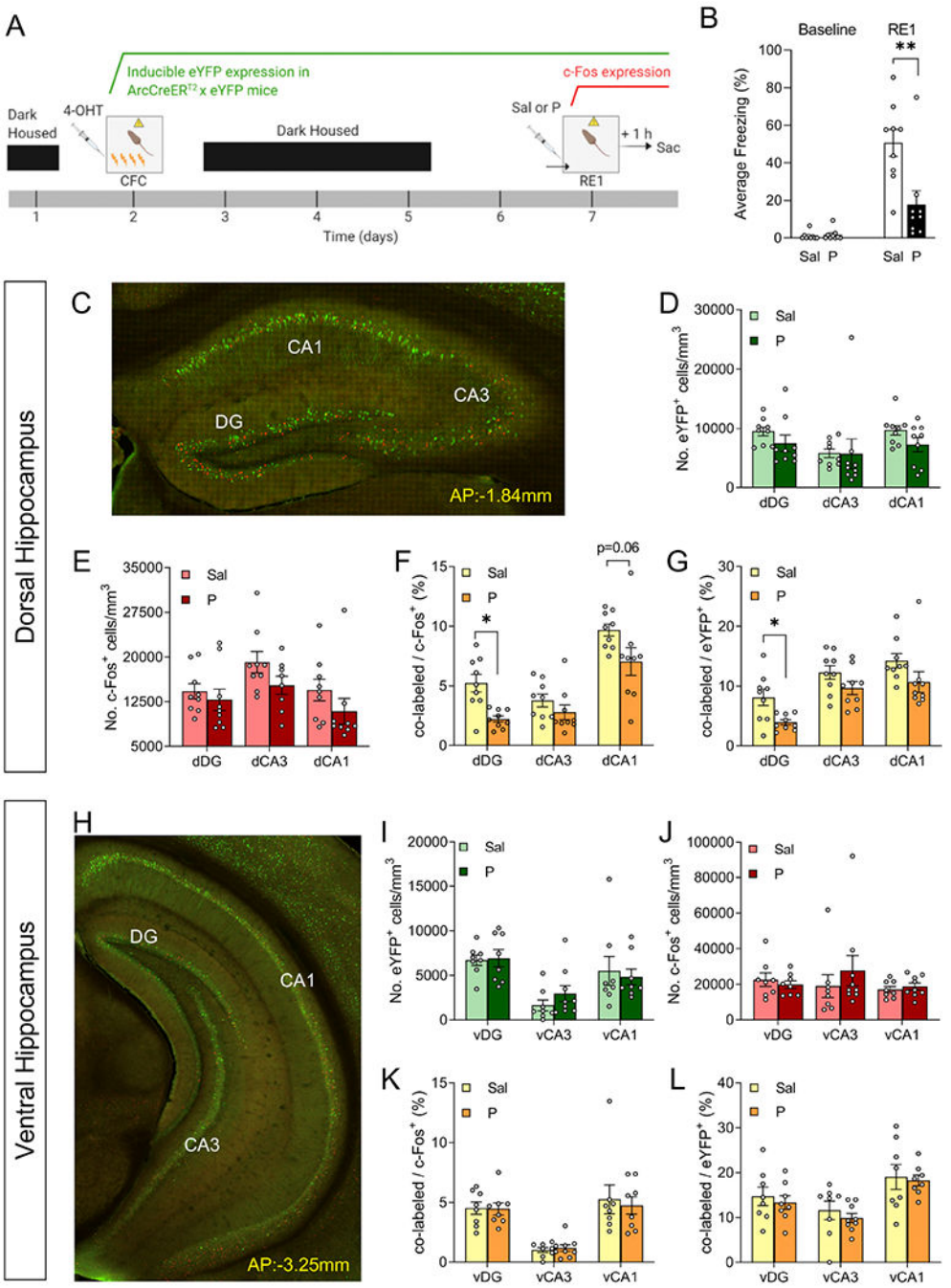

Figure 4. Administration of propranolol decreases fear expression and alters memory traces in the dorsal dentate gyrus of ArcCreER^{T2} x eYFP mice.

(A) Experimental design. **(B)** Injection of propranolol prior to the RE1 decreases freezing behavior. **(C)** Representative image of the dorsal hippocampus. The number of **(D)** eYFP$^+$ cells or **(E)** c-Fos$^+$ cells does not differ in dDG, dCA3, or dCA1 following administration of propranolol. The percentage of **(F)** co-labeled/c-Fos$^+$ cells and **(G)** co-labeled/eYFP$^+$ cells significantly decrease in the dDG following administration of propranolol, but not in dCA3 or dCA1. **(H)** Representative image of the ventral hippocampus. The number of **(I)** eYFP$^+$

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cells or **(J)** c-Fos$^+$ cells does not differ in vDG, vCA1, or vCA3 following administration of propranolol. The percentage of **(K)** co-labeled/c-Fos$^+$ cells and **(L)** co-labeled/eYFP$^+$ cells does not differ in dDG, dCA1 or dCA3 following administration of propranolol. (n = 9 male mice per group). *p < 0.05, **p < 0.01. Error bars represent ± SEM. eYFP, enhanced yellow fluorescent protein; 4-OHT, 4-hydroxytamoxifen; CFC, contextual fear conditioning; RE1, context re-exposure; sac, sacrifice; Sal, saline; P, propranolol; dDG, dorsal dentate gyrus; dCA1, dorsal CA1; dCA3, dorsal CA3; vDG, ventral dentate gyrus; vCA1, ventral CA1; vCA3, ventral CA3.

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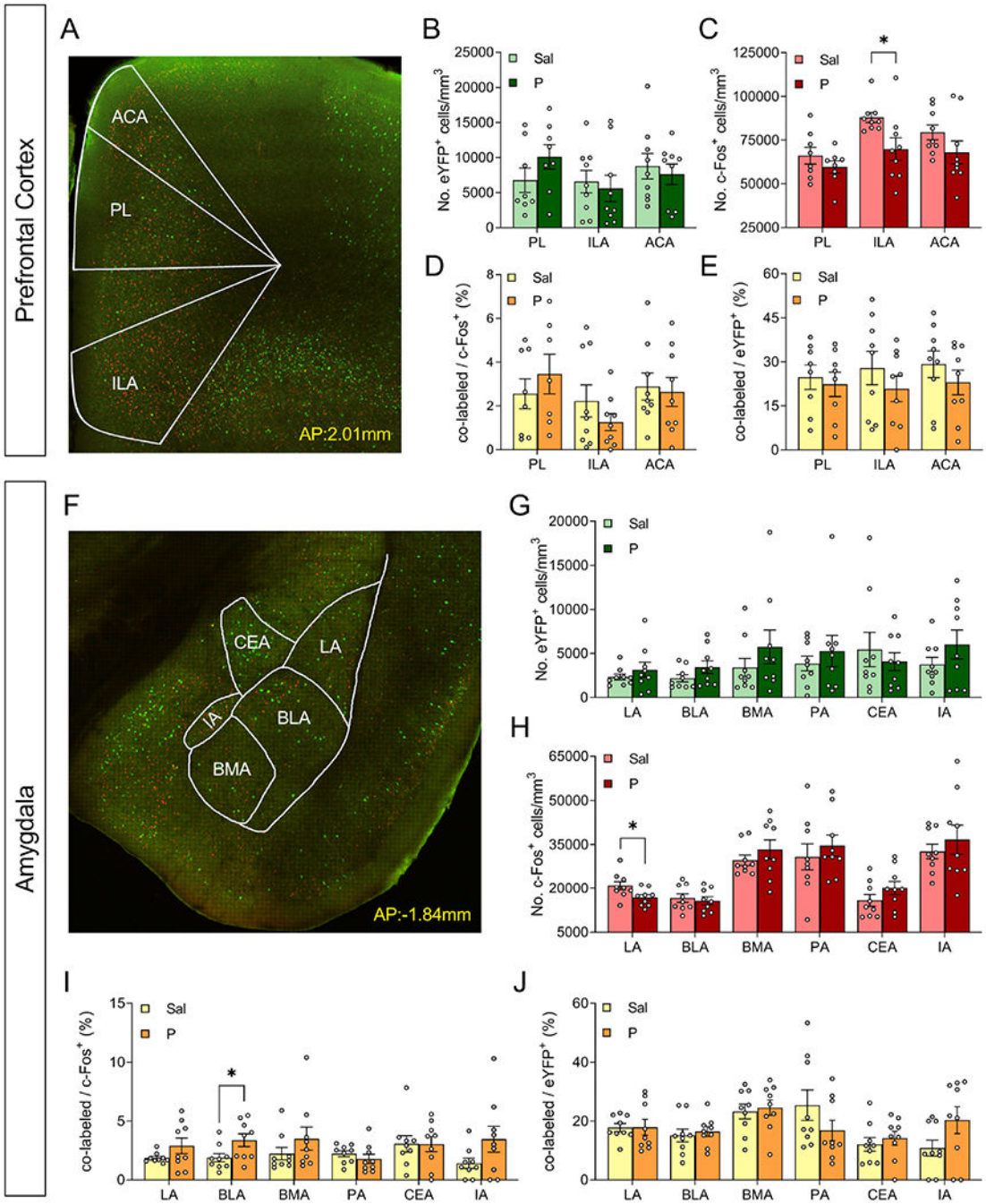

Figure 5. Administration of propranolol decreases activity in the ILA of the PFC and in the LA and alters memory traces in the BLA of ArcCreER^{T2} x eYFP mice.

(A) Representative image of the PFC. **(B)** The number of eYFP$^+$ cells does not differ in PL, ILA, or ACA following administration of propranolol. **(C)** The number of c-Fos$^+$ cells is decreased in the LA of mice that were administered propranolol, but does not differ in PL or ACA. The percentage of **(D)** co-labeled/c-Fos$^+$ cells and **(E)** co-labeled/eYFP$^+$ cells is unchanged in the PLA, ILA, and ACA after propranolol. **(F)** Representative images of the amygdala. **(G)** The number of eYFP$^+$ cells in amygdalar nuclei does not change following

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propranolol administration. **(H)** The number of c-Fos$^+$ cells is decreased in the LA for the propranolol group but does not differ in the remaining nuclei of the amygdala. The percentage of **(I)** co-labeled/c-Fos$^+$ cells was altered in the BLA following propranolol administration, but the percentage of **(J)** co-labeled/eYFP$^+$ cells does not differ in any amygdalar nuclei following administration of propranolol. (n = 9 male mice per group). *$p < 0.05$. Error bars represent ± SEM. eYFP, enhanced yellow fluorescent protein; Sal, saline; P, propranolol; PL, prelimbic area; ILA, infralimbic area; ACA, anterior cingulate area; LA, lateral amygdalar nucleus; BLA, basolateral amygdalar nucleus; BMA, basomedial amygdalar nucleus; PA, posterior amygdalar nucleus; CEA, central amygdalar nucleus; IA, intercalated amygdalar nucleus.

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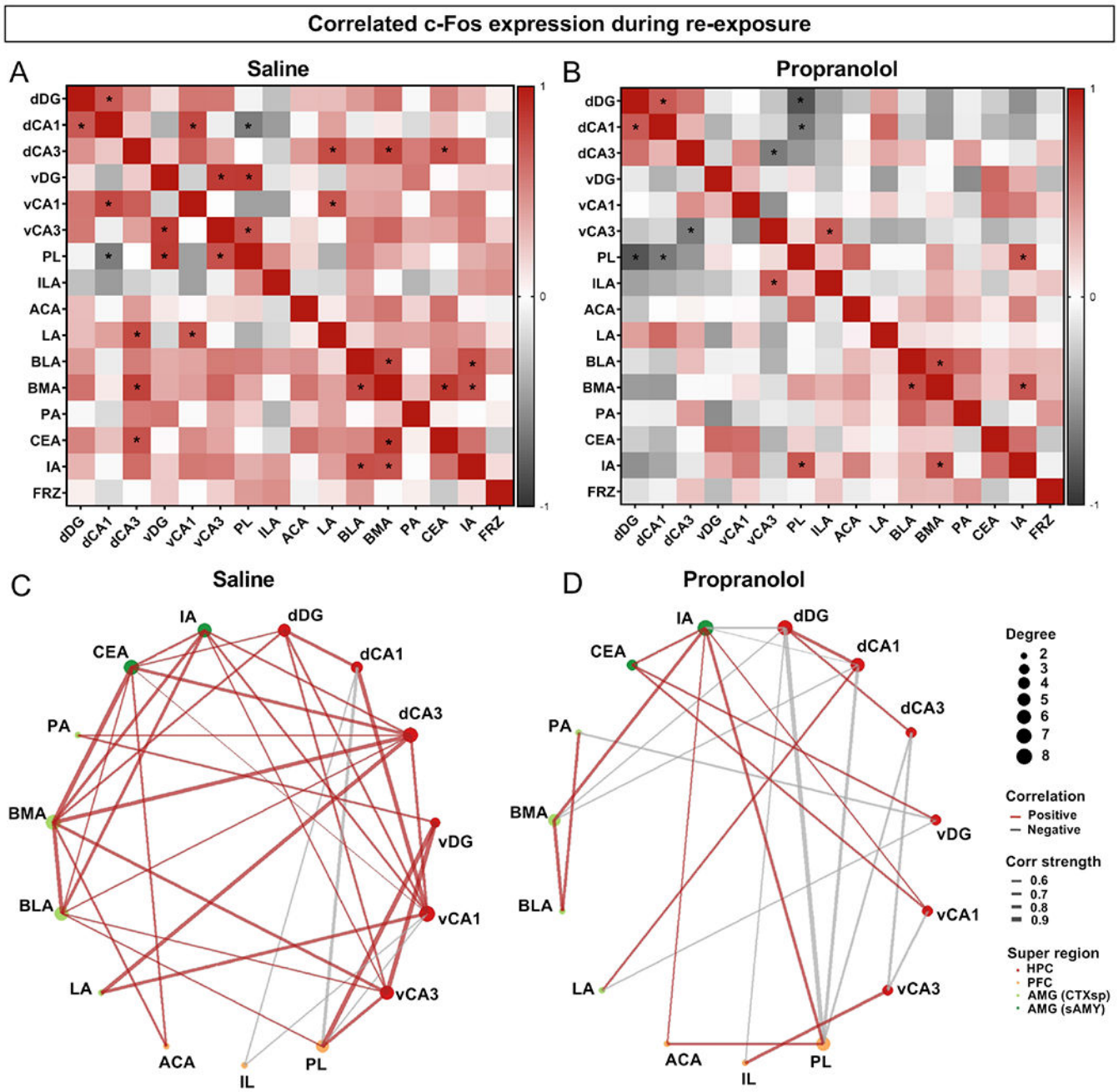

Figure 6. Propranolol alters correlated activity between hippocampal, prefrontal and amygdalar regions and the correlation of memory trace reactivation with freezing levels.

(A) Correlation analysis of c-Fos^{+} activity across brain regions in the saline group. **(B)** Correlation analysis of c-Fos^{+} activity across brain regions in the propranolol group. Square color reflects the Pearson correlation coefficient and asterisks represent a significant correlation. Correlations with R>0.5 (in red) or R<−0.5 (in grey) are displayed in **(C)** for mice that received saline and in **(D)** for mice that received propranolol, with line thickness being proportional to strength of correlation (R value). (n = 9 male mice per group). Sal, saline; P, propranolol; dDG, dorsal dentate gyrus; dCA1, dorsal CA1; dCA3, dorsal CA3;

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vDG, ventral dentate gyrus; vCA1, ventral CA1; vCA3, ventral CA3; ILA, infralimbic area; ACA, anterior cingulate area; LA, lateral amygdalar nucleus; BLA, basolateral amygdalar nucleus; BMA, basomedial amygdalar nucleus; PA, posterior amygdalar nucleus; CEA, central amygdalar nucleus; IA, intercalated amygdalar nucleus.

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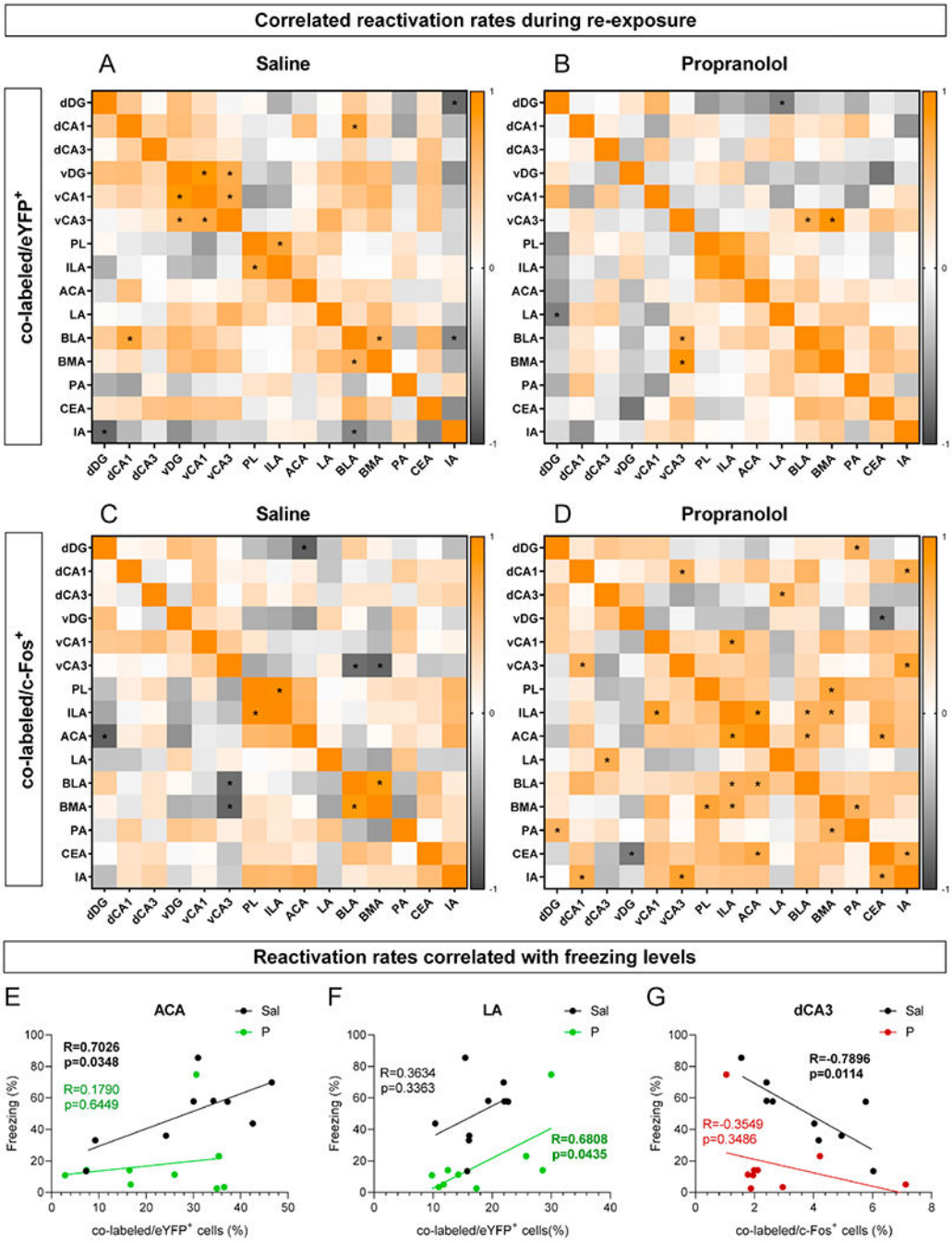

Figure 7. Correlations between memory reactivation rates across regions and with behavior.

(A) Correlation of percentage of co-labeled/eYFP$^+$ cells during context re-exposure after administration of saline. **(B)** Correlation of percentage of co-labeled/eYFP$^+$ cells during context re-exposure after administration of propranolol. **(C)** Correlation of percentage of co-labeled/c-Fos$^+$ cells during context re-exposure after administration of saline. **(D)** Correlation of percentage of co-labeled/c-Fos$^+$ cells during context re-exposure after administration of propranolol. Square color reflects the Pearson correlation coefficient and asterisks represent a significant correlation. **(E)** Correlation between percentage of co-

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labeled/c-eYFP^{+} cells in the ACA and freezing levels. **(F)** Correlation between percentage of co-labeled/eYFP^{+} cells in the LA and freezing levels. **(G)** Correlation between percentage of co-labeled/c-Fos^{+} cells in the dCA3 and freezing levels. (n = 9 male mice per group). Sal, saline; P, propranolol; dDG, dorsal dentate gyrus; dCA1, dorsal CA1; dCA3, dorsal CA3; vDG, ventral dentate gyrus; vCA1, ventral CA1; vCA3, ventral CA3; ILA, infralimbic area; ACA, anterior cingulate area; LA, lateral amygdalar nucleus; BLA, basolateral amygdalar nucleus; BMA, basomedial amygdalar nucleus; PA, posterior amygdalar nucleus; CEA, central amygdalar nucleus; IA, intercalated amygdalar nucleus.

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KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use "this paper" if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Antibody	Chicken polyclonal anti-GFP	Abcam, Cambridge, MA	Cat.#ab13970	concentration 1:500
Antibody	Rabbit polyclonal IgG anti-c-Fos	SySy, Goettingen, Germany	Cat.#226 003	concentration 1:5000
Antibody	Alexa 647 conjugated Donkey Anti-Rabbit IgG	Life Technologies, Carlsbad, CA	Cat.# A-31573, RRID: AB_2536183	concentration 1:500
Antibody	Cy2 conjugated Donkey Anti-Chicken IgG	Jackson ImmunoResearch, West Grove, PA	Cat.# 703-225-155, RRID: AB_2340370	concentration 1:250
Mounting medium	Fluoromount G	Electron Microscopy Sciences, Hatfield, PA	Cat.# 17984-25	
Organism/strain	Mouse: 129S6/SvEv	Taconic (Hudson, NY)	Cat.# 129SVE	
Organism/strain	Mouse: ArcCreERT2(+) x eYFP	doi:10.1016/j.neuron.2014.05.018	Non applicable	
Chemical Compound or Drug	Propranolol hydrochloride	Sigma-Aldrich, St. Louis, MO	Cat.# PHR1308	
Chemical Compound or Drug	Sotalol hydrochloride	Sigma-Aldrich, St. Louis, MO	Cat.# S0278	
Chemical Compound or Drug	4-Hydroxytamoxifen	Sigma-Aldrich, St. Louis, MO	Cat.# H6278	
Software; Algorithm	ImageJ v.1.52p	http://fiji.sc/		
Software; Algorithm	R Studio v.1.1.423	https://rstudio.com/		
Software; Algorithm	R v.3.6.3	https://www.r-project.org/		
Software; Algorithm	WholeBrain package v.0.1.35	https://github.com/tractatus/wholebrain		