

could be accounted for by changes in the firing rates of neurons alone.

An appropriate model of the visual environment should also capture its temporal dynamics. Therefore, we extended our analysis beyond the purely spatial domain to the temporal domain. We measured the probability of transitioning between any two patterns in a wide range of temporal delays for all conditions and tested the strength and match of temporal correlations by using surrogate distributions as was done in the spatial domain (17). The activity of neurons showed strong temporal correlations up to ~20 ms in both aEA and SA in adult animals (Fig. 3C). A strong prediction of the hypothesis that V1 neural activity reflects a statistically optimal internal model is that these transition probabilities should also be matched between aEA, when V1 processes temporally strongly structured visual input, and SA, when no visual stimulus is provided. Indeed, we found that the match between transition probabilities in aEA and SA significantly improved with age (Fig. 3D, Spearman's $\rho = -0.72$, $P < 0.003$), such that in adult animals the temporal correlations were matched up to delays when they decayed to zero (Fig. 3C).

If the internal model reflected in V1 activity is tuned specifically to the natural visual environment, then the match between aEA and SA should also be specific to using a natural image ensemble for eliciting aEA, and other, "artificial" stimulus ensembles should yield higher divergences between aEA and SA for mature animals. To test this prediction, aEA was collected with two other types of stimulus classes: drifting sinusoid gratings at different orientations and frequencies, as well as dynamic binary block noise that was updated at frame rate (17). Indeed, although in young animals there was no significant difference between the degree of match of SA and aEA, in the oldest age group SA was significantly better matched to neural activity evoked by natural images than that

evoked by the two artificial stimulus ensembles (Fig. 4, A and B, movie versus noise: $m = 16.47$, $P < 0.05$; movie versus grating: $m = 943.07$, $P < 0.002$). Furthermore, the divergence between different aEA distributions did not decrease significantly with age (Fig. 4, B and C, movie versus noise: $\rho = 0.19$, $P = 0.49$, movie versus grating: $\rho = 0.5$, $P = 0.21$, noise versus grating: $\rho = 0.67$, $P = 0.07$), which ruled out the possibility that the decreasing divergence between aEA and SA was due to a general decoupling of V1 from sensory input (see also fig. S3).

Our results suggest that V1 implements an internal model that is adapted gradually during development to the statistical structure of the natural visual environment and that SA reflects prior expectations of this internal model. Although these findings do not address the degree to which statistical adaptation in the cortex is driven by visual experience or by developmental programs, they set useful constraints for both dynamical (24) and functional models (12) of sensory processing. We expect our approach to extend to other brain areas and to provide a general, quantitative way to test future proposals for computational strategies used by the cortex.

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Supporting Online Material

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Electrical Synapses Control Hippocampal Contributions to Fear Learning and Memory

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The role of electrical synapses in synchronizing neuronal assemblies in the adult mammalian brain is well documented. However, their role in learning and memory processes remains unclear. By combining Pavlovian fear conditioning, activity-dependent immediate early gene expression, and in vivo electrophysiology, we discovered that blocking neuronal gap junctions within the dorsal hippocampus impaired context-dependent fear learning, memory, and extinction. Theta rhythms in freely moving rats were also disrupted. Our results show that gap junction-mediated neuronal transmission is a prominent feature underlying emotional memories.

Unlike chemical synapses, the role of electrical synapses in fear learning and memory remains largely unknown (1–3). In the

adult mammalian brain, gap junctions formed by connexin 36 (Cx36) couple γ -aminobutyric acid-releasing (GABAergic) interneurons that partici-

pate in the generation of synchronized oscillations (2–4). Cx36 expression has been localized within the amygdala-hippocampus-cortical axis (4, 5), and disrupted hippocampal and cortical oscillations have been reported in Cx36 knockout mice (6, 7). Electrical synapses undergo posttranslational modifications and activity-dependent plasticity similar to chemical synapses (8, 9). Thus, we hypothesized that electrical synapses may be important for the formation and maintenance of fear behaviors and memories.

Rats received intraperitoneal injections of the general gap junction blocker carbenoxolone (Cbx) (10, 11) or the selective Cx36 blocker mefloquine (Meflo) (12) and were fear-conditioned using three pairings of a neutral tone (conditional stimulus, CS) with an aversive footshock (uncon-

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ditional stimulus, US) (Fig. 1, A and B). All animals exhibited equal levels of freezing when tested 24 hours later for their tone fear memories (Fig. 1C). However, both drugs significantly reduced context fear ($F_{2,34} = 31.1, P < 0.0001$; Bonferroni corrected post hoc tests at $P < 0.05$ indicated that both drugs were different from vehicle but not from each other) (Fig. 1D). During training, all rats froze similarly during tone presentations and the intertrial interval, indicating that the drugs did not interfere with short-term memory or the ability to freeze [3 (drug) \times 3 (trial) analysis of variance (ANOVA) for freezing during tone: trial, $F_{2,82} = 133.2, P < 0.0001$; drug, $F_{2,82} = 1.82, n.s.$; interaction, $F_{4,82} = 1.19, n.s.$] (Fig. 1B; see fig. S1 for intertrial interval). Locomotor activity and shock reactivity were identical in all groups, ruling out indirect effects on sensori-

motor processes (figs. S2 and S3). To determine whether the drugs affected acquisition, consolidation, or expression of context fear, we injected Cbx and Meflo posttraining, pretest, or both pretraining and pretest (Fig. 1E). Posttraining injections of Cbx and Meflo attenuated later context fear expression (one-way ANOVA, $F_{2,25} = 9.78, P < 0.001$) (Fig. 1G). Pretest manipulations did not affect fear expression (Fig. 1G), indicating that once context memories were consolidated, they became resistant to disruption of electrical communication. The deficit seen in the pretraining group was maintained in the pretraining and pretest group, ruling out a state-dependent effect (Fig. 1G).

Because the drugs prevented context fear learning, tone fear may have been acquired in a context-independent manner and thus might be more susceptible to extinction (13). Rats were fear-

conditioned as previously described and were given 2 days of extinction training in a novel context (Fig. 1F). On extinction day 1, all groups initially exhibited similar tone fear responses (Fig. 1H). However, 40 tones across two extinction days were required for the vehicle group to fully extinguish, whereas in the drug groups, one session was sufficient to induce an accelerated decrease in freezing [3 (drug) \times 4 (trial block) ANOVA; drug, $F_{2,140} = 10.86, P = 0.0002$; block, $F_{4,140} = 75.15, P < 0.0001$; interaction, $F_{8,140} = 6.291, P < 0.0001$] (Fig. 1H). When tested for extinction memory on extinction day 2, freezing in the Cbx and Meflo groups had already reached baseline levels (percent time spent freezing, average of the first four tone presentations: Cbx, 21.6 ± 6.1 ; Meflo, 18.5 ± 4.9 ; vehicle, $66.7 \pm 7.4; P < 0.0001$) (Fig. 1H).

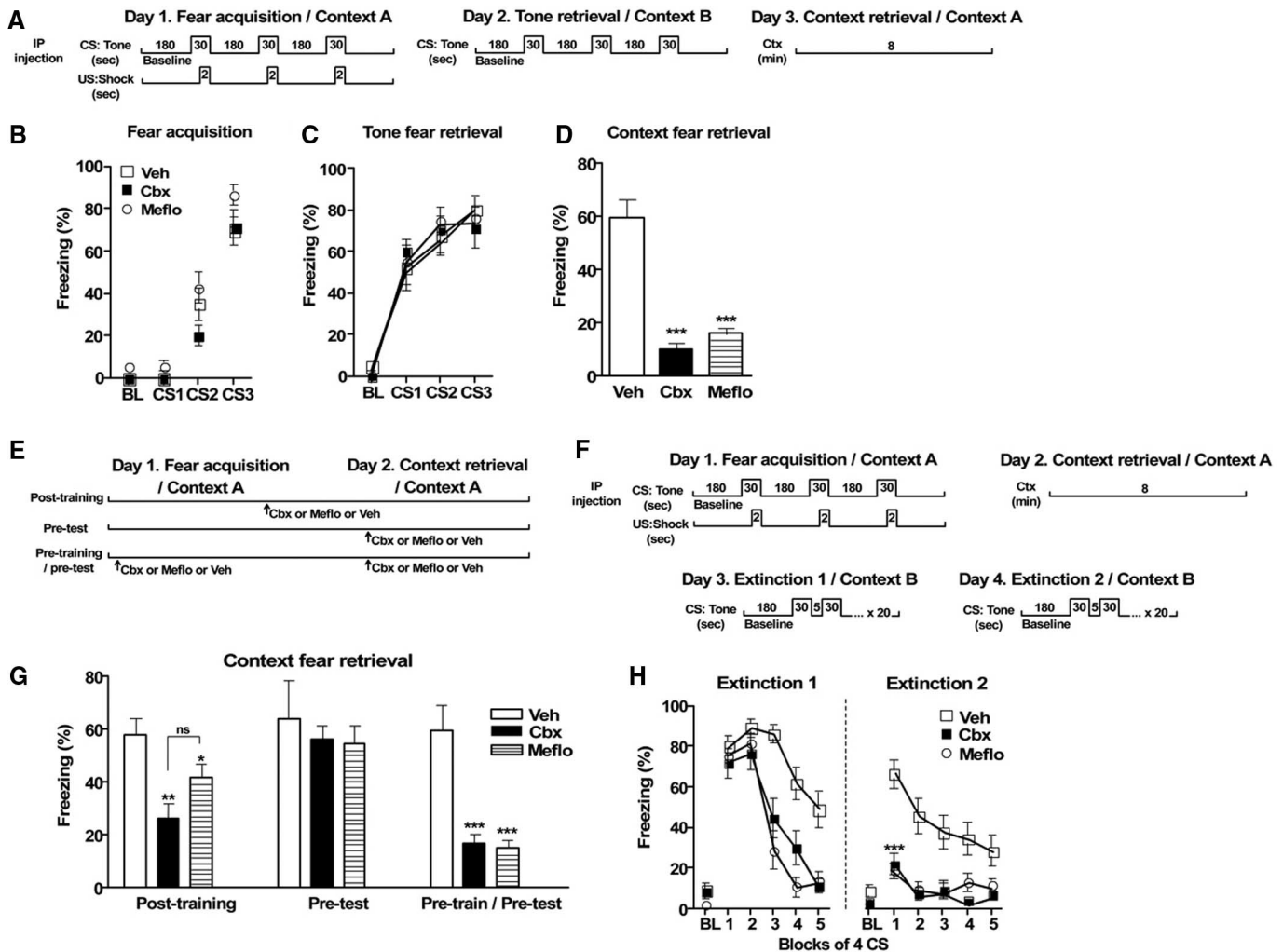


Fig. 1. Systemic blockade of gap junctions impairs context-dependent memories and accelerates extinction. (A) Experimental design. (B) Fear acquisition [vehicle (Veh), $n = 20$; Cbx, $n = 13$; Meflo, $n = 11$]. (C) Tone fear memory was intact in all groups (Veh, $n = 14$; Cbx, $n = 13$; Meflo, $n = 11$). (D) Context fear retrieval was impaired in the drug groups (Veh, $n = 14$; Cbx, $n = 13$; Meflo, $n = 11$). (E) Experimental design for posttraining, pretesting, and dual pretraining and pretest injections. (F) Posttraining injections of Cbx and Meflo impaired consolidation of context fear memories (Veh, $n = 10$; Cbx, $n = 10$; Meflo, $n = 8$). Pretesting injections did not affect context fear

retrieval (Veh, $n = 12$; Cbx, $n = 8$; Meflo, $n = 6$). Dual pretraining and pretesting injections showed no drug state dependency (Veh, $n = 10$; Cbx, $n = 6$; Meflo, $n = 6$). (G) Experimental design for extinction experiment. (H) Cbx and Meflo groups exhibited rapid reduction in freezing on days 1 and 2 (Veh, $n = 15$; Cbx, $n = 13$; Meflo, $n = 10$). Between-group differences: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; n.s., not significant. Results are presented as means \pm SEM. BL, baseline; CS, conditional stimulus; Ctx, context; US, unconditional stimulus. Contexts A and B refer to two different conditioning chambers.

Given that extinction is context-dependent, it is possible that the accelerated loss of tone fear resulted from impaired contextual learning and that the drugs' effects were mediated by the dorsal hippocampus (DH) (14). Thus, we blocked gap junctions specifically in the DH with Cbx, Meflo, and the mimetic connexin peptides GAP27 and GAP36 (15) (Fig. 2), which were injected at both pretraining and posttraining to ensure blockade during acquisition and consolidation. Similar to systemic injections, DH microinfusions reduced context fear memories ($F_{4,51} = 17.14, P < 0.0001$) (Fig. 2, A and B) and accelerated extinction [5 (drug) \times 4 (trial block) ANOVA; drug, $F_{4,172} = 10.74, P = 0.0001$; block, $F_{4,172} = 121.7, P < 0.0001$; interaction, $F_{16,172} = 4.35, P < 0.0001$] (Fig. 2C). Post hoc tests revealed a significant reduction in context fear and facilitated extinction in all drug groups, with no differences between peptides and blockers (Fig. 2, B and C). When animals were reconditioned to a white noise in a novel context and tested for context fear the next day, all groups exhibited similar amounts of context freezing (fig. S5), ruling out permanent DH damage from the infusion.

Because fear renewal is also susceptible to DH manipulations (16), we hypothesized that contextual information encoded in the presence of gap junction blockers and the subsequent enhancement of extinction might prevent renewal of fear. As predicted, fear renewal measured in the original context was compromised in the drug and peptide groups ($F_{4,37} = 8.82, P < 0.0001$) (Fig. 2, A and D). To distinguish between an effect on contextual encoding proper and encoding in the presence of an aversive experience, we used the immediate shock deficit paradigm (17, 18). Cbx or GAP36 infused before a shock-free pre-exposure session abolished the preexposure's ability to rescue the immediate shock deficit ($F_{3,20} = 9.8, P < 0.0001$) (Fig. 2, E and F).

If electrical synapses in the DH are required to form and consolidate contextual representations, then rats may not recognize a context previously explored under blockade of gap junctions and treat that environment as novel. We thus examined c-fos expression as an indication of context familiarity (19, 20). Rats exposed to the testing context for the first time served as a novel

environment control ("1st expo" group). In both the CA1 and CA3 regions of the hippocampus, c-fos expression was higher in the vehicle group than in the home cage controls (HC) but was lower than in animals placed in the context for the first time (1st expo, Fig. 3). However, in rats initially trained under Cbx, c-fos expression was similar to the 1st expo but higher than the vehicle and HC groups (one-way ANOVA on vehicle, CBX, 1st expo, and HC, per region: CA1, $F_{3,12} = 65.96, P < 0.0001$; CA3, $F_{3,12} = 31.84, P < 0.0001$) (Fig. 3). The 1st expo group did not receive any aversive experience in that context; this suggests that rats in the Cbx group, when reintroduced to the training context, exhibited neuronal activity similar to a first-time contextual exposure. Contrasting results were found in the basolateral amygdala (BLA), the site where context-shock associations are formed (21) ($F_{3,13} = 38.64, P < 0.0001$). Post hoc tests indicated reduced c-fos expression in both the Cbx and 1st expo groups relative to the vehicle group. There were no differences between the Cbx and 1st expo groups. However, the Cbx group showed higher c-fos

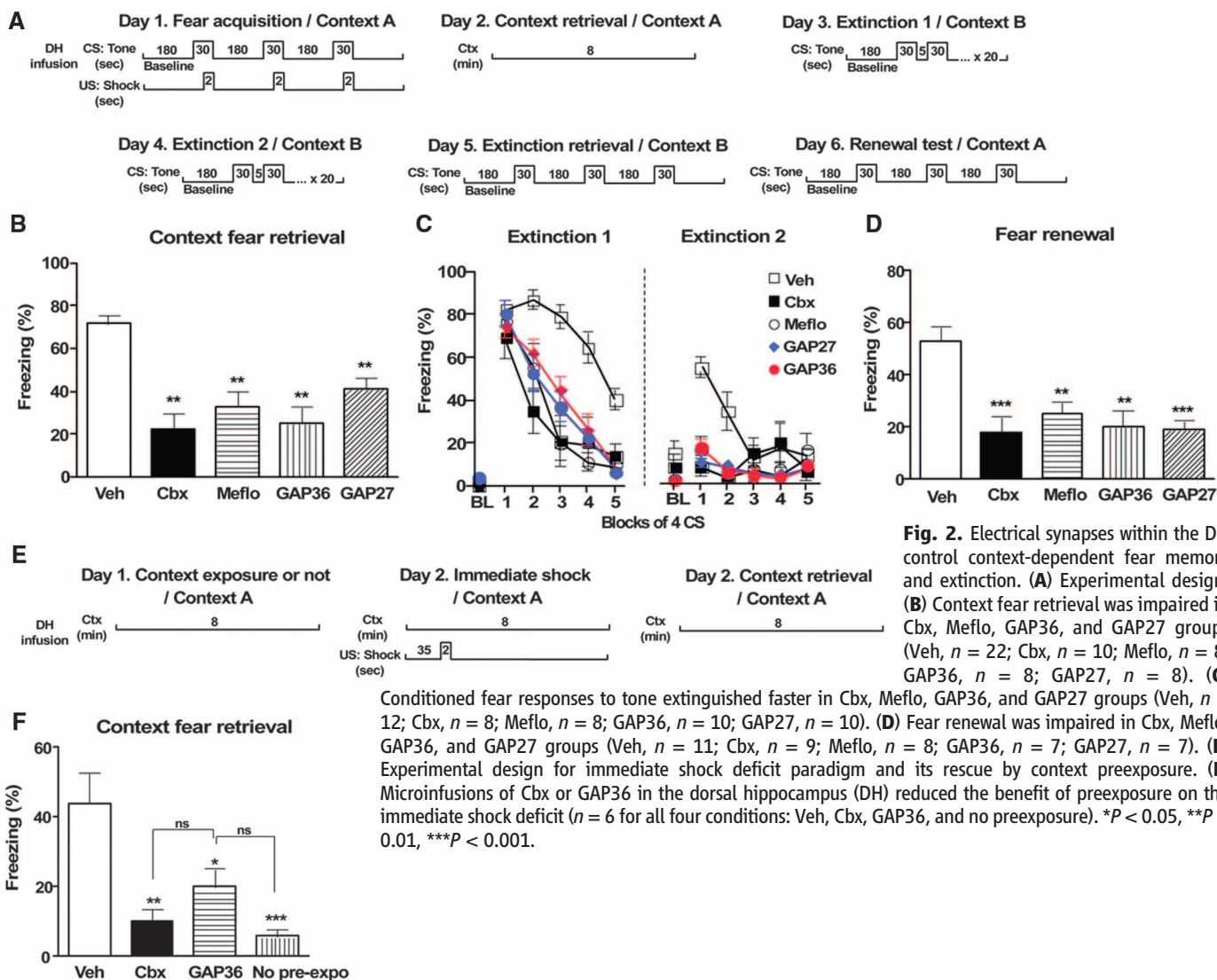


Fig. 2. Electrical synapses within the DH control context-dependent fear memory and extinction. (A) Experimental design. (B) Context fear retrieval was impaired in Cbx, Meflo, GAP36, and GAP27 groups (Veh, $n = 22$; Cbx, $n = 10$; Meflo, $n = 8$; GAP36, $n = 8$; GAP27, $n = 8$). (C) Conditioned fear responses to tone extinguished faster in Cbx, Meflo, GAP36, and GAP27 groups (Veh, $n = 12$; Cbx, $n = 8$; Meflo, $n = 8$; GAP36, $n = 10$; GAP27, $n = 10$). (D) Fear renewal was impaired in Cbx, Meflo, GAP36, and GAP27 groups (Veh, $n = 11$; Cbx, $n = 9$; Meflo, $n = 8$; GAP36, $n = 7$; GAP27, $n = 7$). (E) Experimental design for immediate shock deficit paradigm and its rescue by context preexposure. (F) Microinfusions of Cbx or GAP36 in the dorsal hippocampus (DH) reduced the benefit of preexposure on the immediate shock deficit ($n = 6$ for all four conditions: Veh, Cbx, GAP36, and no preexposure). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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expression relative to HC rats, whereas the 1st expo group did not. To distinguish the effects of blocking electrical synapses on contextual encoding versus context-shock associations, we repeated the experiment with shock-free context exposure (fig. S7). Again, c-fos expression was higher in both the Cbx and 1st expo groups in the CA1 and CA3 regions relative to the vehicle group (one-way ANOVA on Veh, CBX, and 1st expo, per region: CA1, $F_{2,9} = 6.49, P < 0.0001$; CA3, $F_{2,9} = 5.90, P < 0.05$) (fig. S7), confirming an effect on the context encoding.

GABAergic interneurons expressing Cx36 in the DH and medial septum drive hippocampal theta rhythms previously linked to investigatory

behaviors (22–25). We recorded theta electroencephalograms (EEGs) from the CA1 of freely moving rats after Cbx intracerebroventricular (icv) infusions, which reproduced the behavioral effects on contextual memories (fig. S8). Cbx specifically disrupted theta oscillations (Fig. 4, A and B). A 3 (speed) \times 2 (Cbx, pre versus post) ANOVA revealed that blocking electrical synapses attenuated the power of theta rhythms (main effect of Cbx: $F_{1,5} = 25.6, P < 0.005$) (Fig. 4C) but did not disrupt the normal positive correlation between theta power and running speed (main effect of speed: $F_{2,10} = 5.78, P < 0.05$; speed \times Cbx interaction: $F_{2,10} = 0.08, n.s.$). Theta rhythm frequency was also increased after Cbx ($F_{1,3} =$

11.54, $P < 0.05$) (Fig. 4D), but the normal positive correlation between theta frequency and running speed was unaffected (main effect of speed: $F_{2,6} = 5.54, P < 0.05$; speed \times Cbx interaction: $F_{2,6} = 0.98, n.s.$). Cbx had no effect on time spent running ($F_{1,5} = 0.17, n.s.$) or on mean running speed recorded at each frequency (Fig. 4D). Vehicle infusions did not affect theta power ($F_{1,5} = 5.25, n.s.$) or frequency ($F_{1,5} = 0.03, n.s.$).

Our c-fos analyses (Fig. 3), the immediate shock deficit experiment, and the disruption of theta rhythms during novel exploration (Fig. 4) all suggest that blocking DH gap junctions disrupts contextual encoding and thereby prevents contextual fear learning (Figs. 1 and 2). Considering that place cells fire in relation to the theta cycle (24), disrupting theta may abolish the temporal code for that location. Furthermore, blocking gap junctions in the DH before or during the consolidation of aversive experiences may disrupt theta-band synchronization within the amygdalohippocampal network required for the consolidation of fear memories (26).

Pretraining blockade of gap junctions in the DH spared tone-shock learning but rendered tone fear memories more prone to extinction and impaired renewal (Fig. 2). This suggests that extinction learning was facilitated by a lack of contextualization of the tone-shock memory. Our findings not only provide new evidence for a functional role for electrical synapses in mechanisms underlying fear learning

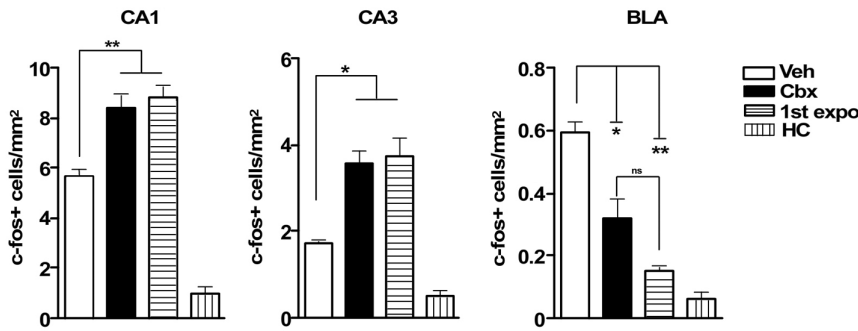


Fig. 3. Blocking of pretraining at gap junctions affects c-fos expression within the amygdalohippocampal network, as shown by quantification of c-fos expression in CA1, CA3, and BLA. * $P < 0.05$, ** $P < 0.01$.

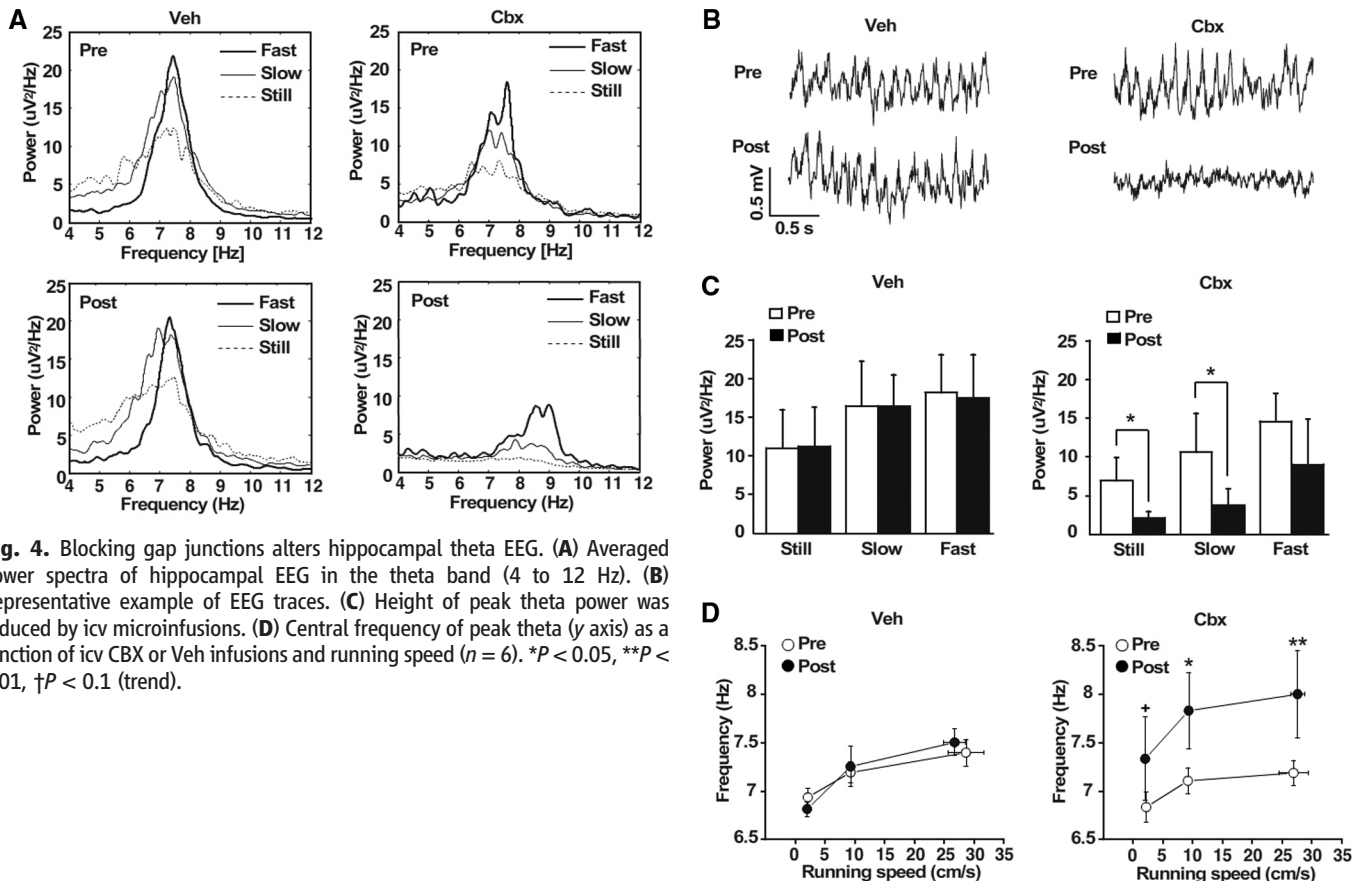


Fig. 4. Blocking gap junctions alters hippocampal theta EEG. (A) Averaged power spectra of hippocampal EEG in the theta band (4 to 12 Hz). (B) Representative example of EEG traces. (C) Height of peak theta power was reduced by icv microinfusions. (D) Central frequency of peak theta (γ axis) as a function of icv CBX or Veh infusions and running speed ($n = 6$). * $P < 0.05$, ** $P < 0.01$, † $P < 0.1$ (trend).

and memory in the adult mammalian brain, but may also point toward new therapeutic avenues for the treatment of trauma and anxiety disorders.

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Supporting Online Material

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Structure of Precursor-Bound NifEN: A Nitrogenase FeMo Cofactor Maturase/Insertase

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NifEN plays an essential role in the biosynthesis of the nitrogenase iron-molybdenum (FeMo) cofactor (M cluster). It is an $\alpha_2\beta_2$ tetramer that is homologous to the catalytic molybdenum-iron (MoFe) protein (NifDK) component of nitrogenase. NifEN serves as a scaffold for the conversion of an iron-only precursor to a matured form of the M cluster before delivering the latter to its target location within NifDK. Here, we present the structure of the precursor-bound NifEN of *Azotobacter vinelandii* at 2.6 angstrom resolution. From a structural comparison of NifEN with des-M-cluster NifDK and holo NifDK, we propose similar pathways of cluster insertion for the homologous NifEN and NifDK proteins.

Nitrogenase is a complex metalloenzyme that catalyzes a key step in the global nitrogen cycle: the reduction of atmospheric dinitrogen to bioavailable ammonia. The Mo-dependent nitrogenase is a two-component system, in which the Fe protein (NifH) mediates the adenosine triphosphate-dependent transfer of electrons to the catalytic MoFe protein (NifDK) during substrate turnover (1). The MoFe protein is an $\alpha_2\beta_2$ tetramer that contains two unusual metal clusters per $\alpha\beta$ dimer, the P cluster and the M cluster. The P cluster is an [8Fe-7S] cluster at the α/β -subunit interface, coordinated by three Cys ligands from the α subunit and three Cys ligands from the β subunit. The M cluster (or FeMo cofactor) is a [Mo-7Fe-9S-X-homocitrate] cluster (where X = C, N, or O) located within the α subunit, coordinated by a His ligand at the Mo

end and a Cys ligand at the opposite Fe atom, with a Lys residue binding to the homocitrate entity (2, 3). During catalysis, the P cluster is thought to mediate the electron flow from the Fe protein to the M cluster, where substrate reduction occurs.

NifEN is an essential player in M-cluster biosynthesis (4–6). It presumably receives a precursor form of the M cluster from NifB and hosts the conversion of this precursor to a mature M cluster before delivering the latter to the MoFe protein (4, 5). A role for NifEN in FeMo cofactor biosynthesis was initially hypothesized based on a considerable degree of similarity between the primary sequences of NifEN and MoFe protein, suggesting that NifEN may contain P- and M-like clusters (6). Subsequently, the NifEN-associated clusters were identified through the biochemical and spectroscopic analyses of three forms of NifEN. The first, apo NifEN, is free of any cofactor species and contains a [4Fe-4S] cluster in place of the [8Fe-7S] P cluster (7). The second, NifEN, contains, in addition to the [4Fe-4S] cluster, an all-iron precursor that closely resembles the Fe/S core of the M cluster (8). The third, holo NifEN, contains a mature M cluster and the [4Fe-4S] cluster (9). NifEN could be readily converted to holo NifEN by Fe protein-mediated insertion

of Mo and homocitrate into the precursor (10), and holo NifEN could directly serve as a cofactor donor for the apo MoFe protein (11). These observations not only establish the role of NifEN in cofactor biosynthesis but also illustrate the dynamic nature of the cofactor site in NifEN during the assembly process. However, the mechanistic details of the biosynthetic events on NifEN have remained unclear without structural information on this protein. In this study, the structure of the precursor-bound NifEN of *Azotobacter vinelandii* has been solved to 2.6 Å resolution (12). From a structural comparison of NifEN with des-M-cluster MoFe protein (apo NifDK) (11) and holo MoFe protein (NifDK) (2, 3), we propose similar pathways of cluster insertion for the homologous NifEN and NifDK proteins.

The crystal structure determination of NifEN is summarized in tables S1 and S2. Like the $\alpha_2\beta_2$ -tetrameric NifDK, NifEN consists of a pair of $\alpha\beta$ dimers that are related by a molecular twofold rotation axis (Fig. 1A). The α and β subunits of NifEN, like those of NifDK and apo NifDK, are composed of three domains each— α I, α II, and α III, and β I, β II, and β III, respectively. All domains of NifEN, as for NifDK and apo NifDK, are organized around a common core of a four-stranded, parallel β sheet flanked with α helices and additional β strands (13). Moreover, NifEN contains two types of clusters that correspond to the P and M clusters in NifDK: one, termed the O cluster, is a [4Fe-4S] cluster that is coordinated by Cys³⁷, Cys⁶², Cys¹²⁴, and Cys¹⁴⁴ at the same site in the α/β -subunit interface as the P cluster; the other, termed the L cluster, is an iron-only precursor form of the M cluster that is at least ligated by Cys²²⁵ at one end (Fig. 1B) (14). Although the electron density is not sufficiently well resolved to unambiguously establish the structure of the L cluster, the shape and extent of the density is compatible with the core geometry of the M cluster and, therefore, consistent with the previously proposed 8Fe model (8) of this M-cluster precursor (Fig. 1B).

Strikingly, although the L and M clusters are both positioned at the junction between the α I,

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Electrical Synapses Control Hippocampal Contributions to Fear Learning and Memory

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