Cellular Neuroscience, Spring 2010. Worksheet for section 4 classes, Gahtan. 4/21/2010

Phenomenon being studied: How vasopressin signaling in the amygdala regulates emotional responses, particular in the domain of social approach or avoidance

Levels of analysis (write down explanations at each level)

Adaptation- Social approach is a necessary instinct in any sexually reproducing species but it is fraught with risks, so CNS mechanisms exist to guide 'approach vs avoid' decisions

Behavioral- CNS mechanisms controling social approach vs avoid decisions are assayed in different ways. In rodents, they include social recognition, intruder responses, maternal care levels. In humans, engagement in creative dance, monetary investment, etc.

Systems- distinct neural systems for social inhibition and social approach

Circuit- Afferents from the cortex and limbic system to the lateral amygdala carry processed sensory input. Some release oxytocin, which indirectly inhibits amygdala output to the HPA and inhibits stress, some carry vasopressin, which directly excites amygdala output to the HPA

Cell biological (including electrophysiological)- CNS AVP signaling mechanisms are not well understood. In peripheral AVP signaling, metabotropic AVP1AR receptors activates PLC-b and IP3, resulting in release of intracellular Ca++ and membrane depolarization. In amygdala, different signaling mechanisms must exist because AVP has different electrophysiological responses on different neurons (Raggenbass paper). Neuropeptides may be coreleased with amine or glutamate transmitters.

Genetic- In gene association studies, polymorphisms in the AVP1AR promoter region leads to different expression levels and/or distribution of AVP1R's, with consequences on behavior in humans and rodents.

Computational- The theorized function of AVP and OXT signaling in the amygdala is to modulate the responses of primary neurons to glutamate, serotonin, dopamine, or other transmitters. From a computational perspective, these peptides place primary neurons in a different equilibrium state.

Other- for humans, social decisions (approach or avoid) are experienced as being motivated by emotion, but these emotions may be the *consequences* of the neuromodulatory struggle over amygdala excitation between AVP and OXT

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Phenomenon being studied:

Levels of analysis (write down explanations at each level)

Adaptation-

Behavioral-

Systems- distinct learning systems for a. contextual fear b. cued fear c. short term fear memory d. long term fear memory

Circuit-

Cell biological (including electrophysiological)-

Genetic-

Computational-

Other- Methods: great when a lesion causes an improvement in performance since non-specific effects of the lesion (sick mouse) are less likely as explanation of the effect in that case.

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Identification of a Signaling Network in Lateral Nucleus of Amygdala Important for Inhibiting Memory Specifically Related to Learned Fear

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Bombesin

From Wikipedia, the free encyclopedia

Bombesin is a 14-amino acid peptide originally isolated from the skin of a frog. It has two known homologs in mammals called neuromedin B and gastrin releasing peptide. It stimulates gastrin release from G cells. It activates three different G-protein-coupled receptors known as BBR1 2 & 3. It also activates these receptors in the brain. Together with cholecystokinin, it is the second major source of negative feedback signals that stop eating behaviour.

Bombesin is also a tumor marker for small cell carcinoma of lung, gastric cancer, and neuroblastoma.

CNS Neurol Disord Drug Targets. 2006 Apr;5(2):197-204.

Gastrin-releasing peptide receptor as a molecular target for psychiatric and neurological disorders.

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Abstract

The mammalian bombesin (BB)-like peptide gastrin-releasing peptide (GRP) stimulates cell proliferation, displays a range of neuroendocrine activities, and acts as a growth factor in the pathogenesis of several types of human cancer. Several lines of evidence have indicated that GRP and its receptor (GRPR) might also be involved in the neurochemical alterations associated with psychiatric and neurological disorders. GRP and GRPR are distributed throughout the mammalian central nervous system (CNS). Altered levels of BB-like peptides have been found in the CNS of patients with schizophrenia and Parkinson's disease. Dysfunctions in GRPR-induced cellular calcium signaling have been reported in fibroblasts from patients with Alzheimer's disease. A translocation in the GRPR gene has been associated with autism. Pharmacological and genetic studies in rodents have shown that GRPRs in brain areas such as the dorsal hippocampus and amygdala are importantly involved in regulating synaptic plasticity and aspects of behavior that might be altered in disorders such as anxiety, schizophrenia, depression, autism and dementia. Behaviors modulated by the GRPR in rodents include grooming, food intake, stereotypy, social behavior, and emotionally-motivated learning and memory. Together, these findings support the view that the GRPR should be considered a therapeutic target for a subset of CNS diseases.

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Research report

Immunohistochemical localization of gastrin-releasing peptide receptor in the mouse brain

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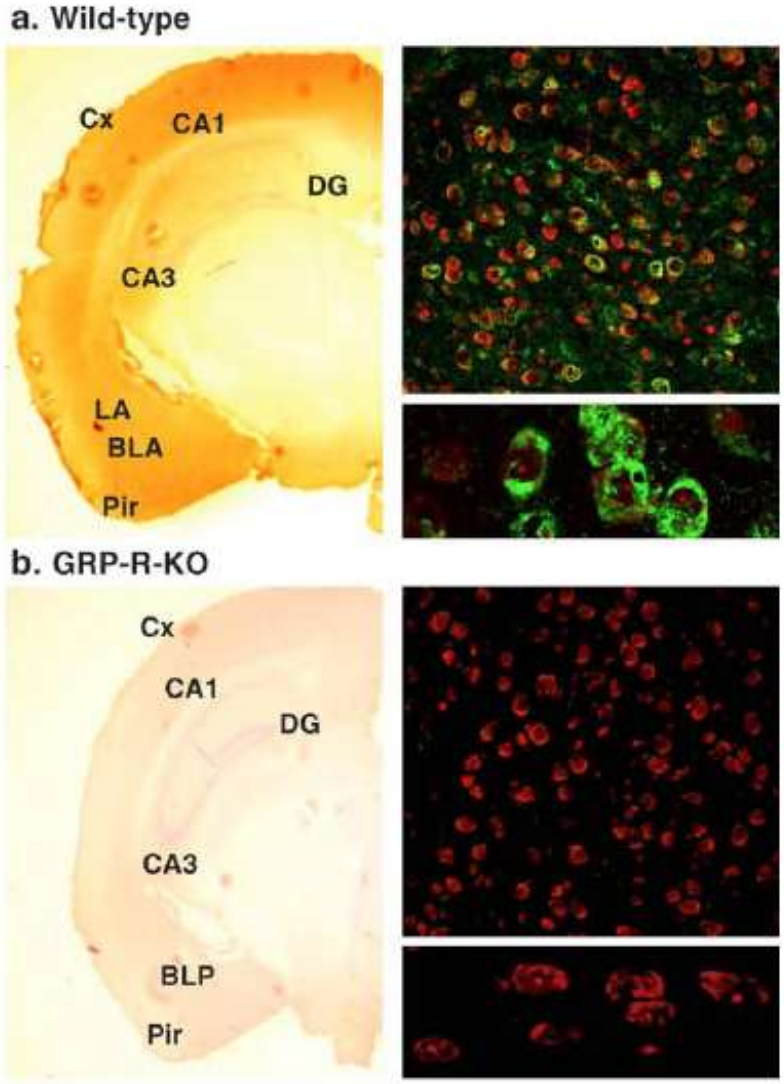

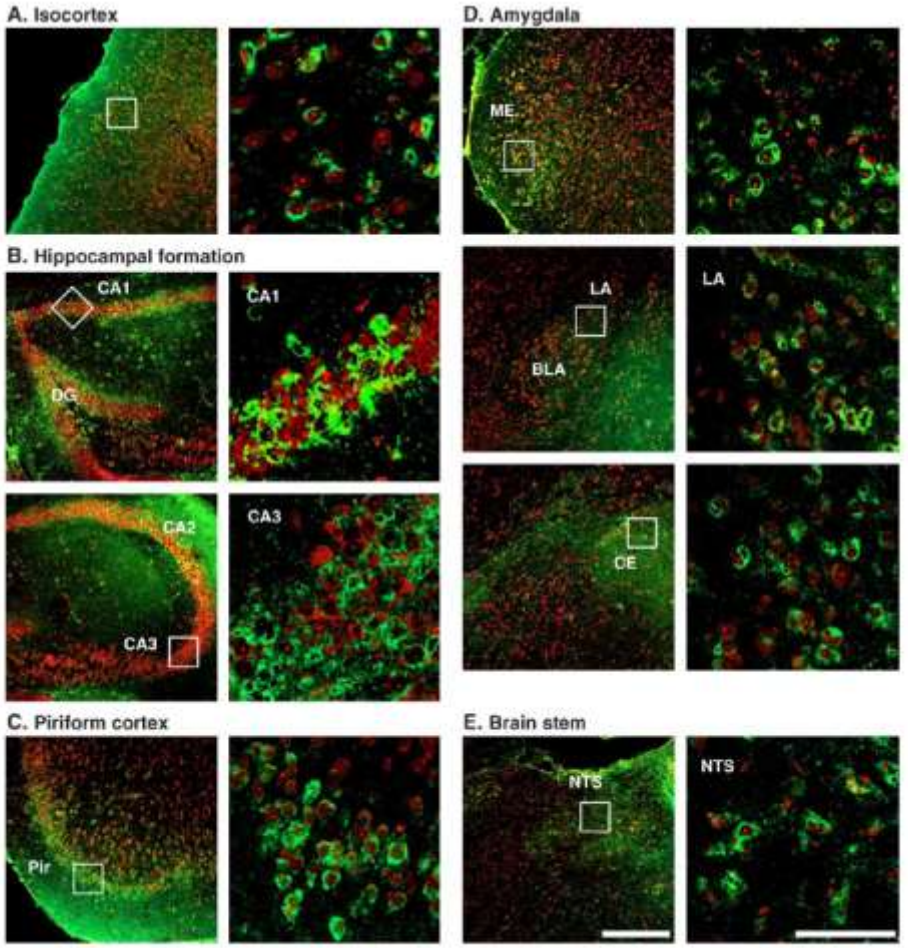

Fig. 4. Localization of GRP-R in the wild-type mouse brain. Left columns show low-powered microscope images and squared regions were magnified in the right columns. After immunosignals for GRP-R antibody (green) were observed, sections were stained with propidium iodide (red) to know nucleus of the

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Identification of a Signaling Network in Lateral Nucleus of Amygdala Important for Inhibiting Memory Specifically Related to Learned Fear

Introduction

Fear is a basic, evolutionally conserved, emotion

Q: What is an emotion?

Q: What is the importance of evolutionary conservation?

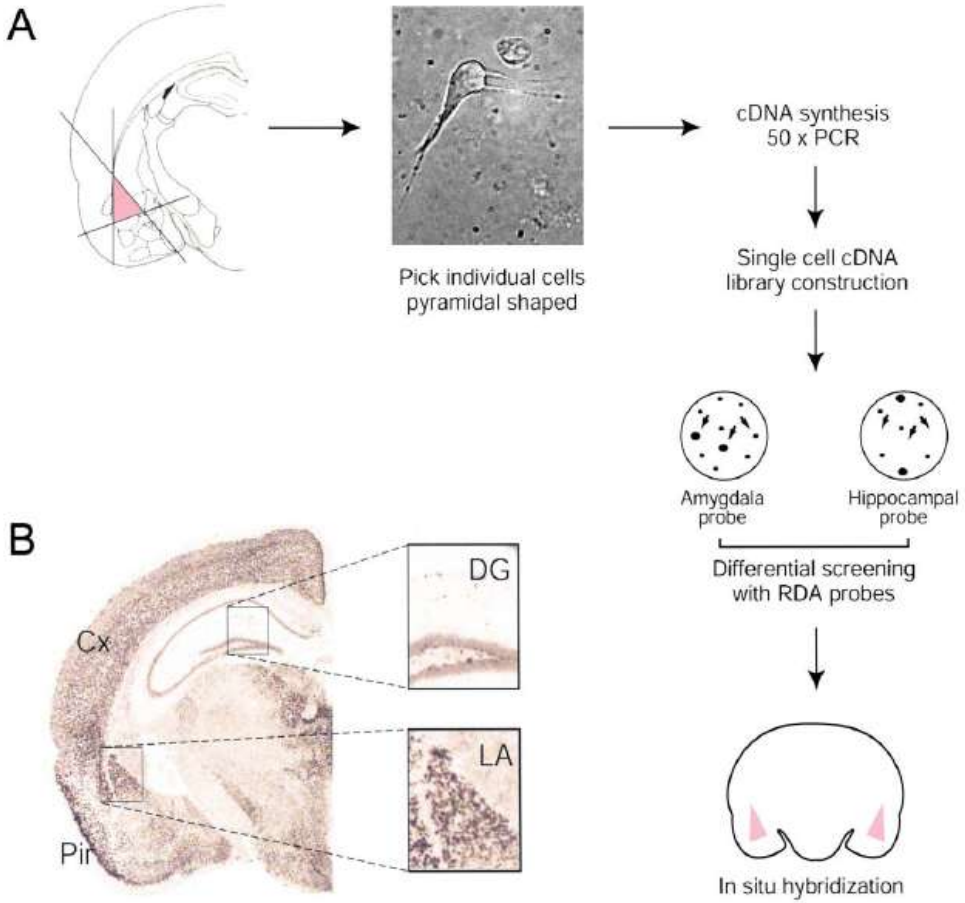
The lateral nucleus is the input region within the amygdala, where the association of learned information about CS and US occurs during auditory fear conditioning. The sensory information that mediates the CS—the auditory tone—reaches the lateral nucleus by way of two neural pathways, both of which are essential for learned fear (Romanski and LeDoux, 1992). One pathway, the direct thalamo-amygdala pathway, originates in the medial geniculate nucleus (MGm) and in the posterior intralaminar nucleus (PIN) of the thalamus. The second pathway, the indirect cortico-amygdala pathway, extends from the auditory thalamus to the auditory cortex (TE3 area) and includes a further projection that relays the processed auditory information from the cortex to the lateral amygdala. After these two inputs are processed in the lateral nucleus, the signal is distributed to other amygdaloid nuclei (Pitkanen et al., 1997), including the central nucleus of the amygdala (CeA), which projects in turn to areas in the brainstem that control autonomic (heart rate) and somatic motor centers (freezing) involved in the expression of fear.

Q: What is track tracing important for cellular neuroscience?

In contrast to the detailed cellular physiological information that is becoming available, the molecular machinery that underlies synaptic plasticity in amygdala-dependent learned fear is largely unknown. Toward this

Q: What is the difference between the cellular and molecular levels of analysis being referred to here?

ing fear learning. We isolated neurons using acute dissociation, which preserves their processes and allows cell identification based on neuronal morphology under the microscope (Yu and Shinnick-Gallagher, 1997). Simi-



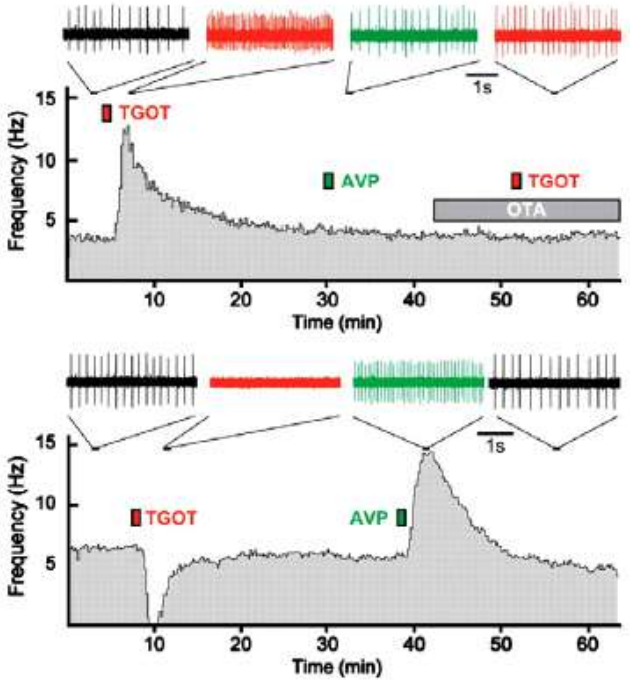


Figure 1. Strategy for Isolating Amygdala-Enriched Genes

(A) Scheme of the differential screening of single cell cDNA libraries from amygdala neurons (with a representative neuron after acute dissociation of the rat amygdala).

(B) *Op18/Stathmin* RNA in situ hybridization on a coronal section of mouse brain. Insets show strong expression in the lateral nucleus of the amygdala and weak expression in the hippocampus.

Q: Why go to the trouble of ID'ing individual neurons?

Q: How did they isolate genes with enriched expression in the amygdala?

Explain the figure…

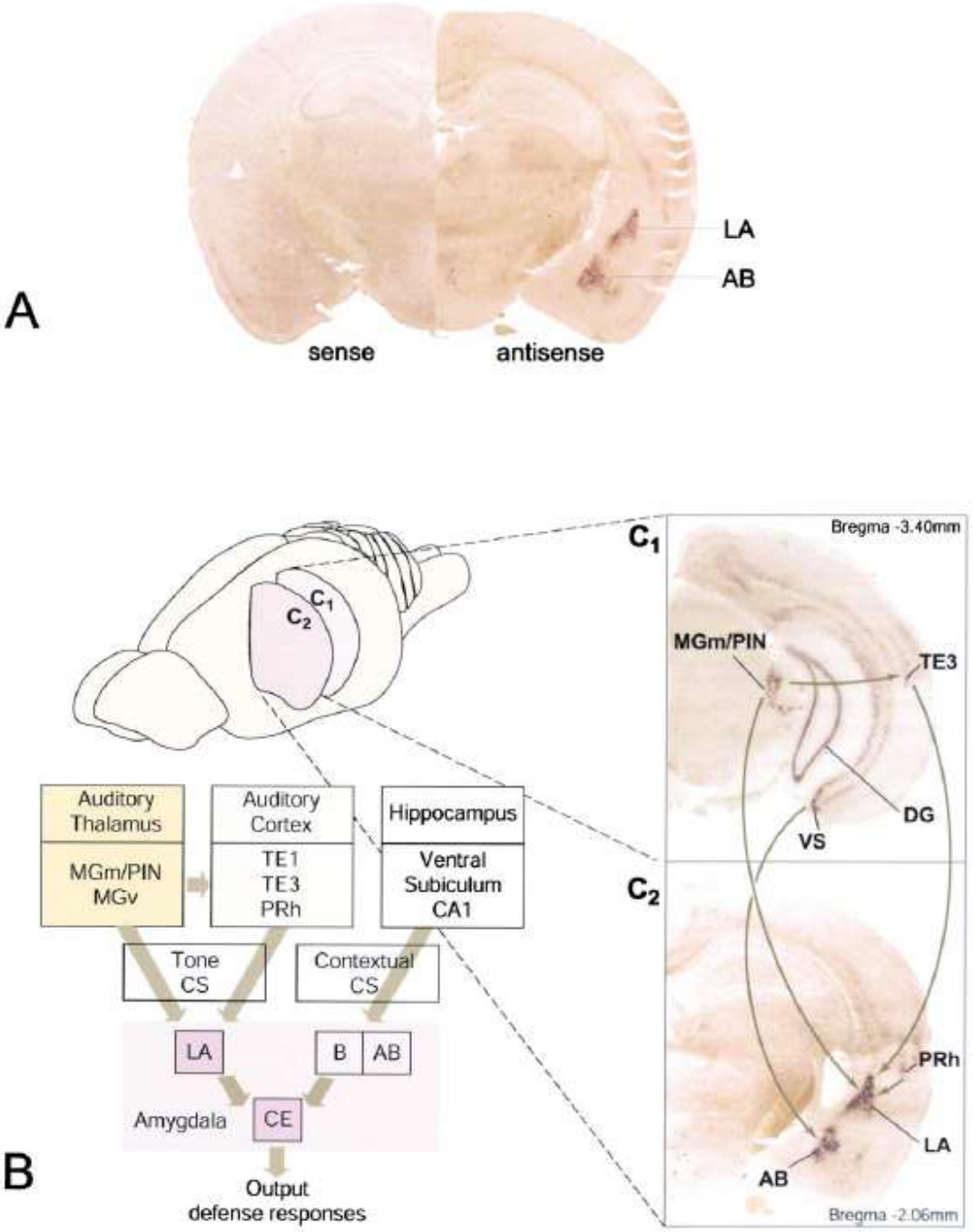


Figure 2. The *Grp* Gene Is Specifically Expressed in the Lateral Nucleus/AB of the Amygdala and in the Cued and Contextual CS Pathways to the Amygdala

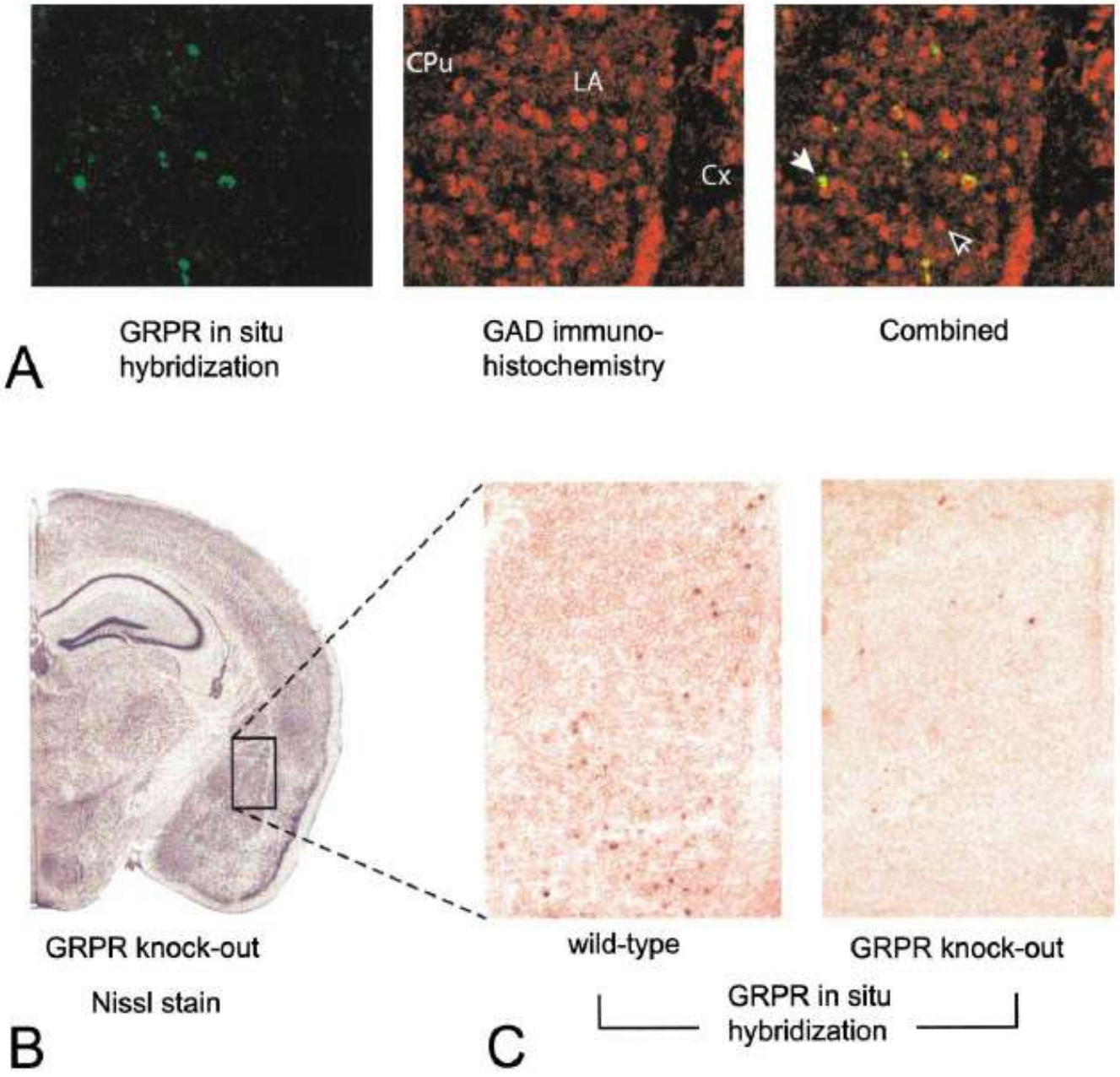
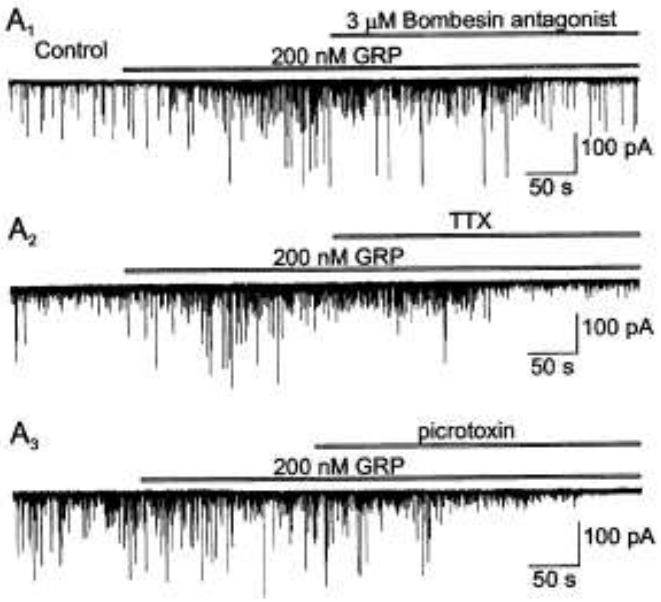


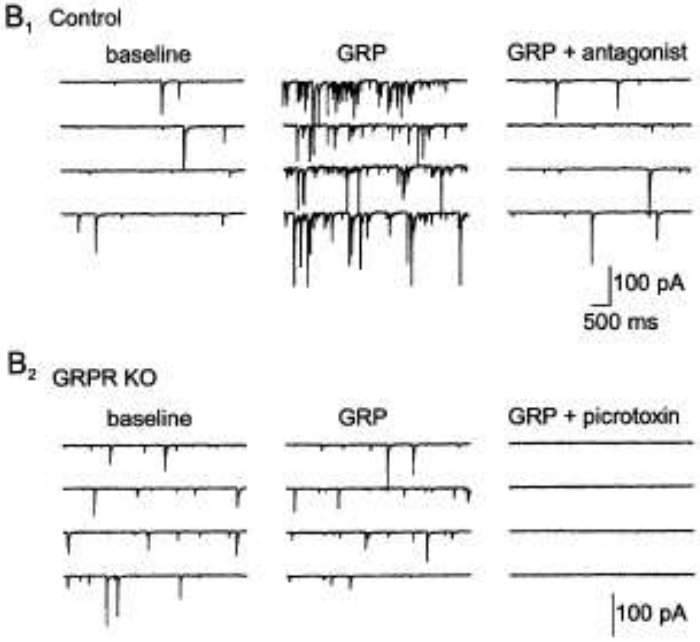
Figure 3. Expression of the *Grpr* Gene in the Amygdala

Explain the figure…

What is their basis for concluding that GRPR is selectively expressed on interneurons?

What is the point of the immunolabeling in the k/o?

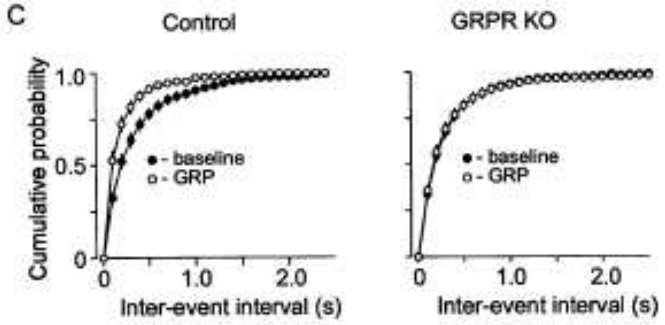




What is the overall conclusion of this figure?

How do the individual data plots support that conclusion?

Why were recorded cells treated with CNQX and D-APV in all of these trials (and Fig 5)?



dione, 20 μM). To increase the inhibitory signals, we inverted the inhibitory currents so that they had an inward direction by dialyzing the postsynaptic cells with a chloride-based intrapipette solution.

Interpret this sentence…

Figure 4. GRP Receptors Are Functionally Expressed in Interneurons of the Lateral Nucleus of the Amygdala

(A_1) Bath application of GRP (200 nM) increased frequency of sIPSCs in a pyramidal cell from a control mouse. The effect was blocked by 3 μM bombesin antagonist (n = 6), thus suggesting that the GRP-induced enhancement of GABAergic tonic inhibition was specifically linked to the activation of the GRP receptors.

(A_2) Effect of GRP on the frequency of sIPSCs is TTX-sensitive, and thus is dependent on action potential firing in interneurons.

(A_3) GRP failed to increase the frequency of the picrotoxin-sensitive sIPSCs in GRPR knockout mice.

(B_1) Representative sIPSCs recorded in a pyramidal cell from a control mouse at a holding potential of −70 mV under baseline conditions (left), during GRP application (center), and after the GRPR antagonist was added (right).

(B_2) Representative sIPSCs recorded in a pyramidal neuron from GRPR knockout mouse under baseline conditions (left), during GRP application (center), and after picrotoxin was added (right).

(C) Cumulative amplitude histograms of sIPSCs recorded under baseline conditions (filled symbols) and after GRP was applied (open symbols) in slices from control (left) and GRPR knockout mice.

Explain the figure…

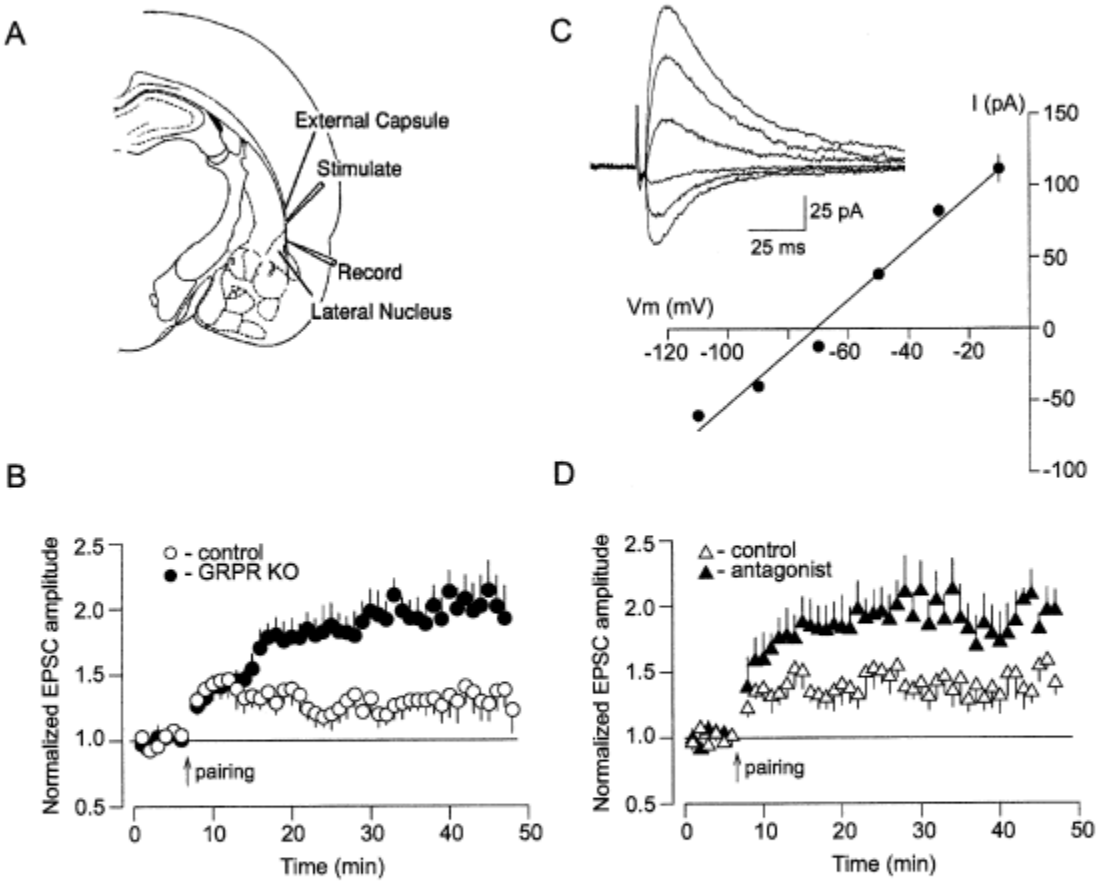


Figure 5. Pairing-Induced LTP Is Enhanced in GRPR Knockout Mice

(A) A schematic representation of a brain slice containing the amygdala that shows position of the recording and stimulation pipettes.

(B) LTP of whole-cell EPSCs recorded in the lateral amygdala neuron in response to the cortical input stimulation in slices from control (open symbols) or GRPR knockout (filled symbols) mice. For induction of LTP, the lateral amygdala neuron was held at +30 mV, and 80 presynaptic stimuli were delivered at 2 Hz to the external capsule fibers (arrow).

(C) Current-voltage plot of the $GABA_A$ receptor IPSCs at holding potentials of −110 mV to −10 mV. Reversal potential of the IPSC mediated by the $GABA_A$ receptors was −71 mV. Synaptic currents were recorded in the presence of the AMPA receptor antagonist CNQX (20 μM) and NMDA receptor antagonist D-APV (50 μM). Inset shows $GABA_A$ receptor IPSCs recorded at holding potentials of −110 mV to −10 mV. Traces are averages of 10 IPSCs recorded at each holding potential.

(D) Pairing-induced LTP of whole-cell EPSCs recorded in the lateral amygdala in wild-type mice under control conditions (open symbols) and in the presence of the bombesin antagonist (3 μM, filled symbols).

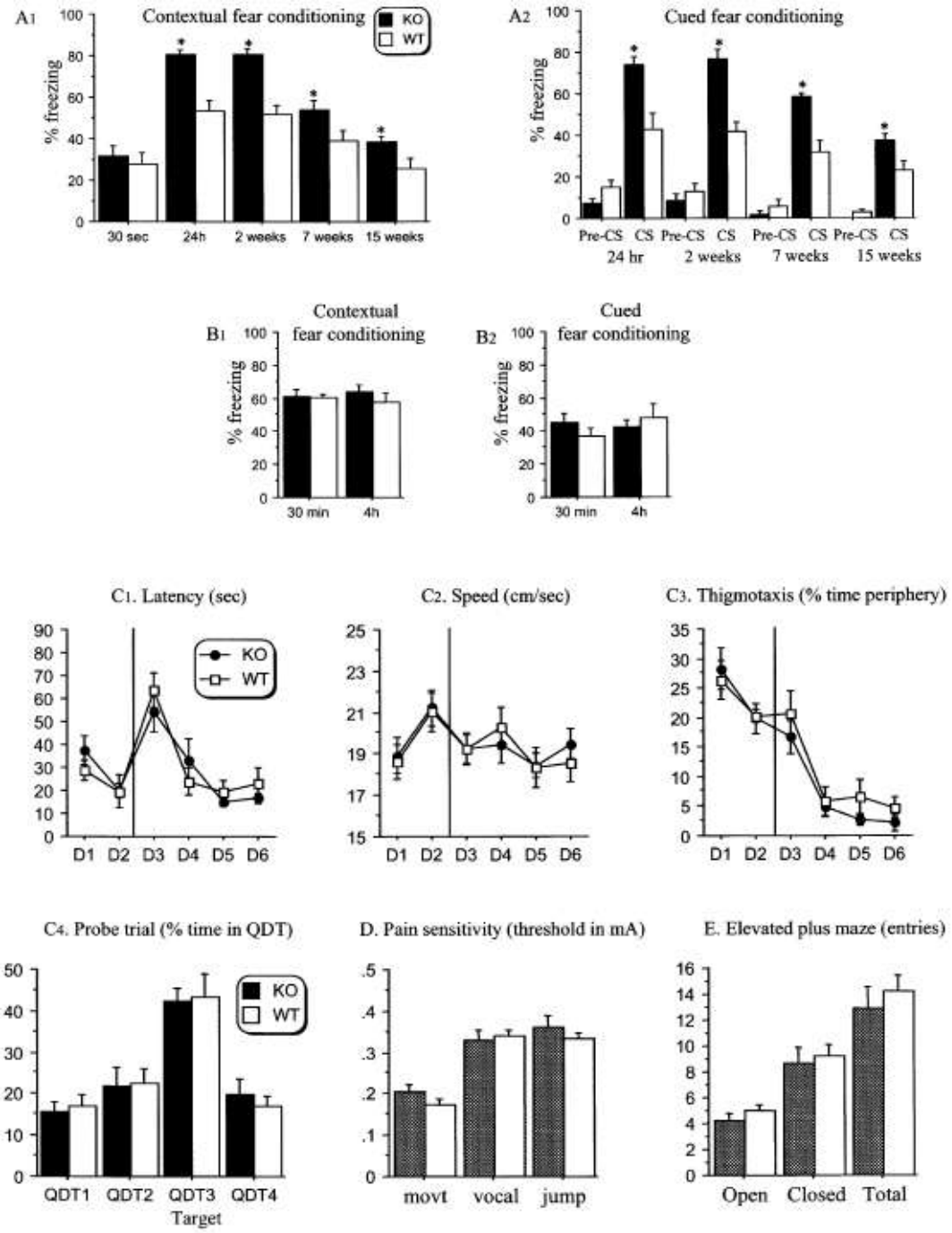


Figure 6. GRPR-Deficient Mice Have Enhanced and Resistant Long-Term But Not Short-Term Amygdala-Dependent Fear Memory

Explain the figure…

Explain the figure…

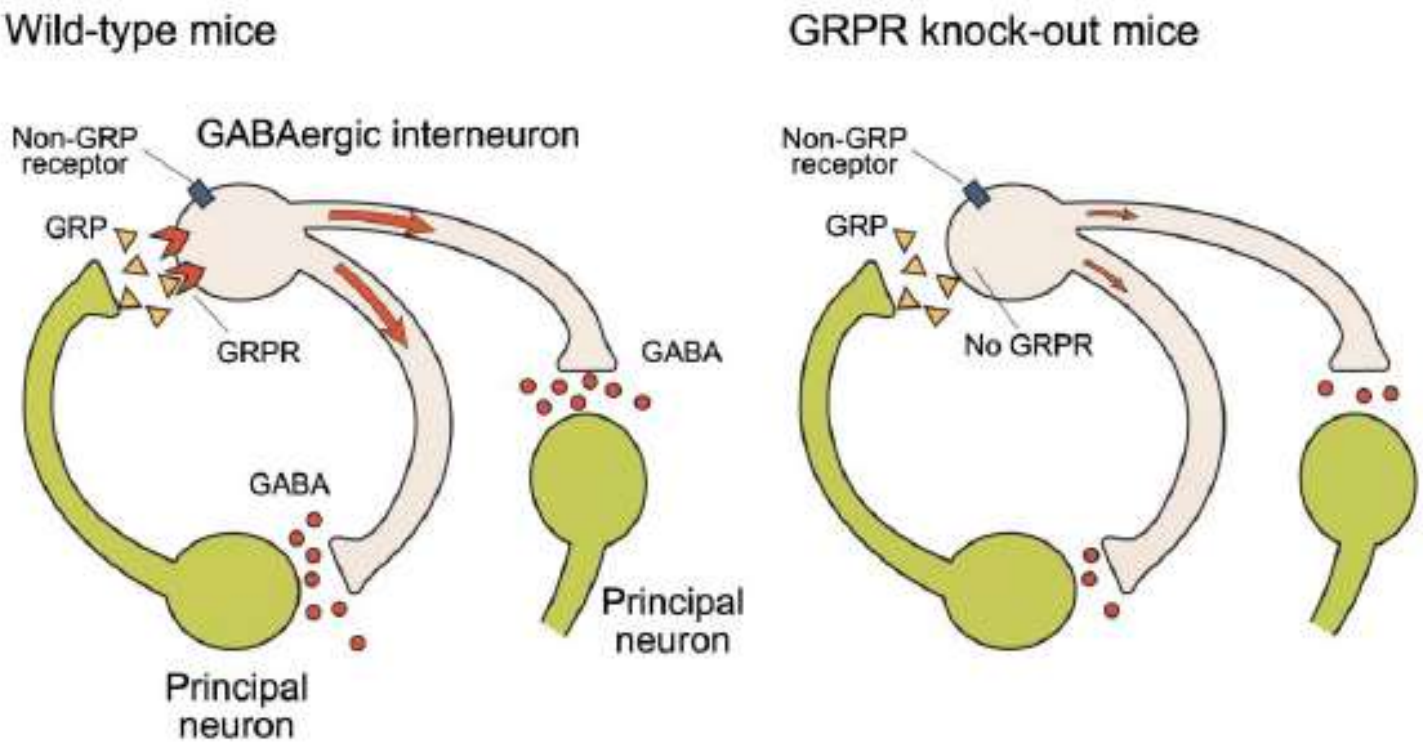


Figure 7. A Model for GRP-Dependent Negative Feedback to Principal Neurons in the Amygdala in Wild-Type and GRPR Knockout Mice

Q: What is GRP co-released with? Does it act directly on the same cells as that neurotransmitter? Explain.

(Wang et al., 2001). The observed pattern of the *Grp* and *Grpr* genes expression (see Figures 2 and 3) suggested to us that GRPR exerts a functional role in modulating the balance between excitation and inhibition in the local neuronal networks related to learned fear.

nucleus. Recent pharmacological and genetic studies have shown that the establishment of a balance between glutamatergic excitatory and GABAergic inhibitory functions is critical for processing of information in the amygdala (Bast et al., 2001; Krezel et al., 2001). Based on

Q: What is a neuromodulator?

Q: would you like to be a genetic mutant with GRPR over expression?