Differential regulation of pain and anxiety behaviors by CeA neurons

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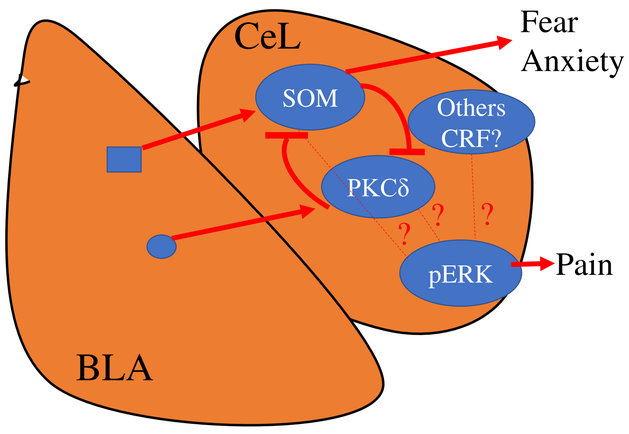
Abstact:

Amygdala nuclei play important roles in emotional responses, fear, depression, and pain processing. However, the identity of the amygdala neuronal subtypes involved in the pain signal is not completely understood. The lateral subdivision of amygdala central nucleus (CEl) contains two major subpopulations of GABAergic neurons which express somatostatin (SOM+) and protein kinase Cδ (PKCδ+). It has been demonstrated that ERK was activated in amygdala central nucleus (CEL) in different pain models. In this study, we showed most of the ERK positive neurons were colocolized with PKC δ+ neurons in different pain models in mice. Optogenetic activation of PKC δ+ neurons was sufficient to induce hyperalgesia without changing the anxiety behaviors in naïve mice. And also, chemogenetic inhibition of the PKC δ+ neurons showed significantly reduce the acute pain response induced by 5% formalin injection. Conversely, activation of SOM+ neurons changed the anxiety behaviors but did not affect the pain behavior. Taken together, our data suggest that CEl PKCδ+ neurons play an important role in mediating the pain signals.

Objectives:



Methods:

1. **Inflammation pain model (20 ul 5% intraplantar injection)**
2. **Immunostaining (ERK, SOM, PKCδ)**
3. **Genetic mice (Prkcd-glc-1/CFP,-cre, SOM-IRES-Cre)**
4. **Optogenetic tool (ChR2, NpHR)**
5. **Chemogenetic tool (hM4D)**
6. **Electrophysiology**

Results:

CEL PKC-δ+ neurons are activated by 5% formalin intraplantar injection

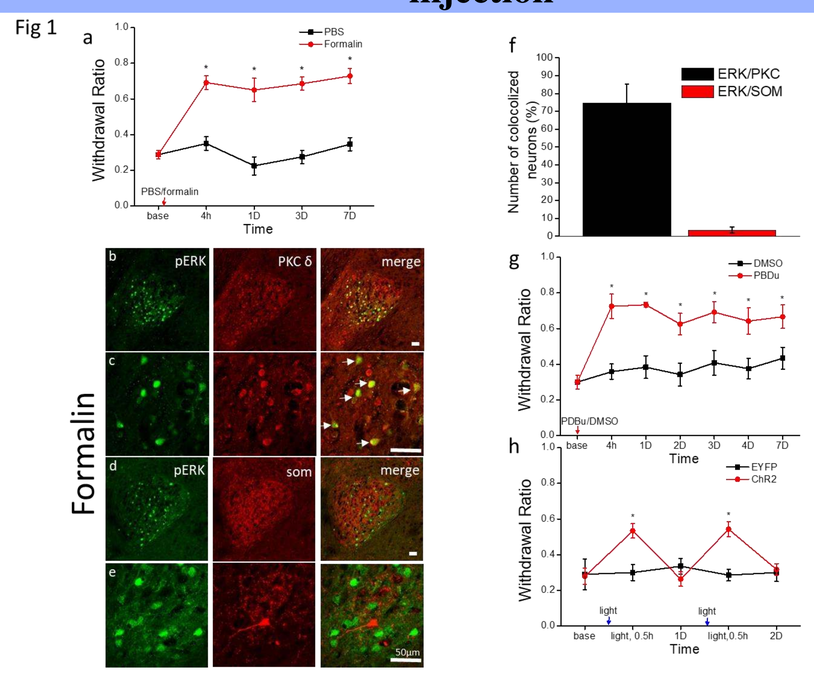


Fig1. (a) Mechanical responses from 5% formalin or PBS intraplantar injection mice (b) Immunoreactivity of pERK and PKCδ in CEA after 5% formalin intraplantar injection 90 min (c) Higher magnification (d) Immunoreactivity of pERK and somatostatin antibodies in CEA after 5% formalin intraplantar injection 90 min (e) Higher magnification (f) Quantification of pERK and PKCδ neurons colocolized percentage or pERK and somatostatin neurons colocolized percentage (g) Mechanical responses from DMSO or PDBu intra-amygdala injection (h) Mechanical responses with optogenetic activation of CEA neurons. White arrowhead indicates the colocolized neurons. Red arrowhead indicates the drug infusion timepoint. Blue arrowhead indicates the light stimulation timepoint. *P < 0.05, Scale bar = 50 μm.

Activation of PKCδ+ neurons in CeL produce chronic hyperalgesia

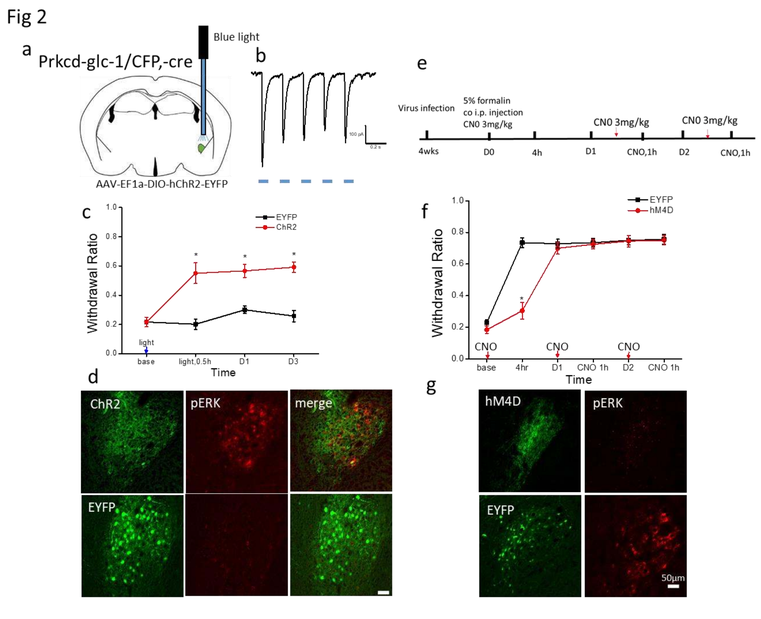


Fig 2. (a) Diagram illustrating the optogenetic activation experiments (b) Inward currents elicited by blue light stimulation from PKCδ+ neurons (c) Mechanical responses with optogenetic activation of CEA PKCδ+ neurons (d) Brain slices from PKCδ+ neurons expressing hChR2 or EYFP were co-stained with pERK after blue light stimulation 10min (Scale bar = 50 μm) (e) Schematic of experimental design showing the timeline for formalin injection and CNO i.p. injection (f) Mechanical responses with chemogenetic silencing of PKCδ+ neurons after 5% formalin intraplantar injection (g) Brain slices from PKCδ+ neurons expressing hM4d or EYFP were co-stained with pERK and PKCδ. Red arrowhead indicates the drug infusion timepoint. Blue arrowhead indicates the light stimulation timepoint. *P < 0.05, scale bar = 50 μm

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Activation of PKCδ+ neurons does not directly alter the anxiety behavior

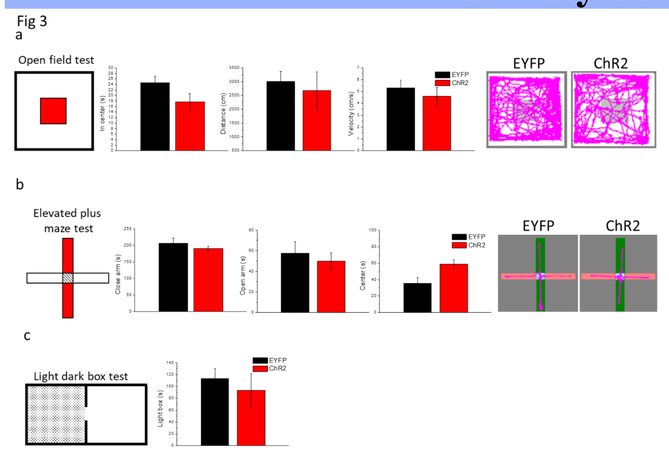

Fig 3. Optogenetic activation of PKC+ neurons during behavior (a) open filed test and quantification of behavioral parameters (b) Elevated plus maze test and quantification of behavioral parameters (c) Light dark box test and quantification of behavioral parameters.

Inhibition of somatostatin neurons induced chronic hyperalgesia

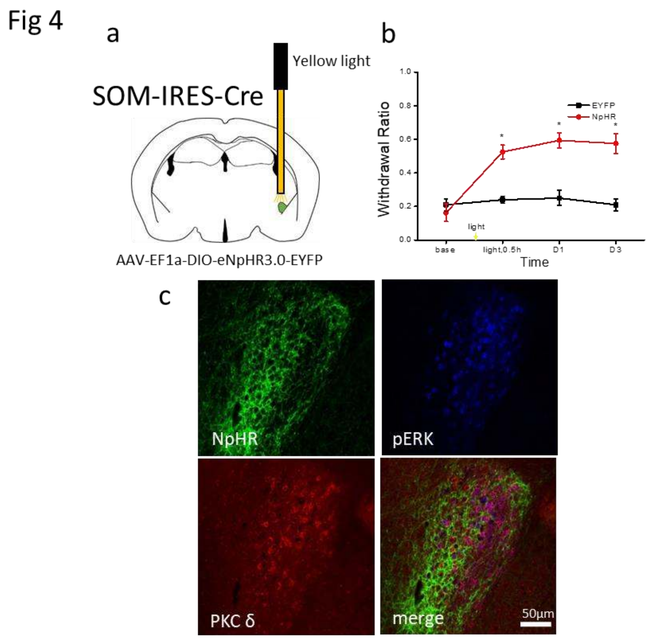

Fig 4. (a) Diagram illustrating the optogenetic inhibition experiments (b) Mechanical responses with optogenetic inhitbition of CEA somatostatin+ neurons in naïve mice. (c) Brain slices from som+ neurons expressing eNpHR3.0 were co-stained with pERK and PKCδ. *P < 0.05, scale bar = 50 μm

Activation of the somatostatin neurons does not change the mechanical hyperalgesia but affect the mice anxiety behavior

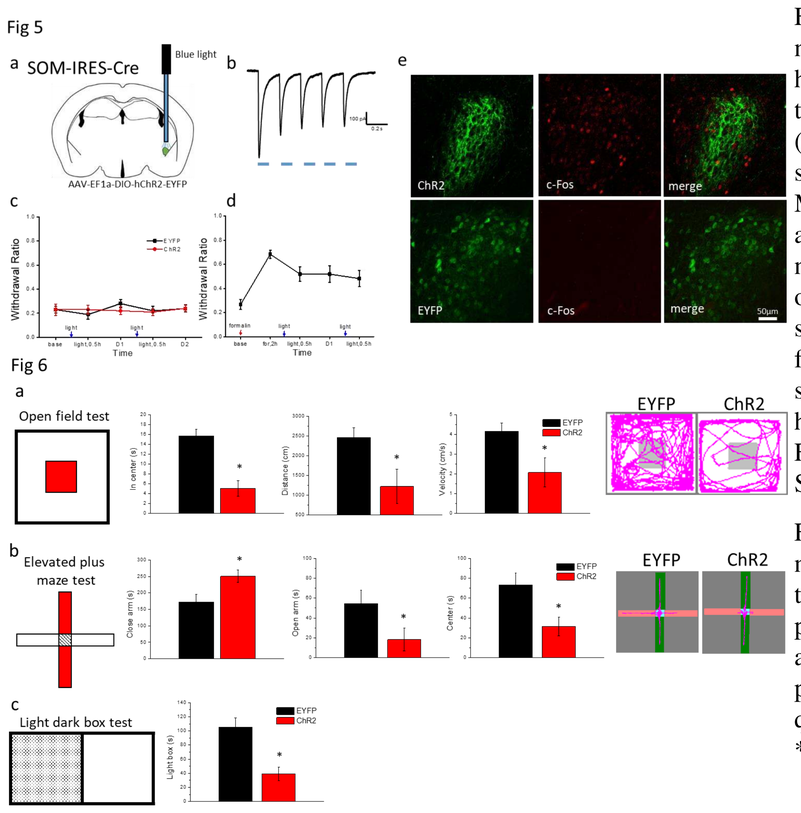

Fig 5. Activation of the somatostatin+ neurons does not change the mechanical hyperalgesia. (a) Diagram illustrating the optogenetic activation experiments (b) Inward currents elicited by blue light stimulation from som+ neurons (c) Mechanical responses with optogenetic activation of CEA somatostatin+ neurons (d) Mechanical responses with optogenetic activation of CEA somatostatin+ neurons after 5% formalin intraplantar injection (e) Brain slices from som+ neurons expressing hChR2 or EYFP were co-stained with c-Fos after blue light stimulation 10min. Scale bar = 50 μm

Fig 6. Optogenetic activation of som+ neurons during behavior (a) open filed test and quantification of behavioral parameters (b) Elevated plus maze test and quantification of behavioral parameters (c) Light dark box test and quantification of behavioral parameters. *P < 0.05

Formalin induced inflammation model does not significantly increase the mice anxiety behavior

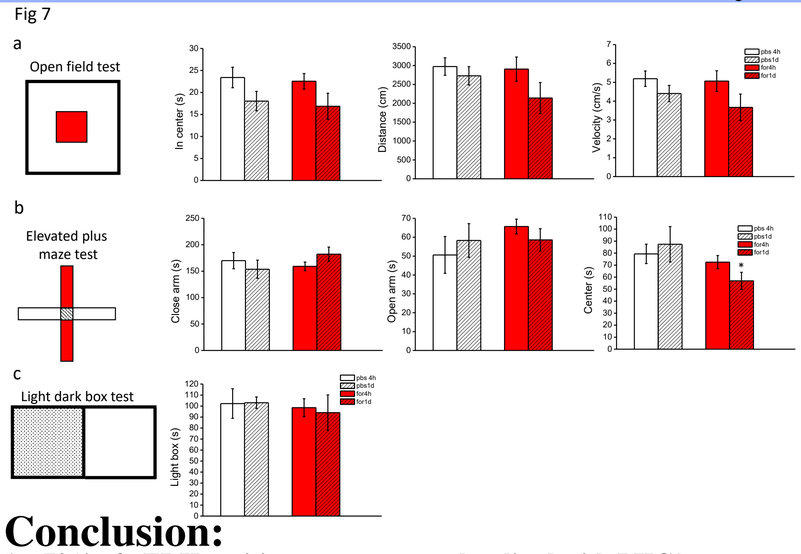

Fig 7. (a) open filed test and quantification of behavioral parameters (b) Elevated plus maze test and quantification of behavioral parameters (c) Light dark box test and quantification of behavioral parameters. *P < 0.05

Conclusion:

1. **70% of pERK positive neurons are colocolized with PKCδ+ neurons in inflammation model.**
2. **Activation of CeL PKCδ+ neurons produce chronic hyperalgesia in naïve mice.**
3. **Chemogenetic silencing the CeL PKCδ+ neurons reduce the formalin-induced inflammation pain.**
4. **Activation of CeL SOM+ neurons produce anxiety behavior in naïve mice.**

References:

1. Cai H, Haubensak W, Anthony TE, Anderson DJ. Nat Neurosci. 2014 Sep;17(9):1240-8. doi: 10.1038/nn.3767. Epub 2014 Jul 27. Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic.
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