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Julie Carcaud

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Stress-induced plasticity of a CRH/GABA projection disrupts reward behaviors in mice

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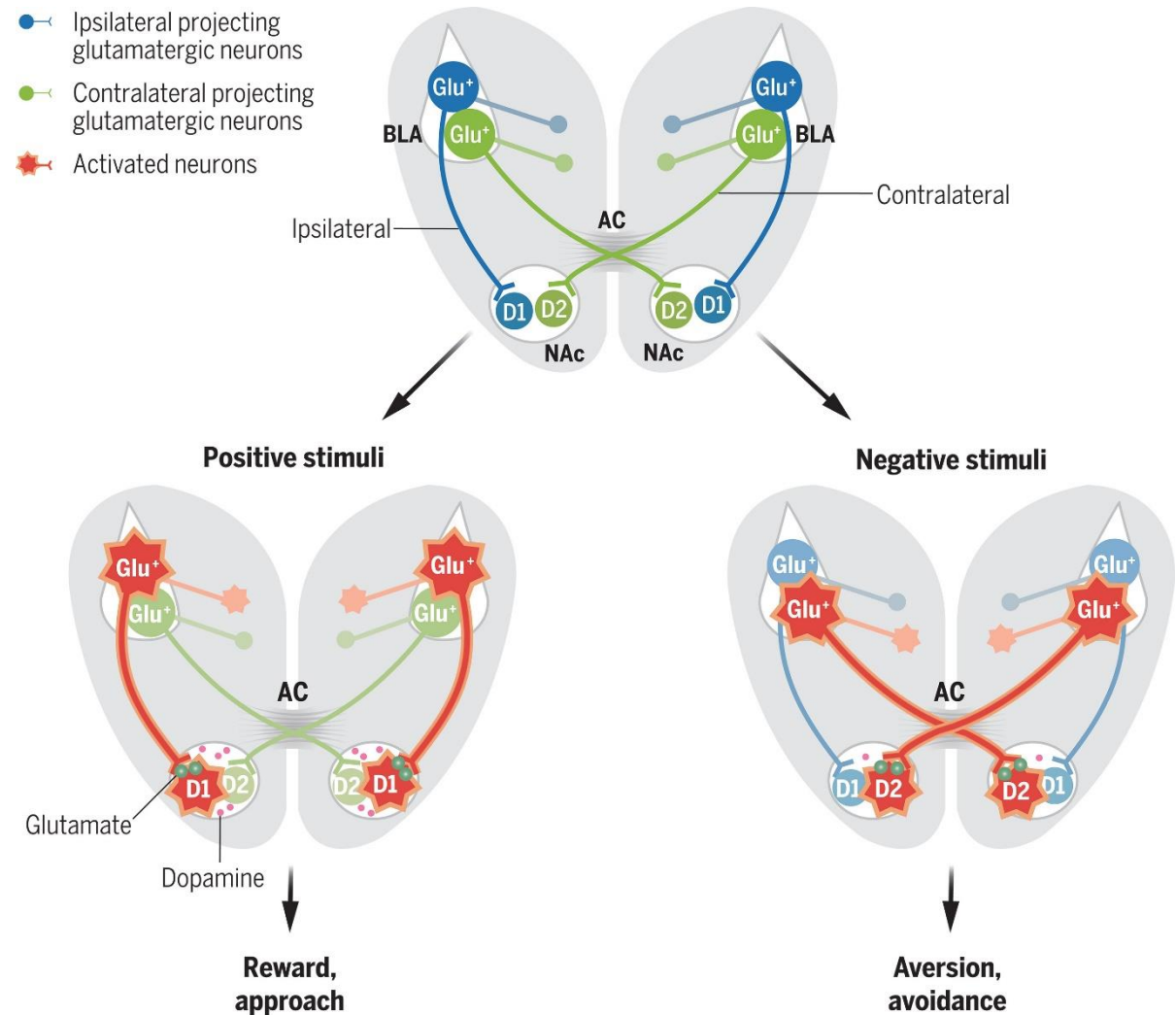
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Matthew T. Birnie ^{1,2,5}, Annabel K. Short ^{1,2,5}, Gregory B. de Carvalho ²,
Lara Taniguchi ^{1,2}, Benjamin G. Gunn ², Aidan L. Pham^{1,2}, Christy A. Itoga ²,
Xiangmin Xu², Lulu Y. Chen ², Stephen V. Mahler ³, Yuncai Chen ^{1,2} ✉ &
Tallie Z. Baram ^{1,2,4} ✉

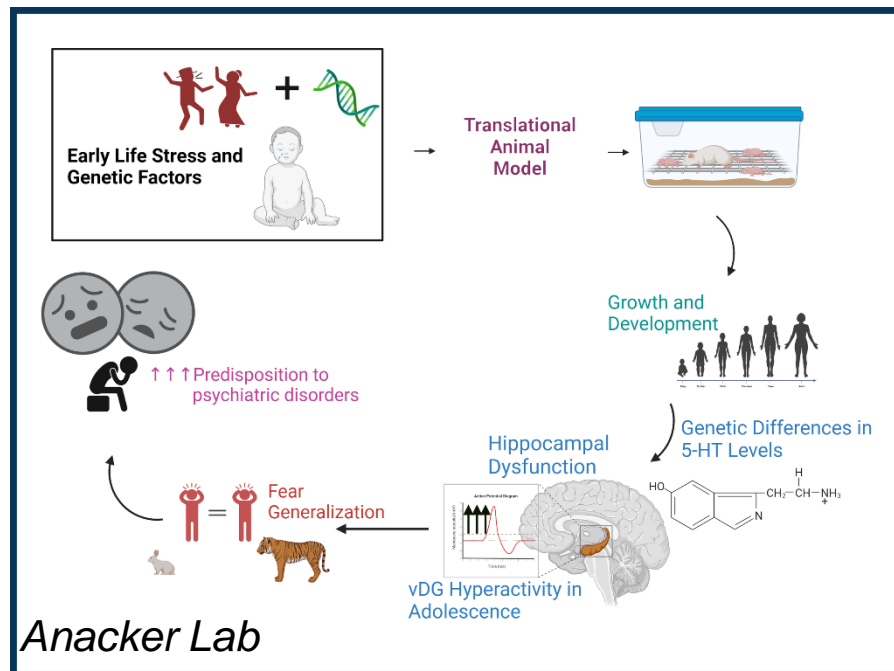
Introduction

- Nucleus accumbens (Nac) involved in pleasure and motivation processes
- Multiple afferents to Nac: including basolateral amygdala (BLA)
- BLA mediates associative learning
- Glutamatergic BLA projections to the Nac facilitates reward behavior

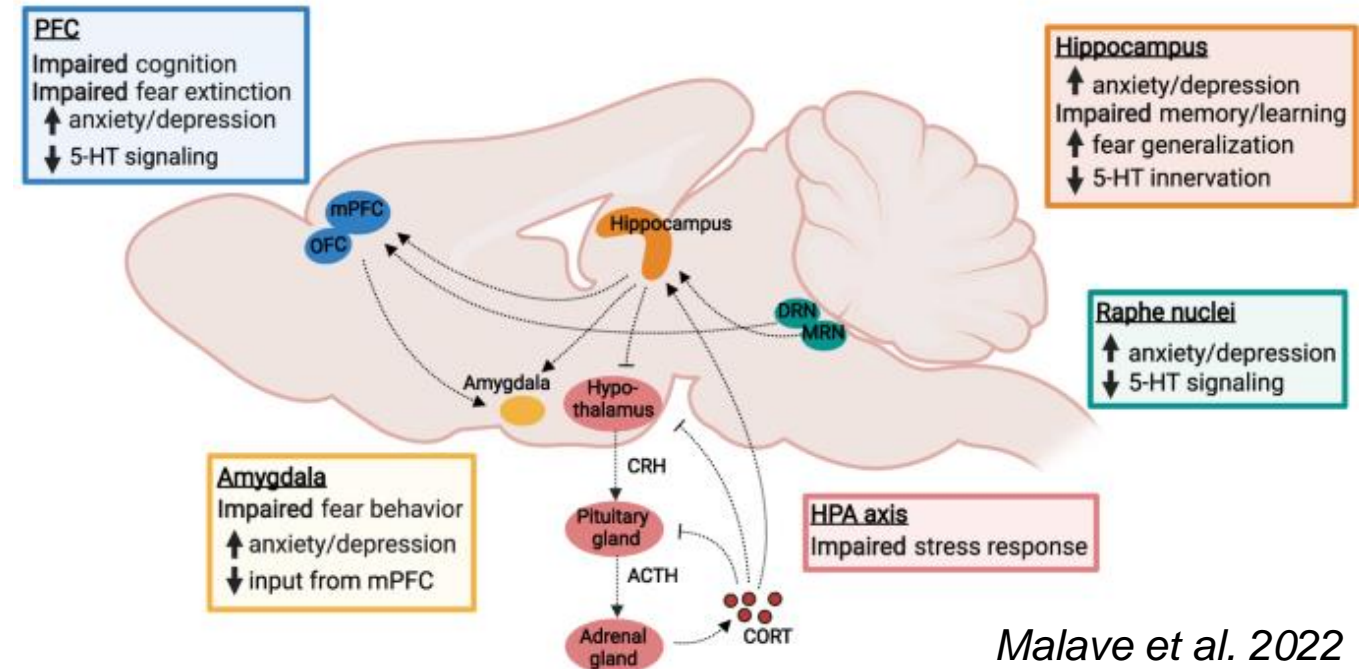


Introduction

- Stress during early-life periods can result in mental health problems
- Neuropeptide CRH (corticotropin-releasing hormone) is a modulator of Nac functions in a stress-dependent manner
- CRH expression and function influenced by early-life adversity (ELA)



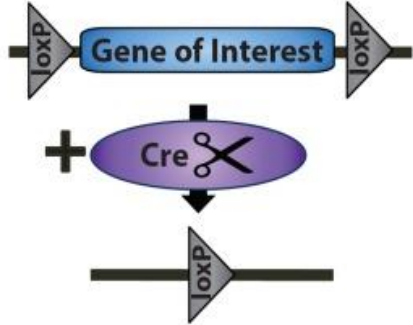
ELA reared



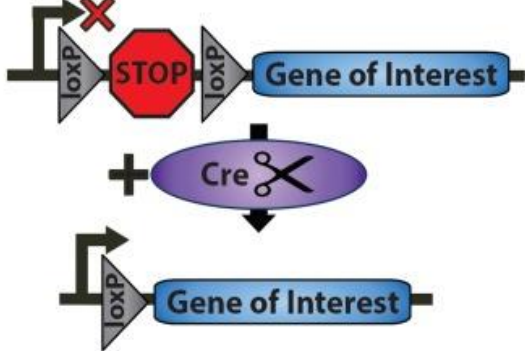
Malave et al. 2022

Cre-Lox system

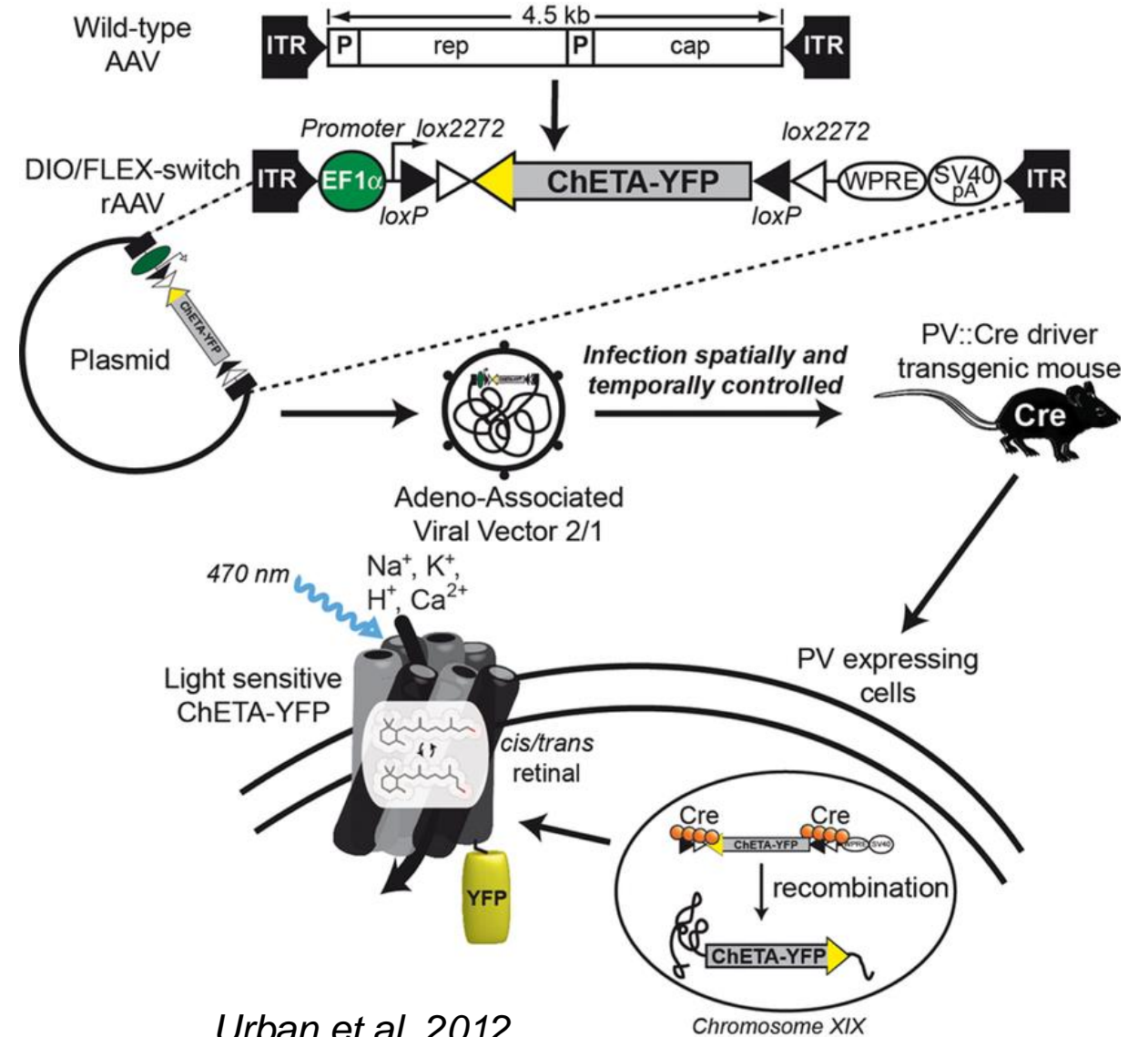
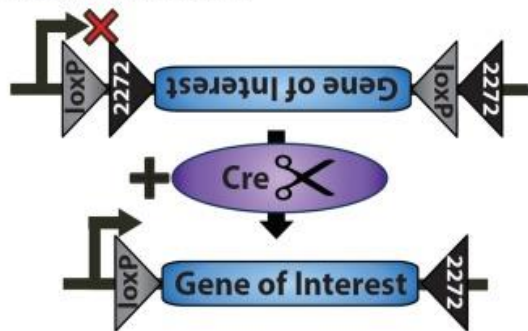
A. Cre-induced deletion



B. Cre-induced expression



C. DIO/FLEX vectors



Sup. Figure 1

Supplementary Figure 1

Colocalized Cre expression in BLA neurons expressing endogenous CRH in the CRH-ires-Cre mouse line

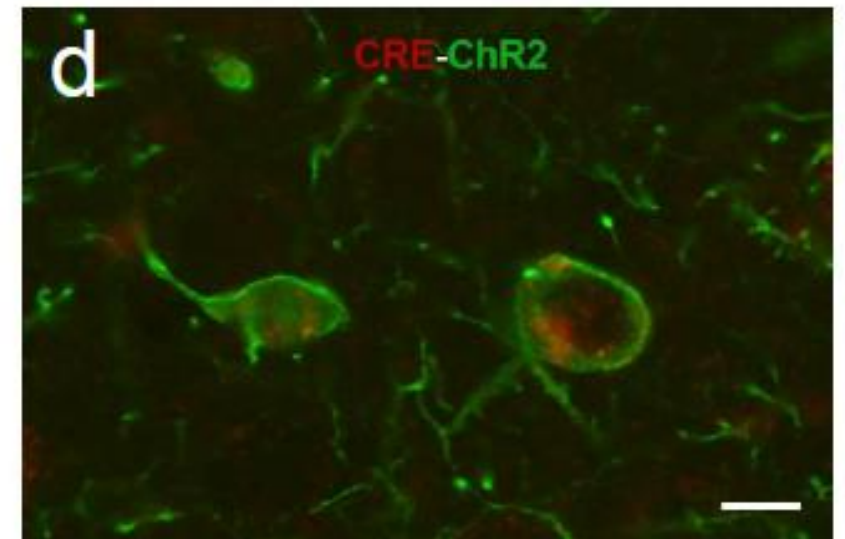
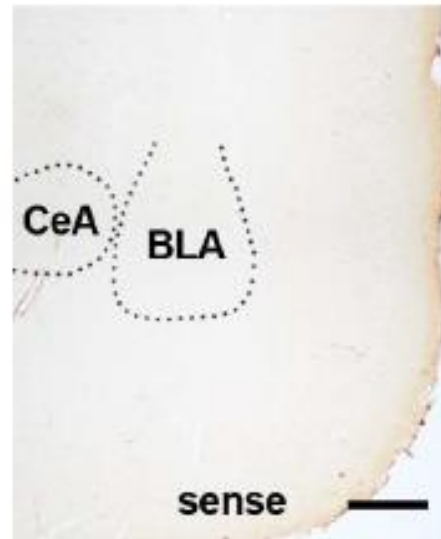
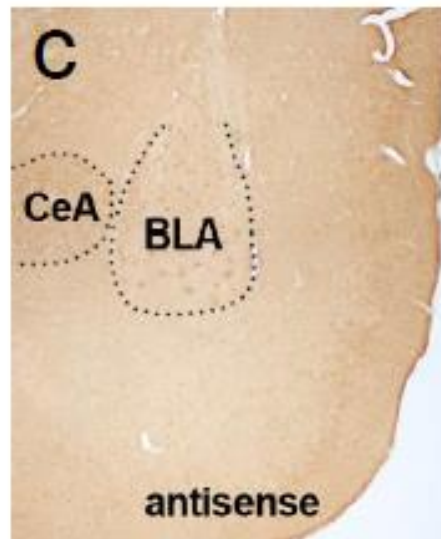
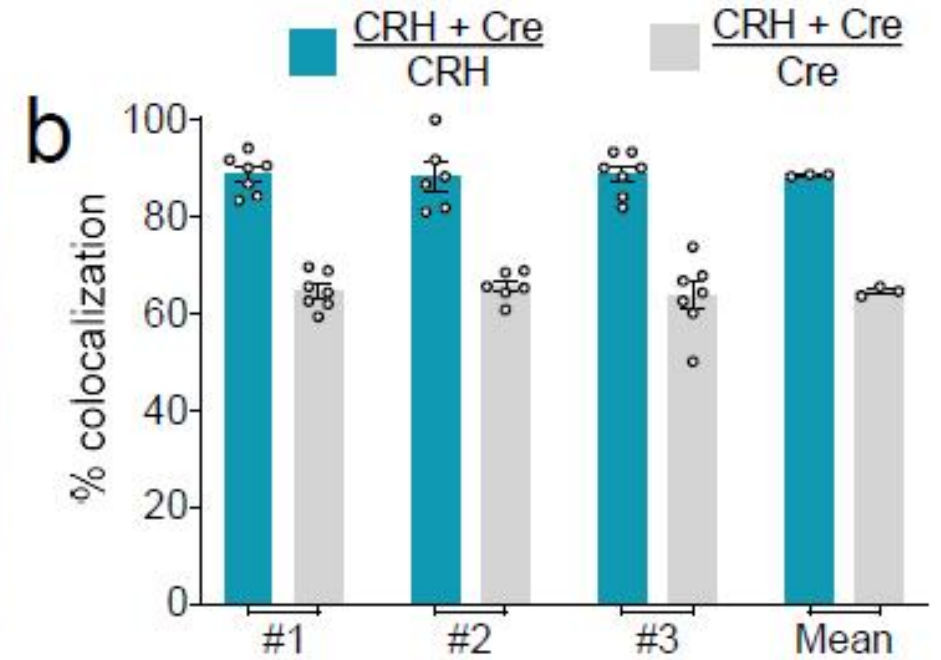
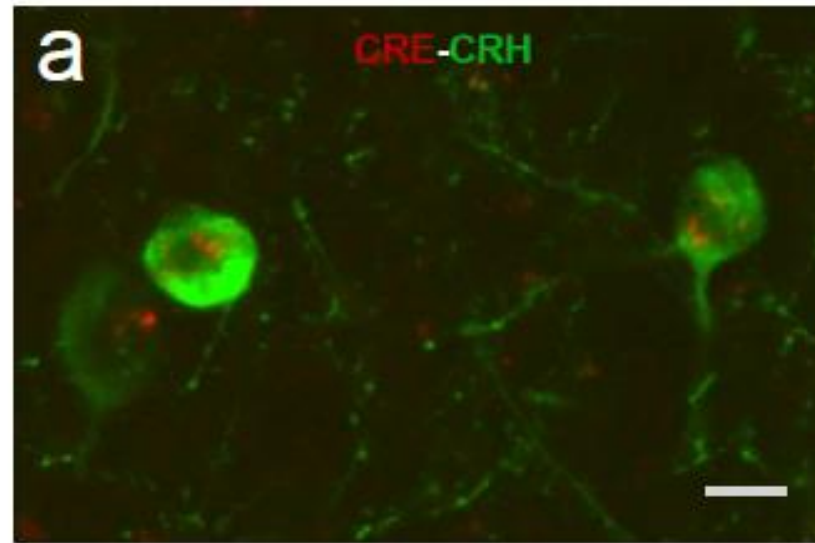
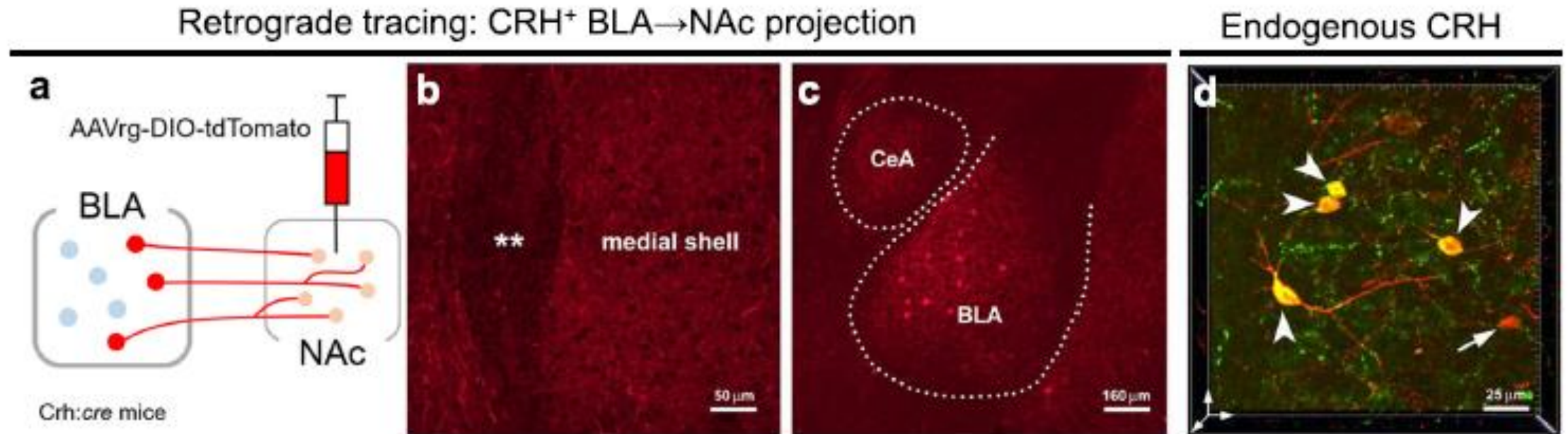


Figure 1

A projection of CRH/GABA neurons in the medial BLA to the medial NAc shell

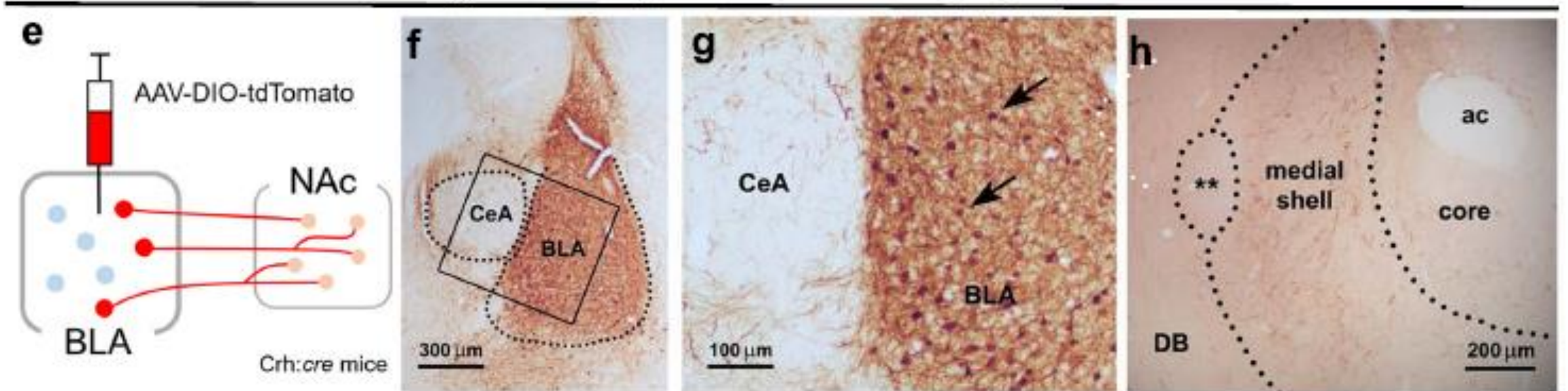


- Injection of CRE-dependent retrograde virus into the medial NAc shell
- Retrogradely infect projection neurons, including those originating in the BLA
- The specificity of CRE-dependent viral expression to CRH⁺ neurons (colocalization of endogenous CRH in viral-infected BLA cell bodies)

Figure 1

A projection of CRH/GABA neurons in the medial BLA to the medial Nac shell

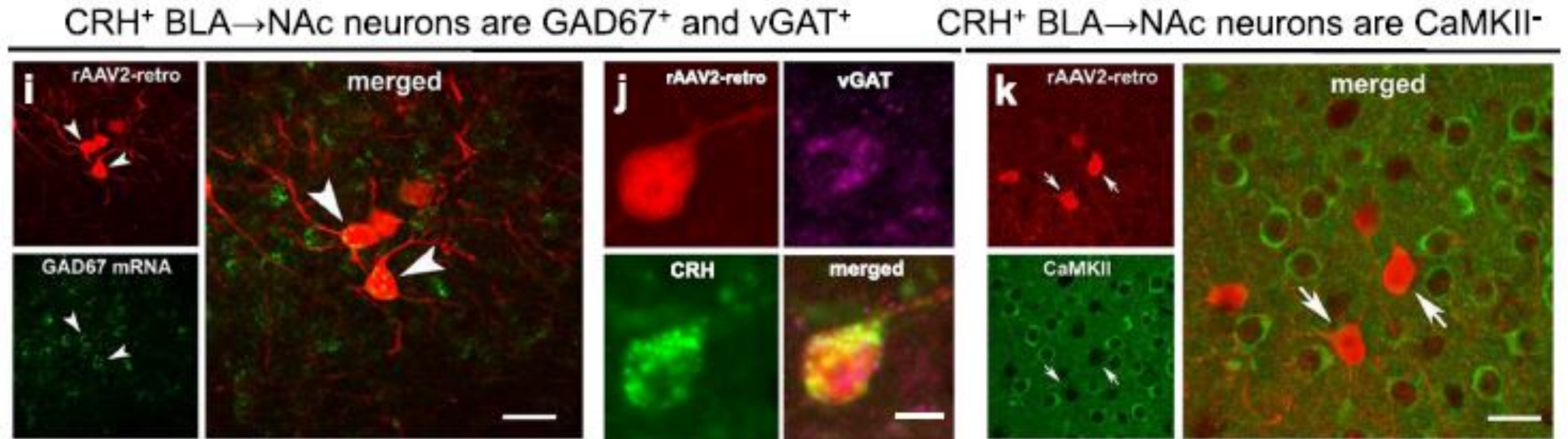
Anterograde tracing: CRH⁺ BLA→Nac projection



- Define the neuroanatomical distribution of the BLA-origin CRH⁺ projection in the NAc
- injected a CRE-dependent anterograde virus into the medial BLA
- identified CRH⁺ BLA projection fibers and terminals in the medial NAc shell

Figure 1

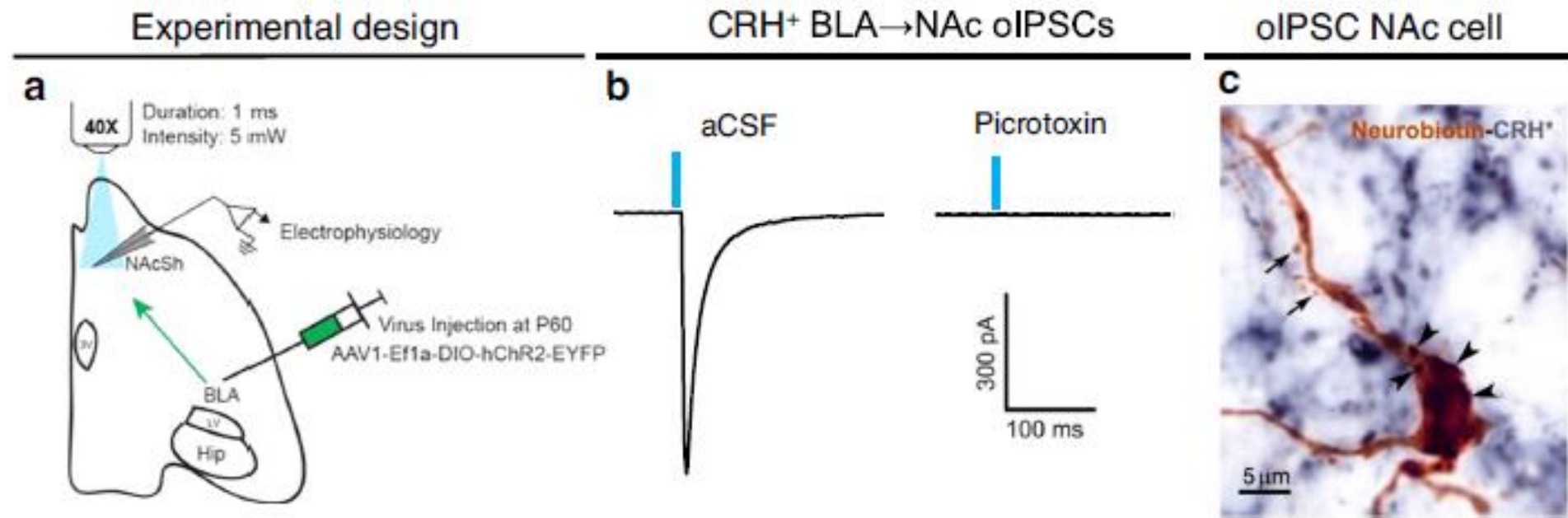
A projection of CRH/GABA neurons in the medial BLA to the medial Nac shell



- Combination of viral reporter, fluorescent in situ hybridization and immunostaining identified this population of CRH₊ neurons as GABAergic, rather than glutamatergic
- CRH₊ neurons coexpress two defining GABA markers: Gad67 and the vesicular GABA transporter-vGAT
- In contrast, they do not express a defining glutamatergic marker-CaMKII

Figure 2

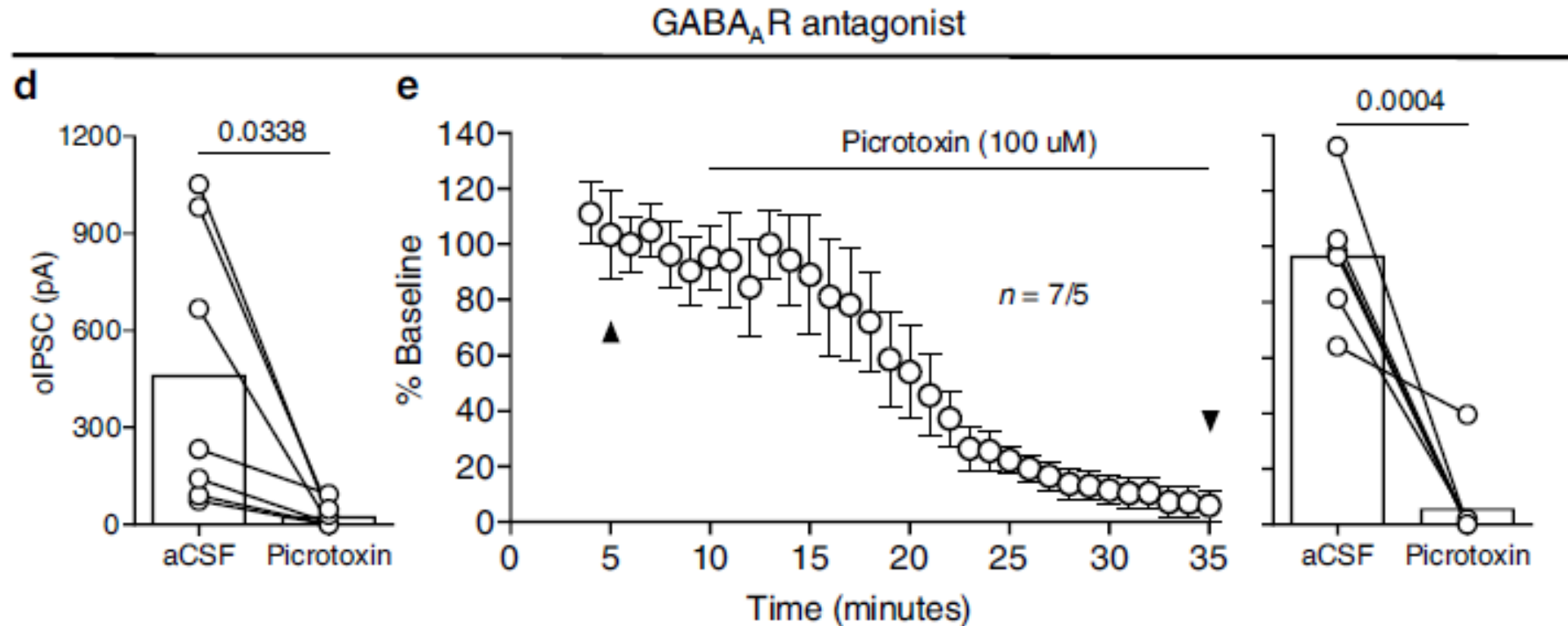
Optical stimulation of CRH+ BLA-origin axons in the NAc evokes exclusively IPSCs



- To substantiate that the CRH⁺ BLA → NAc projection evokes inhibitory currents, we performed whole-cell patch-clamp recordings in the medial NAc shell of CRH-ires-CRE mice injected with a CRE-dependent ChR2-EYFP in the BLA
- Optical stimulation of CRH⁺ BLA-origin fibers in the NAc reliably evoked inhibitory postsynaptic currents (oIPSCs) in medium spiny neurons (MSNs)

Figure 2

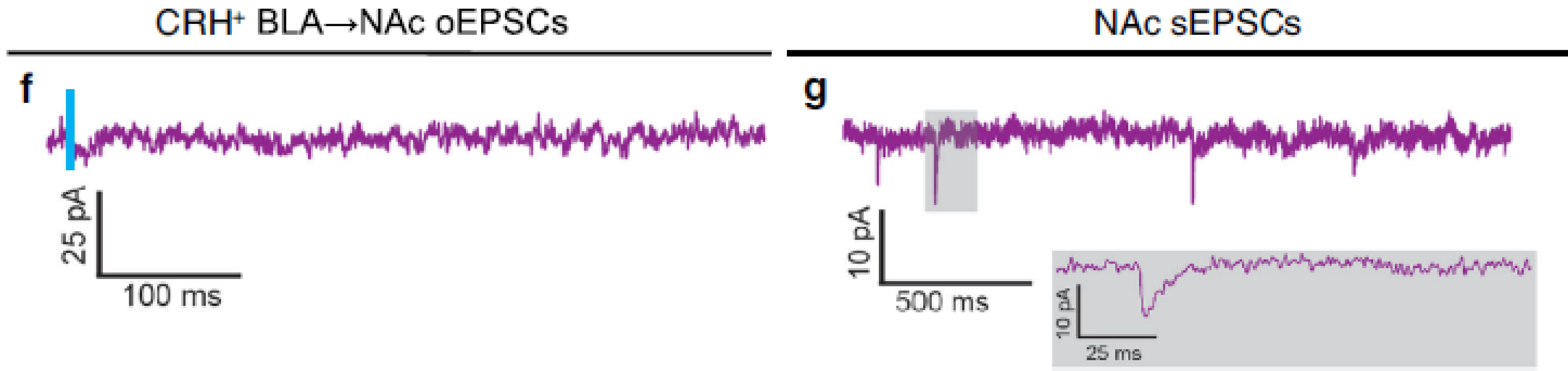
Optical stimulation of CRH+ BLA-origin axons in the NAc evokes exclusively IPSCs



- optically evoked inhibitory postsynaptic responses were consistently blocked by superfusion with the GABA_A receptor antagonist, picrotoxin (100 μM)

Figure 2

Optical stimulation of CRH+ BLA-origin axons in the NAc evokes exclusively IPSCs



- In a confirmed oIPSC recorded cell, optical stimulation did not yield excitatory postsynaptic currents (oEPSCs)
- even though spontaneous EPSCs were apparent

these data identify an inhibitory CRH/GABA BLA → NAc projection that is distinct from the well-described glutamatergic BLA → NAc projection

Figure 3

Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice

Utilisation des virus DREADD

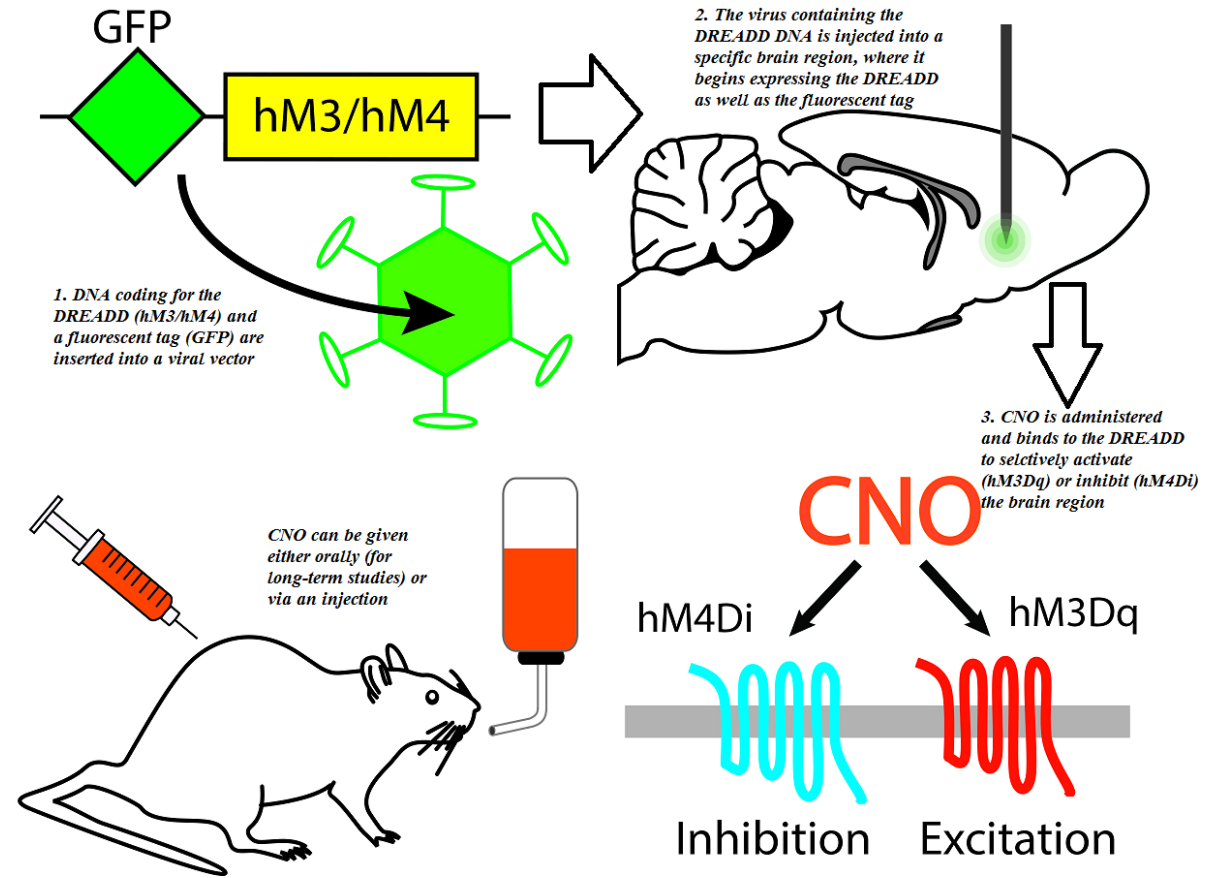
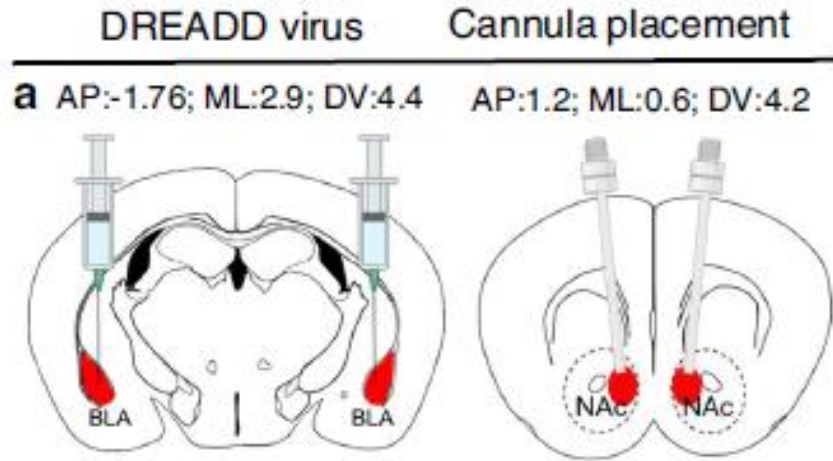


Figure 3

Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice

Utilisation de stimulation optogénétique

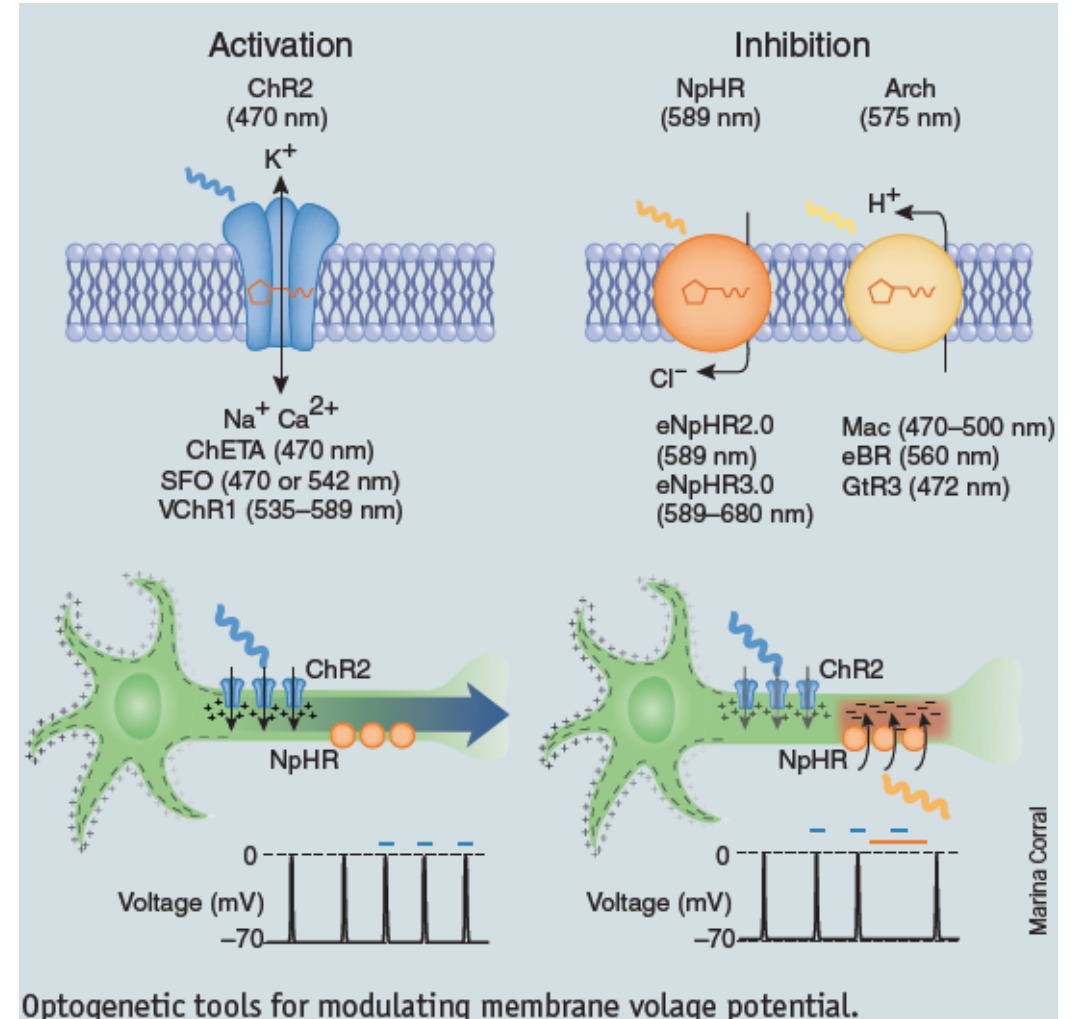
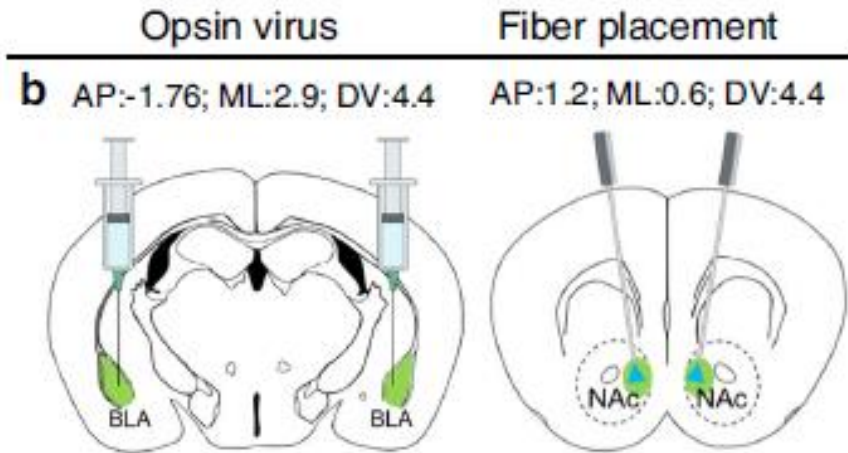


Figure 3

Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice

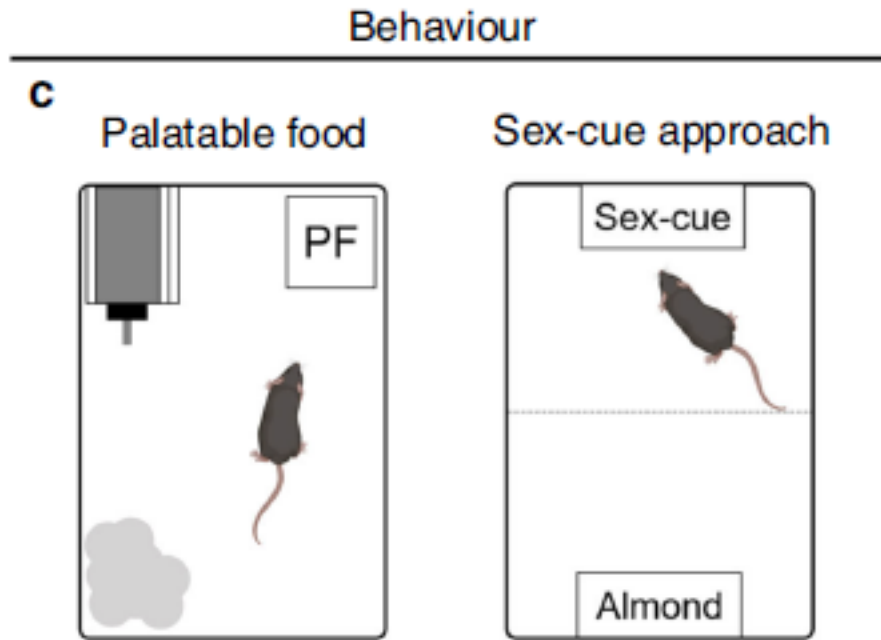
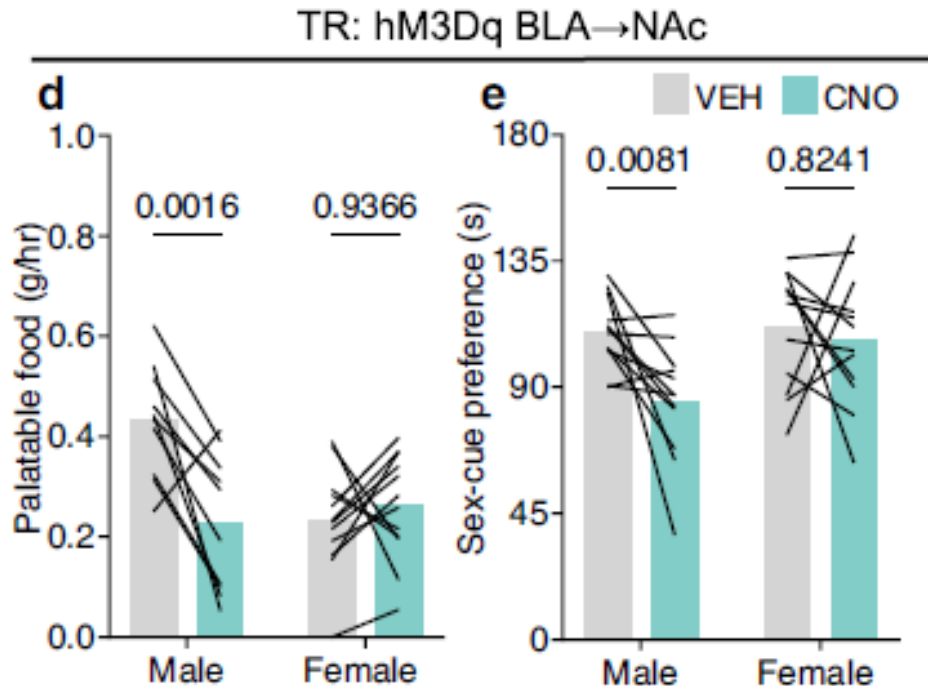


Figure 3

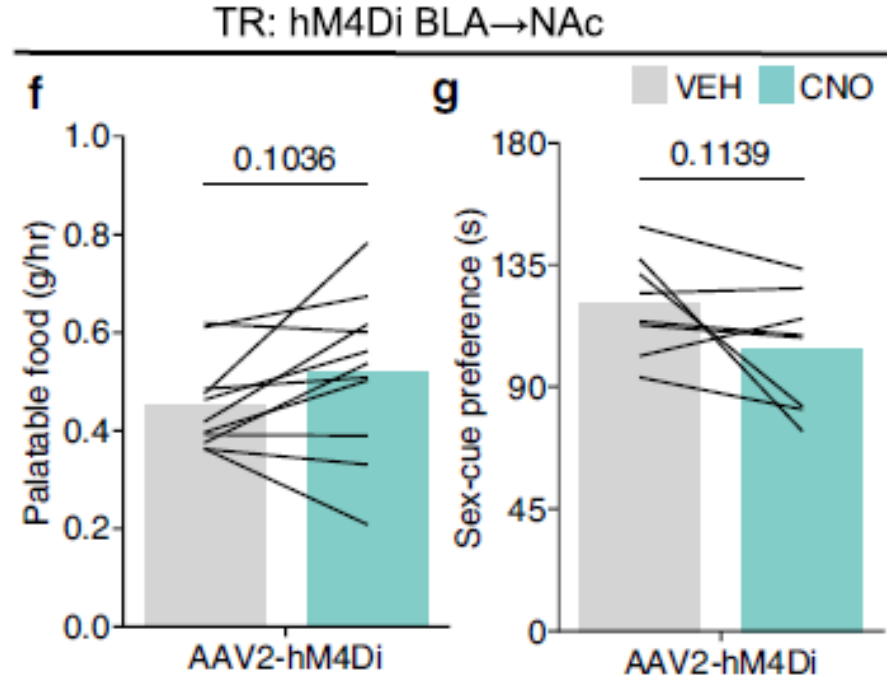
Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice



- in typically reared male and female mice, we microinjected CNO into the medial NAc shell
- stimulated the hM3Dq⁺ CRH/GABA BLA-origin fibers in the NAc during two different reward tasks
- Bilateral microinfusion of CNO (1mM) into the medial NAc shell reduced palatable food consumption in male but not female mice
- This suppression, unique to males was also evident in the sex-cue task

Figure 3

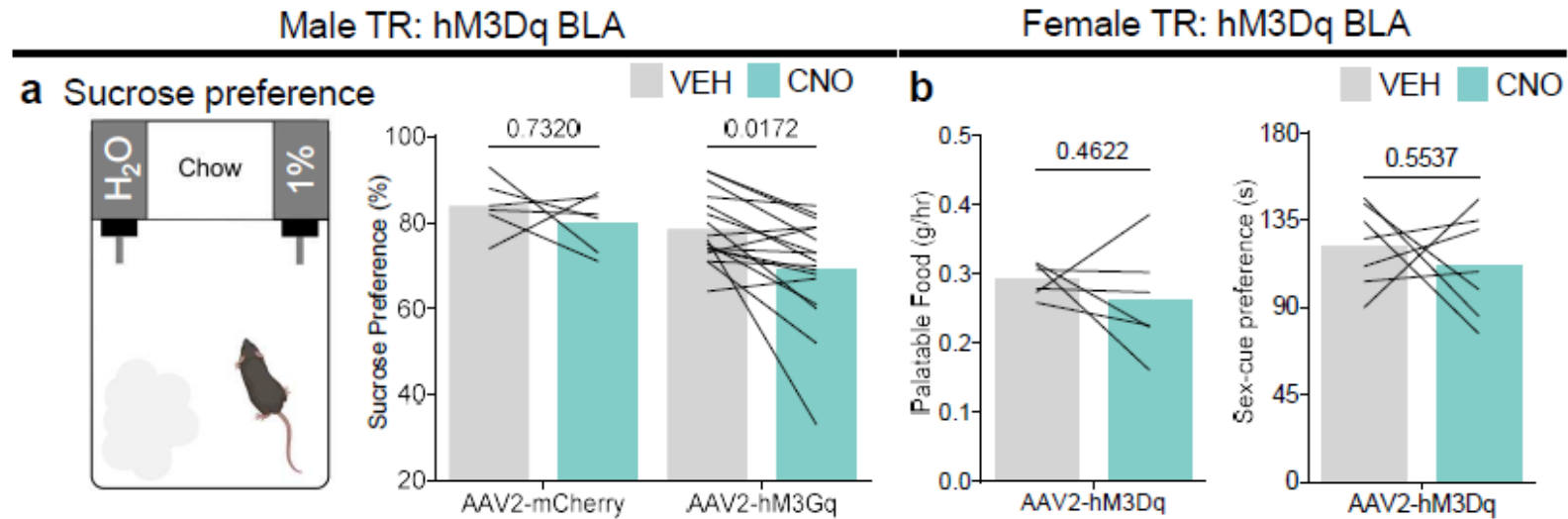
Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice



- Inhibition of the projection in TR male mice had no effect
- specifically did not increase palatable food consumption or the preference for a sex cue
- Contrôles ?

Sup. Figure 2

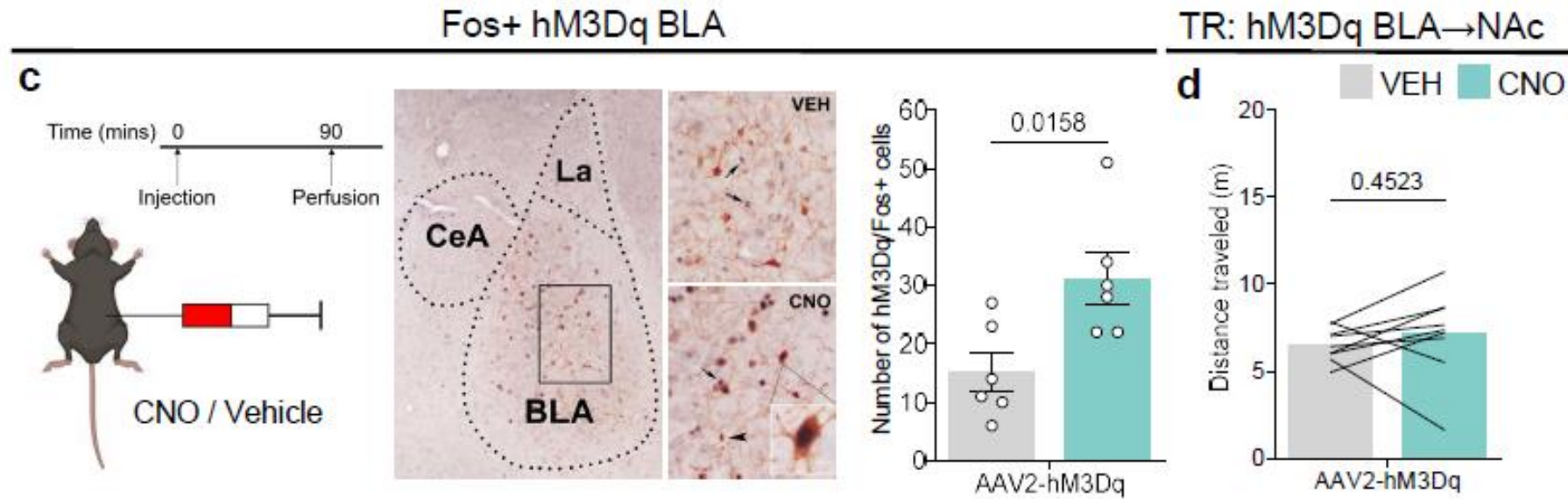
Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice



- Off-targets effects of CNO ?
- Non-reward behaviors unaffected

Sup. Figure 2

Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice

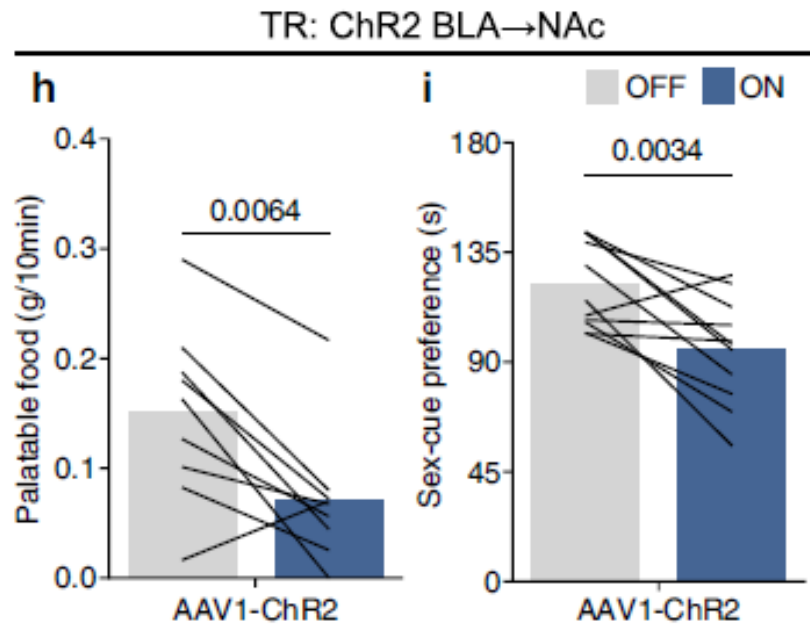


Confirmation of the activation of hM3Dq-expressing CRH⁺ neurons by CNO via quantification of selective Fos expression in the BLA 90min following injection

Figure 3

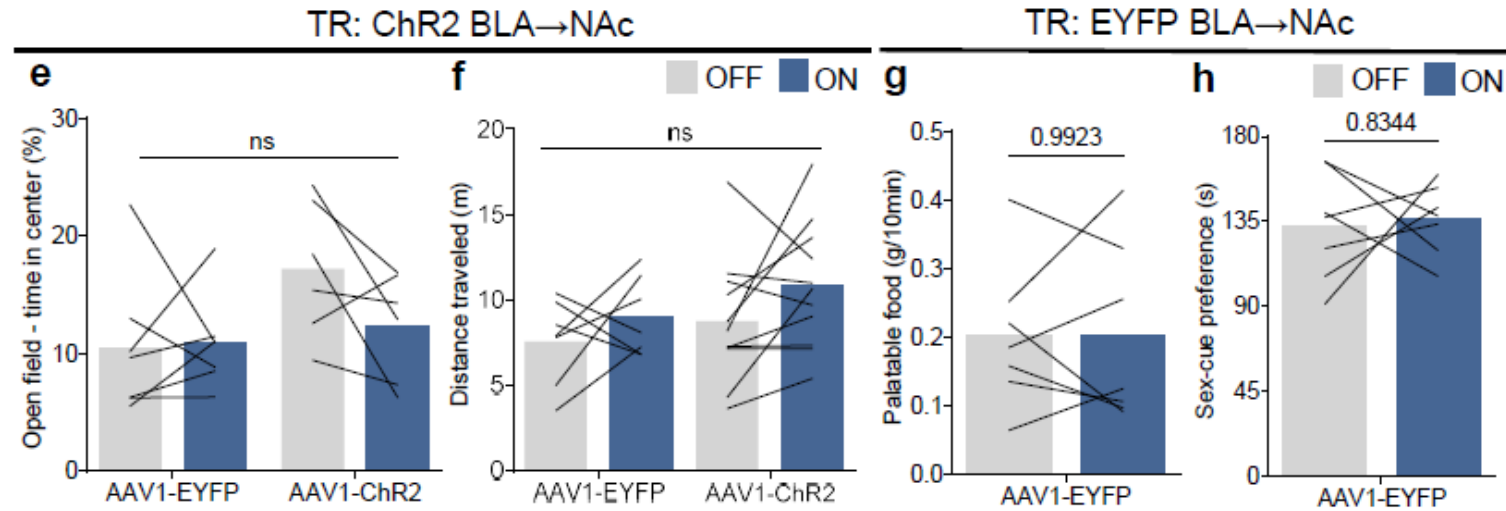
Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice

- used a second, independent technology, optogenetics
- Injection of a CRE-dependent ChR2-EYFP in the BLA bilaterally to CRH-ires-CRE mice, and implanting bilateral optic fibers directly above the medial NAc shell to optically stimulate projection fibers
- Suppressed palatable food consumption and the preference for a sex-cue in males



Sup. Figure 2

Stimulating the CRH/GABA BLA → NAc projection suppresses reward in typically reared male mice



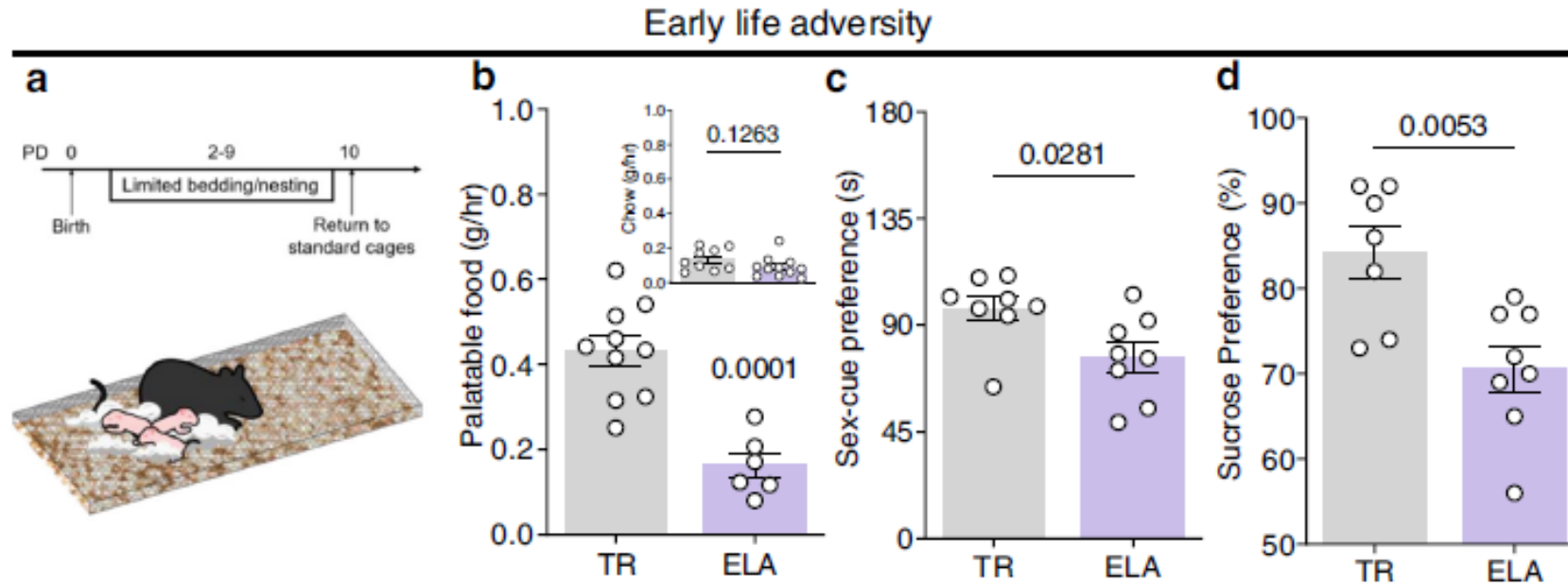
potential off-target effects of virus and/or light stimulation did not contribute to these outcomes

these findings describe an unexpected reward-suppressing role for a CRH/GABA BLA → NAc projection in males, which differs from the well-established, reward enhancing glutamatergic projection connecting the BLA and NAc

This projection might mediate suppressed reward behaviors often observed after adult or developmental stresses

Figure 4

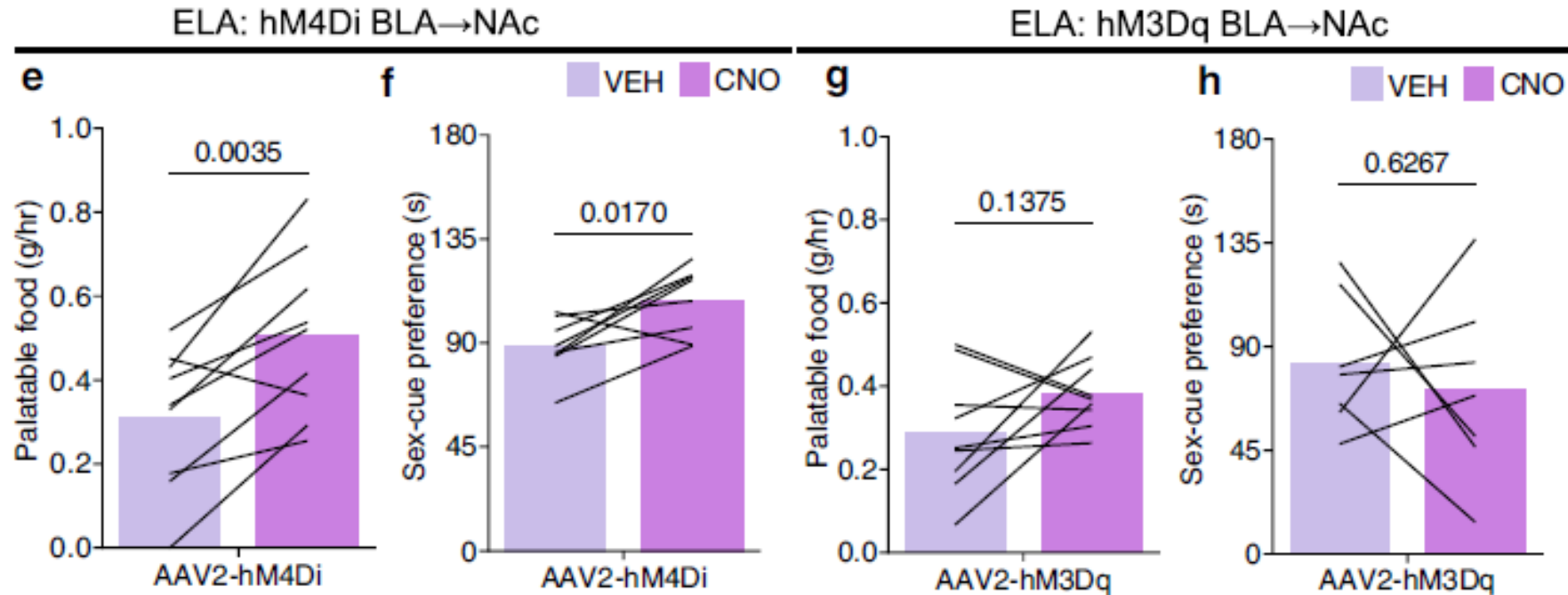
Inhibiting the CRH/GABA BLA → NAc projection rescues reward deficits following early-life adversity (ELA)



- Determination of consequences of ELA on reward behaviors in males: Preference for palatable food, for a sex-cue and sucrose are reduced in adult ELA mice.
- Well-established model of ELA during a sensitive developmental period, in which poverty is simulated via a resource-scarce environment during postnatal days 2–10 in mice.
- The reward deficits in adult ELA mice recapitulated the effects of chemogenetic and optogenetic stimulation of the CRH/GABA BLA → NAc projection in typically reared male mice

Figure 4

Inhibiting the CRH/GABA BLA→ NAc projection rescues reward deficits following early-life adversity (ELA)



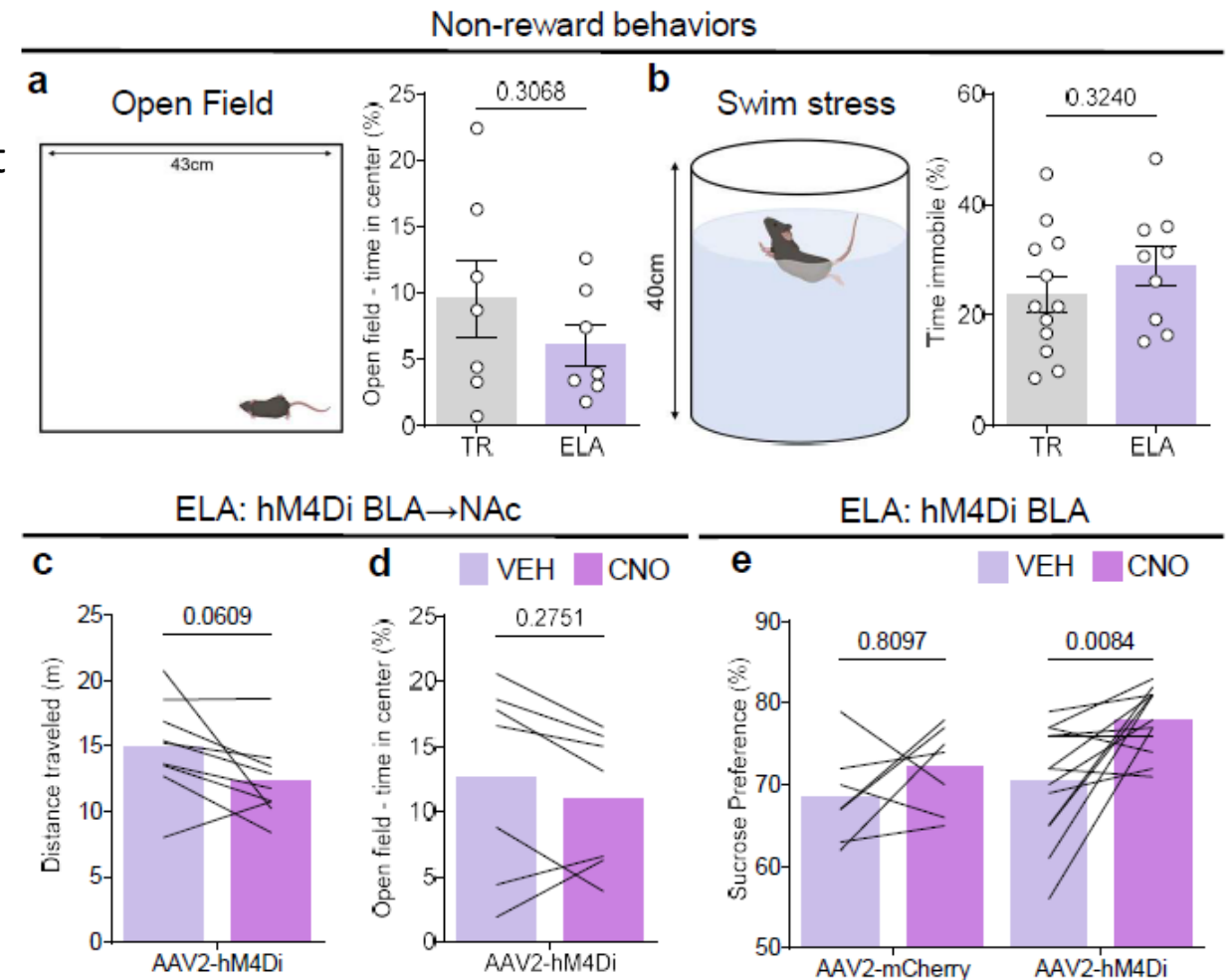
- We inhibited the activity of the CRH/GABA BLA → NAc projection during reward tasks in adult ELA mice
- Inhibition of the BLA-origin fibers in the Nac rescued the consumption of palatable food in adult ELA-experienced mice and restored the preference for a sex-cue
- They assessed if stimulation of this projection further suppressed reward behavior in ELA mice: NO

Sup. Figure 3

Inhibiting the CRH/GABA BLA→NAc projection rescues reward deficits following early life adversity

- Effect of ELA on the open field or swim stress tasks were not apparent
- Non-reward behaviors were not influenced by any manipulation

Inhibiting the CRH/GABA BLA → NAc projection in ELA, but not TR mice, increases reward behavior indicating a selective ELA-induced maladaptive plasticity of this reward-circuit pathway



Sup. Figure 4

Viral injection and cannula/fiber locations in the BLA and NAc

Fig. 3

ChR2 BLA, fiber NAc



TR – hM3Dq BLA, cannula NAc



TR – hM4Di BLA, cannula NAc

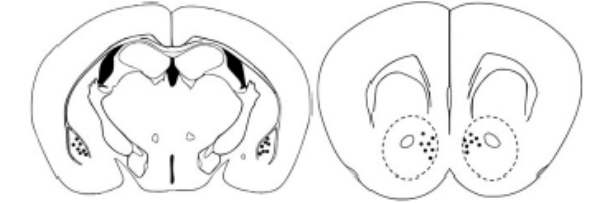


Fig. 4

ELA – hM4Di BLA, cannula NAc



ELA – hM3Dq BLA, cannula NAc



Suppl. Fig. 2

TR - EFYP BLA, fiber NAc



Suppl. Fig. 3

ELA - BLA; mCherry, hM4Di



Questions

- QCM en ligne en fin de semaine sur eCampus
- 24 questions : sur ce papier, sur méthodologie...
- 30 min pour répondre : parfois plusieurs réponses

Si questions sur ce papier :

Julie.carcaud@universite-paris-saclay.fr