Development Of Intersectional Chemogenetic Techniques To Isolate Circuitry Involved In Pain Affect

Carly Fabian[1,2]; Greg Elmer, PhD[1,2]; Paul Shephard, PhD[1,2]
[1]University of Maryland, School of Medicine , [2]Maryland Psychiatric Research Center

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Background

Chronic pain-related depression is hypothesized to result from hyperexcitability of the **lateral habenula (LHb).**

Glutamatergic projections of the LHb strongly innervate GABAergic neurons of the **rostromedial tegmental nucleus (RMTg)**, forming the LHb-RMTg neural circuit.

Intersectional Chemogenetic techniques allows for *in vivo* isolation and transient manipulation of neurons within the LHb-RMTg pathway, and aids in the testing of specific hypotheses.

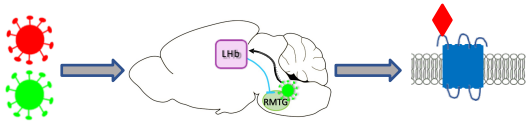


Figure 1. AAVs containing either Cre-dependent DREADD or Cre injected into LHb and RMTg. Following injection, DREADD (mCherry) is expressed in neural membrane at site of injection.

Manipulation achieved by **designer receptor proteins (DREADDs),** mutant muscarinic receptors that are expressed in neural tissue through Adeno-associated viruses (AAVs).

LHb-RMTg circuit can be explicitly targeted using Cre-dependent DREADD in combination with retrograde AAVs containing Cre recombinase.

Methods and Materials

Dual Viral Vector strategy provides specific neural expression, where Cre-dependent DREADD only expressed in cells where Cre is active.

- Successful Intersectional DREADD expression is realized when **mCherry** is expressed in the LHb

Stereotaxic Surgery:

Phase 1: (n=25) OPTIMIZATION
- Fluorochrome Injection into LHb or RMTg

Phase 2: (n=10) DREADD in LHB
- AAV8 encoding excitatory DREADD (hM3Dq-mCherry) injected into LHb

Phase 3: (n=15) DUAL VIRAL VECTOR
- Retrograde AAV2 encoding Cre-recombinase (eGFP) injected into RMTG
- AAV8 expressing Cre-dependent DREADD (hM3Dq-mCherry) in LHb

Histology/ Immunohistochemistry:
- Rats were perfused between 21-30 days post-surgery using an 8-10% formalin solution.
- Brains were removed and sliced at 40-50 µm thickness on the vibratome.
- Sections were then stained with DAPI, mounted on slides, and covered with a fluorescence gel

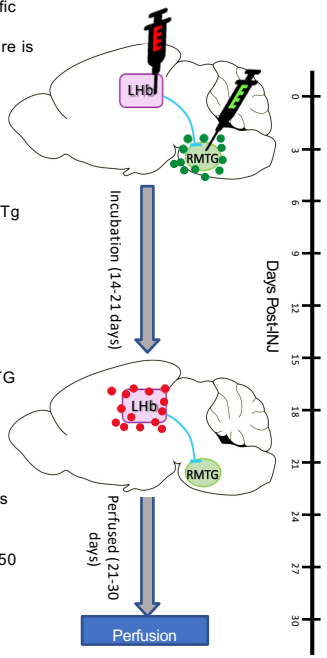


Figure 2. Timeline of the Dual Viral Vector strategy. Animals received injection of retrograde Cre-containing AAV2 (eGFP) in the RMTg, followed by injection of AAV8 encoding Cre-dependent DREADD (hM3Dq-mCherry) into LHb. Following surgery, rats incubate for 14 to 21 days to allow for transport and expression of DREADDs. Perfusion occurred 21-30 days post-injection.

Research Aims

Optimize expression of excitatory DREADDs (hM3Dq-mCherry) in LHb neurons

Inject anterograde and retrograde viral vectors (AAVs) into the LHb and RMTg, to limit expression of excitatory DREADDs to Cre-expressing neurons only.

Determine if Cre-dependent expression of excitatory DREADDs persists in animal models 21-30 days post-injection

Results

Phase 1: (n=25) Optimization

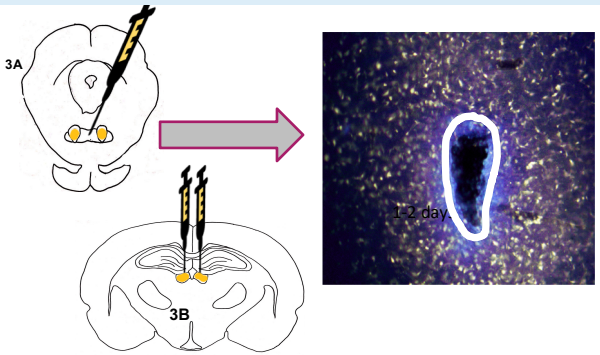
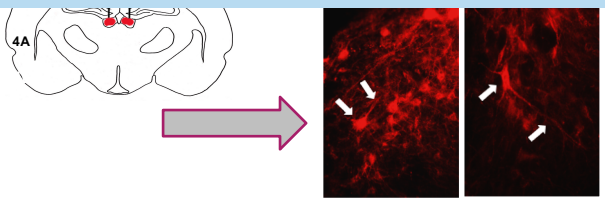


Figure 3: (A/B) Fluorochrome central injection spread into both L/R RMTg and bilateral injection into LHb. **Figure 3C.** Injection site in LHb with surrounding neurons expressing gold fluorochrome (fluorochrome 1-2 days following injection.

Phase 2 : (n=10) Excitatory DREADD expression in LHb



Group 3 (n=15) Dual Viral Vector Strategy

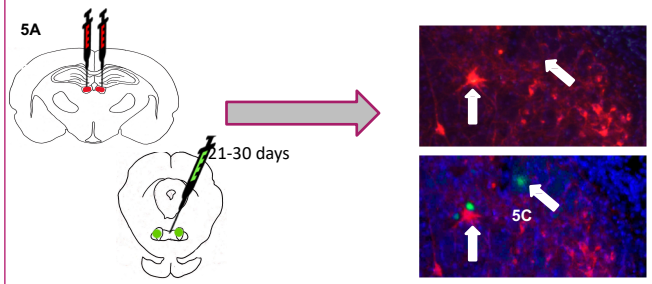
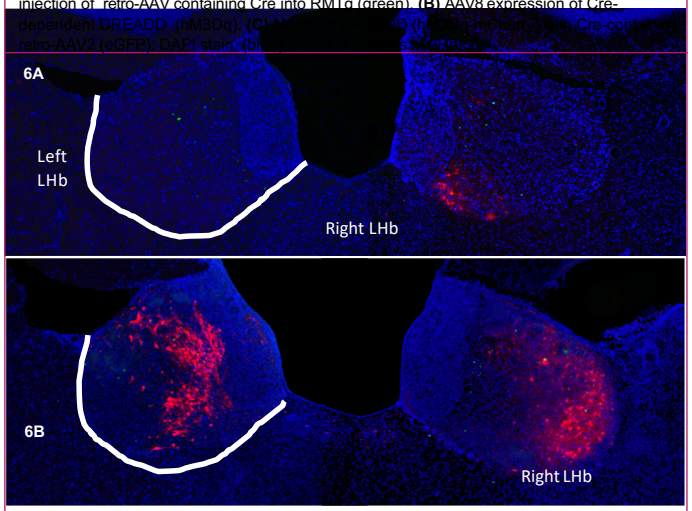


Figure 5: (A) Bilateral injection of Cre-dependent DREADDs into L/R LHb (red); central injection of retro-AAV containing Cre into RMTg (green). **(B)** AAV8 expression of Cre-dependent DREADD (hM3Dq) (C
retro-AAV2 (eGFP) DAPI stain (Bl
Cre-con



Conclusions

- Dual viral vector strategy allowed for successful targeted isolation of neuronal connections between the LHb and its projections into the RMTg
- The combined use of a Cre-dependent DREADD and retrograde AAV containing Cre provided cell-specific isolation of the LHb-RMTg circuit

Future Directions

- Optimize eGFP expression of retrograde virus encoding Cre transgenes
- Transiently activate designer receptors (DREADDS) using Clozapine N. Oxide
  - Confirm activation of DREADDs via cFos expression in RMTg