Mapping the Connectivity of an Olfactory Brain Region, the Dorsal Tenia Tecta, with ABBA

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The Dorsal Tenia Tecta (DTT)

- The dorsal tenia tecta (DTT) is a distinct region that extends from the anterior olfactory nucleus to the genu of the corpus callosum.
- There has been considerable debtate regarding whether the DTT is an extension of the hippocampal formation or is part of the primary olfactory cortex. However, the DTT differs from the hippocampal formation in that it is directly and reciprocally connected with the olfactory bulb.
- Prior work has demonstrated a link DTT signaling and psychosocial stress in rats[4].
- Although prior research has identified specific connection in the rat, a complete connectivity analysis has yet to be performed in any species.

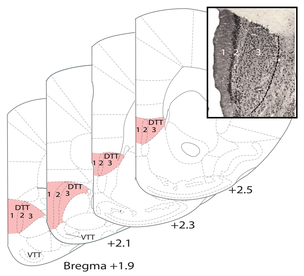

Figure 1: Coronal sections showing the location of the DTT and ventral tenia tecta (VTT). Figure from Paxinos and Franklin (2019) and LaPlante (2013).

Research Goal

To assist in the quantification of the afferent and efferent connections of the DTT.

Tracer Injection into the DTT of Mice

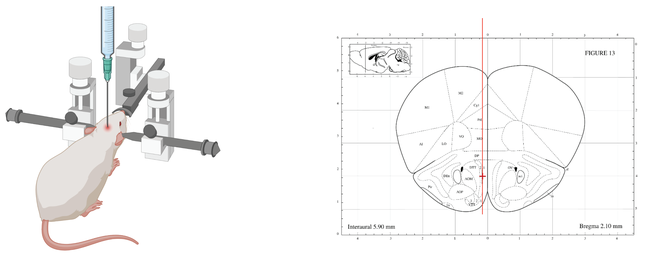

Figure 2: An AAV viral tracer (pAAV.CB7.CI.eGFP.WPRE.rBG) or cholera toxin subunit B (CTB) was injected into the DTT of a C57Bl6/J mouse

Transcardial Perfusions and Brain Collection

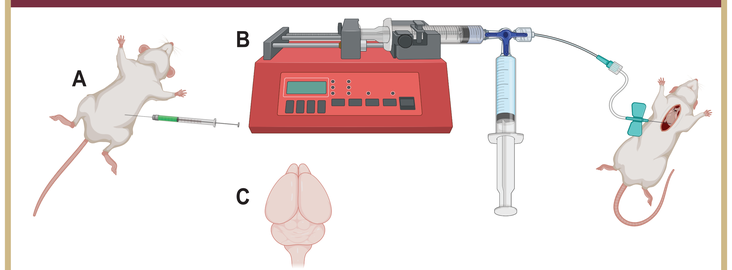

Figure 3: After surgery, mice were allowed to recovered for one - three weeks to allow for optimal expression of the tracer. (A) Mice were then injected with a Somnasol Euthanasia solution, (B) then were transcardially perfused via a syringe pump. (C) The brain was removed and post-fixed overnight. Brains were sunsequently rinsed in PBS and cyroprotected in prepartion for embedding. The tissue was embedded in Tissue Tek optimal cutting temperature (OCT) compound then stored in an -80° C freezer prior to cryostat microsectioning.

Sectioning

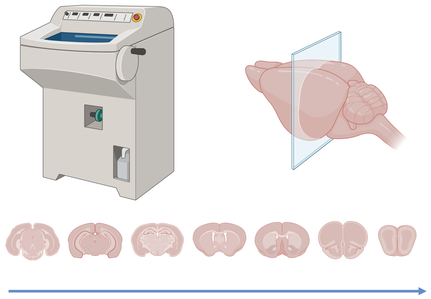

Figure 4: Brains were serially sectioned on a cryostat in 20 µm coronal sections. The resulting slides were stored in the -80° C freezer prior to microscope imaging.

Imaging

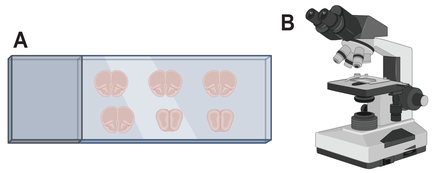

Figure 5: (A) (A) Slides were removed from the -80° C freezer, thawed, counterstained with a fluorescent Nissl Stain (Neurotrace). (B) Slides were imaged using an epifluorescent Zeiss microscope. Using the 10x and 20x objective lenses, images of each section were taken using three different color channels.

ABBA and QuPath Image Processing

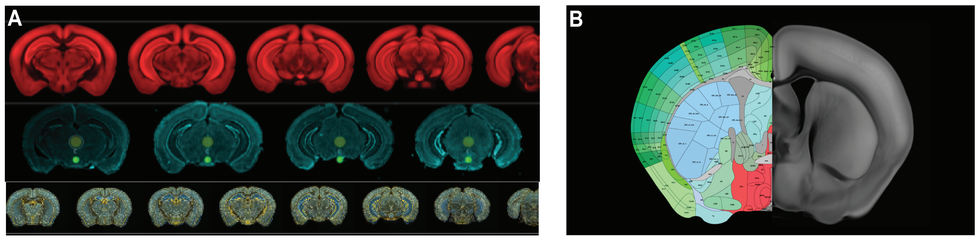

Figure 6: (A) Slide images were registered to the Allen Brain Atlas using ABBA (Aligning Big Brains and Atlases) software. The registered brain sections were imported into QuPath, an imaging software used to analyze the presence and density of fibers in each brain region. (B) Once slide images were aligned in ABBA and processed in QuPath, the exact brain region containing expressing specific fluorescence from the AAV viral tracer was able to be identified (Image from Yongsoo Kim lab, Penn State University).

Preliminary Summary of DTT Connections

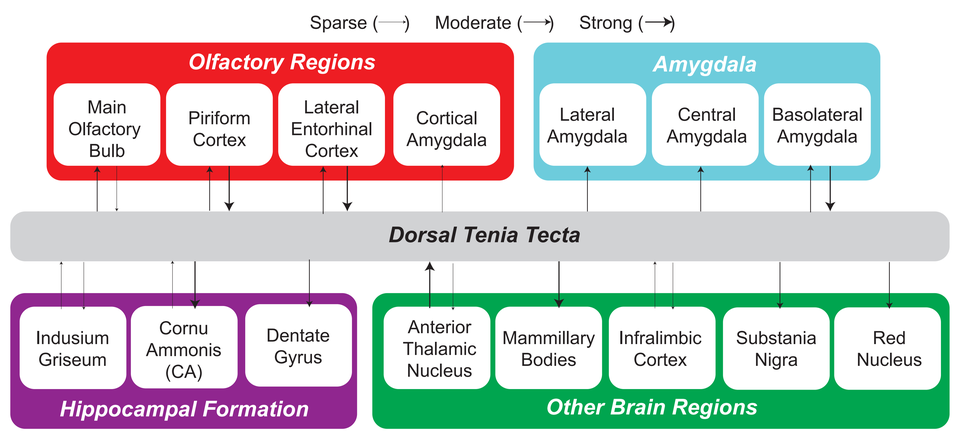

Figure 7: Preliminary summary of the major inputs and outputs of the dorsal tenia tecta (DTT).

Preliminary Results

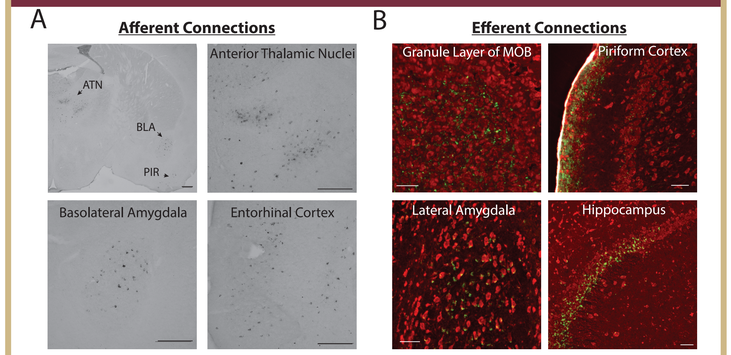

Figure 8: Afferent and efferent connections with the dorsal tenia tecta. (A) Afferent connections are shown with Cholera toxin subunit B (CTB) retrograde tracer. Labeled cell bodies in the anterior thalamic nuclei (ATN), basloateral amygdala (BLA), piriform cortex cortex (PID), and entorinal cortex. Scale bar = 250 um. (B) Efferent connections demonstrated with AAV viral retrograde tracer (green) and NeuroTrace Nissl counterstain (red). (C) Sections were aligned and registered to the Allen Brain Atlas using ABBA. (D) Efferent fiber densities were measured in each section. Plots are mean +/- stdev (N=1).

Summary

- Preliminary analyses indicate that this enigmatic brain region is interconnected with regions involved in stress, memory, and olfactory processing.
- Our preliminary results using a biotin-conjugated CTB tracer (N=1) have yielded afferent connections that are similar to those in rats. Key differences include the absence of the supramammillary nucleus of the hypothalamus and the addition of prominent inputs from the basolateral amygdala and hippocampus as well as a much sparser connection with the median raphe nucleus
- The efferent connections of the DTT (N=1) were also similar to rats. Key differences include the addition of prominent connections with several hippocampal regions, the basolateral amygdala, lateral entorhinal cortex, cortical amygdala, paraventricular nucleus of the thalamus, substania nigra, infralimbic cortex, and red nucleus.

Acknowledgements

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