Investigating the Dorsal Tenia Tecta - an underexplored region of primary olfactory cortex

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Introduction

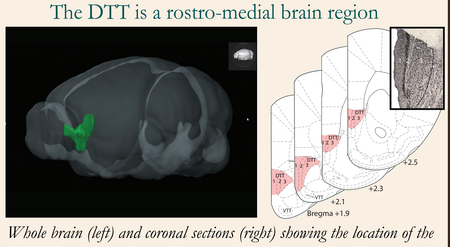
The dorsal tentia tecta (DTT) is a rostro-medial brain region that receives direct input from the main olfactory bulb. Despite its classification as part of the primary olfactory cortex, prior studies have hinted that this region is anatomically and functionally distinct from other olfactory cortical areas. Specifically, the DTT shares many cytoarchitectural similarities with the hippocampus and is distinct from the ventral tenia tecta, another primary olfactory cortical region. The function of this region remains unknown. Prior research have determined that the DTT is odor responsive (Cousens, 2020) and is part of a circuit that mediates psychosocial stress in rodents. The current study will utilize ma-chine learning techniques to investigate the contribution of this region for mouse behavior.

Research Goal

Investigate the contribution of the DTT for mouse behavior

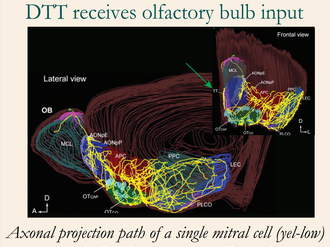
Dorsal Tenia Tecta

The DTT is a rostro-medial brain region



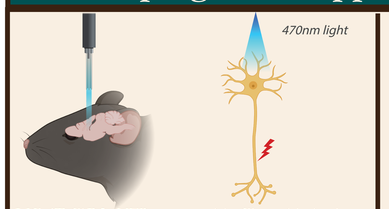
Whole brain (left) and coronal sections (right) showing the location of the DTT. Figures from Brain Explorer (Allen Brain Atlas), Paxinos and Franklin (2019), and LaPlante (2013).

DTT receives olfactory bulb input

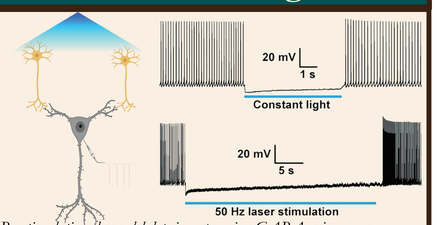


Axonal projection path of a single mitral cell (yel-low) innervating the DTT (green arrow). Figure from Igarashi et al., 2012.

An Optogenetic Approach to Inactivating DTT

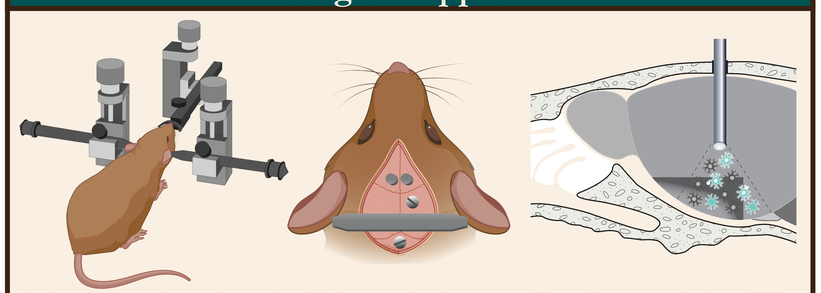


VGAT-ChR2-eYFP mouse model - Channelrhodsopin (ChR2, a light-gated sodium channel) is expressed from GAB-Aergic neurons via the mouse vesicular GABA transporter, VGAT. A yellow fluorescent protein (eYFP) is coexpressed to visualize these neurons.



By stimulating channelrhdopsin expressing GABAergic neurons within the DTT using 470nm blue light, the neurons are depolarized and release the inhibitory neurotransmitter GABA which inactivates connected neurons. Data from Zhao et al., 2011

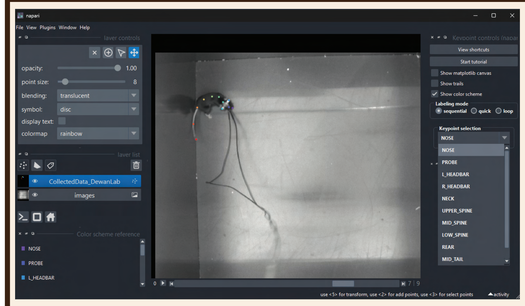
Surgical Approach



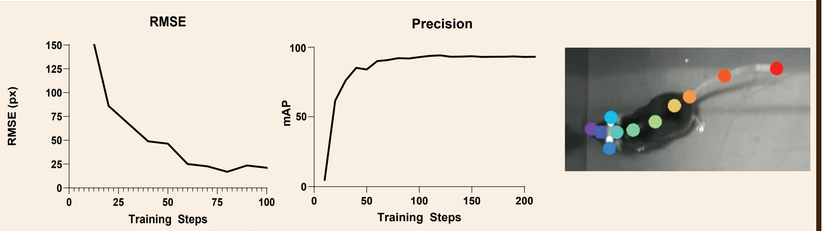
Anesthetized mice are administered an analgesic before being secured within a stereotax. Two small craniotomies directly over DTT is acheived using the Neurostart Robotic Stereotaxic Device. A fiber optic implant is slowly inserted into both the left and right DTT (DV 3.65 from Bregma, inter-implant distance 500 um) until it reaches its target position. A headbar is affixed to the skull with the addition of several microscrews.

Experimental Paradigm and DeepLabCut Analysis

The animal is head-fixed on a wheel and fiber-optic patch cables are attached to the optical probes. The mouse is then allowed to roam freely move around a large plexiglass chamber. The animal will experience 60 10-second trials. Each trial consists of a 4-second pre-stimulus period, a 2-second stimulation period (2.5W continous stimulation), and a 4-second post-stimulus period.

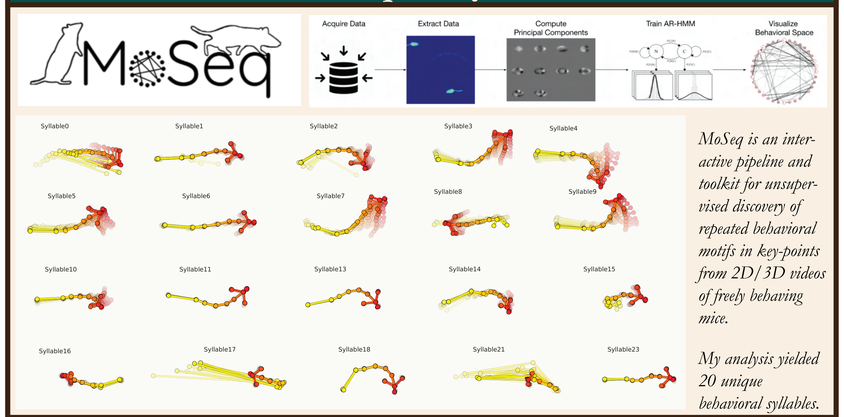


DeepLabCut (DLC) is an open-source marker-less prose-estimation software package that utilizes convolutional neural networks and machine-learning to analyze videos and output coordinates tracking various user-defined points on labaratory animals. DLC allowed us to quantify the position of specific body parts in an automated, high throughput manner. We labeled ~1000 frames from multiple non-experimental videos to iteratively train the model to extract positional keypoints (such as the nose, headbars, neck, tail, etc.) from experimental videos.



The root mean square error (RMSE) decreased as we trained the model, which indicates that the model gets more accurate and is better able to identify and track body parts with each training step. The downward trend indicates that the model is progressively improving with training. The precision also increases with training, indicating that the model is highly reliable and able to detect body parts accurately across multiple frames.

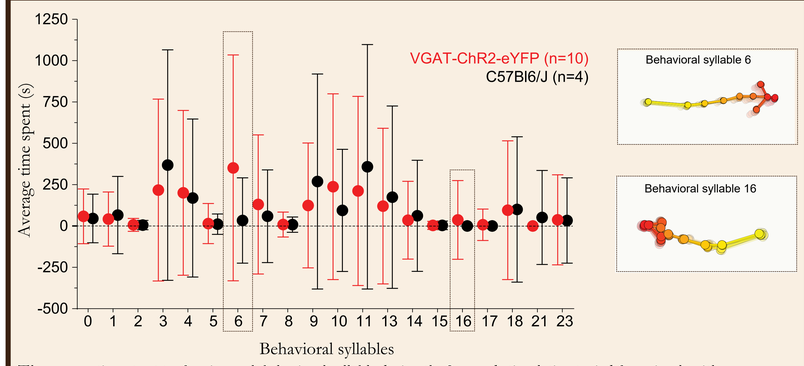
MoSeq Analysis



MoSeq is an interactive pipeline and toolkit for unsupervised discovery of repeated behavioral motifs in key-points from 2D/3D videos of freely behaving mice.

My analysis yielded 20 unique behavioral syllables.

Results



The average time spent performing each behavioral syllable during the 2 second stimulation period for animals with channelrhodopsin (VGAT-ChR2-eFYP) or wild-type controls (C57Bl6/J). Behavioral syllables 6 and 16 trended towards a statistically significant difference resulting from the inactivation of the DTT.

Conclusions

- VGAT-ChR2-eFYP and wild-type (WT) mice were run in an open-field investigation paradigm with periodic optogentic inactivation of the DTT.
- I trained a DeepLabCut model to track 11 key points as the animal roamed freely in the open-field.
- The model was able to accurately track the mouse position throughout the experiment even with the patch cables attached to the fiber optic cable.
- The output from the DeepLabCut model was fed into keypoint MOSEQ to identify and categorize behaviors.
- As a first level of analysis, I limited the data to periods of stimulation for both the VGAT-ChR2-eFYP and WT mice and the twenty most frequent behaviors.
  Comparing this data, I found that the total time spent for behavioral syllable 6 and 16, although not statistically significant, displayed an interesting trend. VGAT-ChR2-eFYP mice performed these behavioral syllables, on average, more often.
- Behavioral syllables 6 and 16 looked similar to olfactory investigation / sniffing behavior - perhaps indicating that the inactivation of this region influences their olfactory system.

Improvements and Next Steps

- Improve the model accuract with targeted refined and additional training.
- Excluding the three tail keypoints may improve the keypoint-MOSEQ model since tracking methods have lower reliability with the tail.
- Due to variability in animal behavior, a larger sample size can be used to make the results more generalizable.
- While the inactivation of the DTT did not significantly influence freely moving behavior, we will conduct further testing to ascertain if it affect odor behavior or sniffing behavior in mice.
- Specifically, we will test whether the inactivation of the DTT influences olfactory perception utilizes the labs's head-fixed operant conditioning approach.

References

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