TAP TO GO BACK TO KIOSK MENU

Stability of Inhibitory Circuits in the Olfactory Bulb

Jared Cohn OMS-II and Gonzalo Otazu, Ph.D
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Introduction

Sensory gating is the process of identifying important information while filtering out supplementary stimuli from the environment. Multiple chronic conditions including schizophrenia and autism spectrum disorder (ASD) are characterized by sensory deficits, specifically in regards to sensory gating (Adler et al.) (Orekhova et al.). For instance, individuals who have schizophrenia have exhibited auditory sensory gating deficiencies, while individuals with ASD have demonstrated auditory, olfactory and visual sensory gating deficits (Sokhazde et al) (Sinclair et al) (Scott et al). It has been proposed that sensory gating may be regulated by inhibitory interneuron circuits of the central nervous system (Cromwell et al 2008). This phenomenon may explain the sensory gating limitations that patients with schizophrenia and autism experience, as dysfunctional inhibitory neuron circuitry have been implicated in both conditions (Inan et al.) (Jung et al). In order to understand the role of inhibitory interneurons in sensory gating, and consequently gain further insight into the above neurological conditions, we studied the stability of inhibitory circuits of the olfactory bulb using a rodent model.

Background

The specific inhibitory pathway that was analyzed is a Dopaminergic/GABAergic pathway located in the olfactory bulb; the specific inhibitory neurons are known as short axon cells. The sequence of the olfactory/inhibitory pathway is as follows. An odor is detected by olfactory sensory neurons (OSNs), located in the nasal epithelia, and the signal is transmitted to the olfactory glomeruli (Woo et al.). The olfactory glomeruli consequently produce an activation pattern that is specific for the odor (ibid). The signal produced by the glomeruli is then propagated by the mitral and tufted cells to the olfactory cortex for processing (ibid). In addition, the signals carried by the mitral and tufted cells are mediated by short axon cells (ibid). The short axon cells receive input from one olfactory glomerulus and consequently relays to Extra tufted (ET cells) and ultimately suppress the activity of the mitral and tufted cells (Banerjee et al). While it has been postulated that the purpose of the inhibitory short axon cells is to decorrelate the signals carried by the mitral and tufted cells to facilitate the process of odor identification, the purpose of the short axon cell inhibitory network still remains unknown (ibid).

Research Question

Previous studies have demonstrated, in neurotypical mice, that the silencing of the short axon cells via optogenetics still leads to suppression of the activity of the mitral and tufted cells, however the phenomenon behind this finding has yet to be understood (Banerjee et al). Nevertheless, this outcome suggests that the inhibitory network may be highly regulated and conserved. Thus the focus of this project was to examine the activity of this pathway, when stimulated by different odors at different time intervals in order to evaluate the stability of the short axon cells.

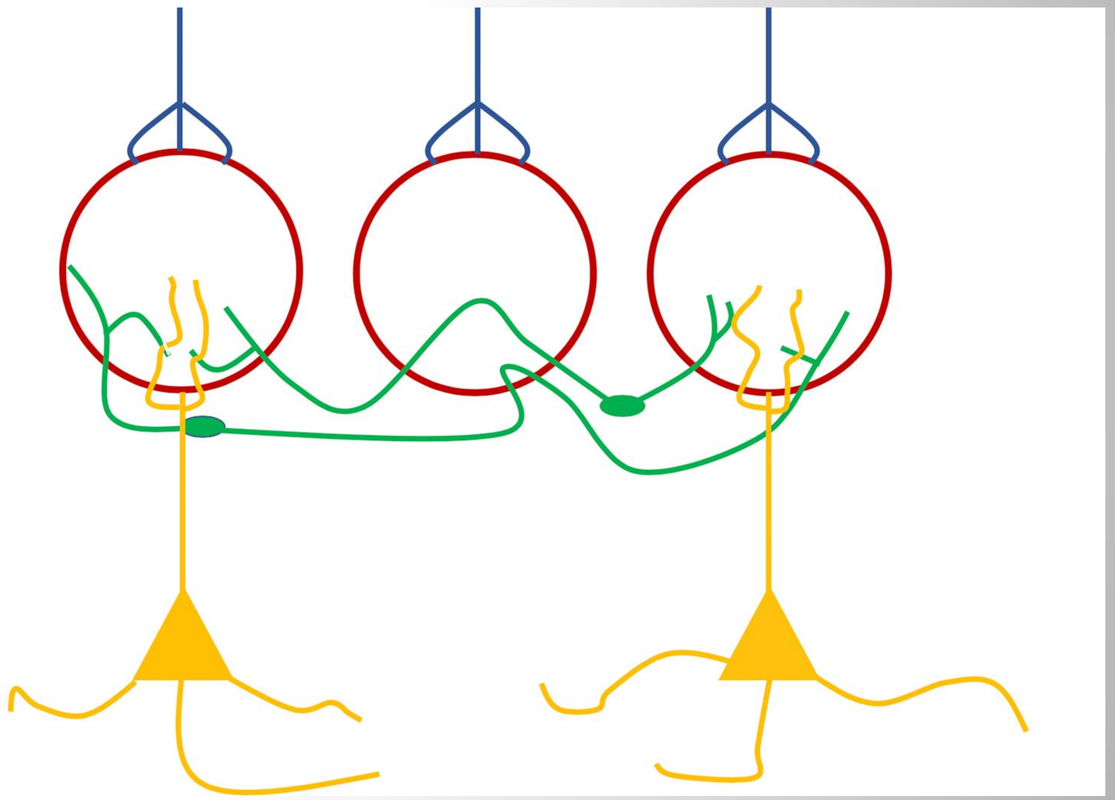

Figure 1: This figure depicts the neural pathway of olfactory sensation. The olfactory sensory neurons (blue) synapse onto the olfactory glomeruli (red) generating a pattern of activation specific to an odor. The glomeruli then transmit this information to the mitral/tufted cells (yellow) and the short axon cells (green). The short axon cells then synapse onto mitral/tufted cells that synapse with other glomeruli in order to inhibit their firing rate.

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Methods

- The experiment involved using four mice with the genetic cross of Dat-Cre/Ai95 to enable the visualization of short axon cell activity
- To analyze whether short axon cell activity would remain consistent when exposed to different odors, the mice were presented with eight different odors individually: limonene, isopropyl butyrate, ethyl valerate, isoamyl acetate, ethyl butyrate, propyl butyrate, ethyl propionate and isobutyl propionate at the day 1 and day 28 imaging sessions.
- Furthermore the mice were also presented with odor combinations. The red mice were presented with a strong odor (ethyl butyrate) paired with each target odors (ethyl propionate, propyl butyrate, isobutyl propionate and isopropyl butyrate). Concurrently, the blue mice were presented with a target odor paired with a weak odor (limonene). The odor combinations were presented for one hour each day for 5 days, between the two imaging session intervals, to further examine the stability of the network
- On the first day of the experiment, glomerular activity was tracked through shining a 780nm light to the mouse's olfactory bulb to illuminate the region. The mouse was then exposed to an odor to stimulate the glomeruli and the activity was recorded.
- To analyze the conservation of short axon cell activity across odors and time, the cellular activity was recorded on day 1 and day 28 of the experiment through the following procedure: First, 480nm light was used to track the activity of the short axon cells: Next, the mouse was presented with an odor to stimulate the glomeruli and, consequently, the short axon cells. Then the stimulation of the short axon cells would open calcium channels, so extracellular calcium would flow into the cell. Finally, calcium would bind to calmodulin to stimulate Gcamp6, which would produce a 509nm beam of light and the response was recorded.

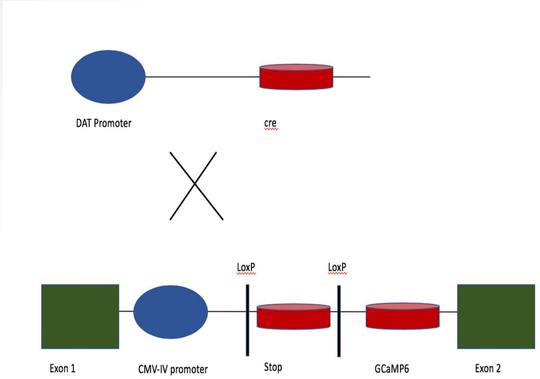

Figure 2: This figure represents the genetic cross between the DAT-Cre and Ai95 mice. The purpose of producing the cross was to visualize the activity of the short axon cells

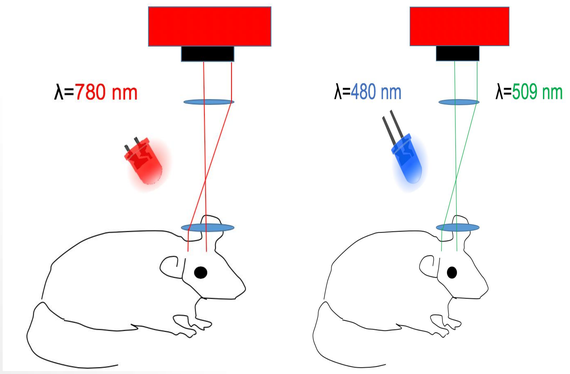

Figure 3: This figure represents the process of recording glomerular and short axel cell activity using the Dat-Cre/Ai95 mouse model. The mouse on the left represents the process of recording glomerular activity while the mouse on the right represents the recording of the short axon cell activity.

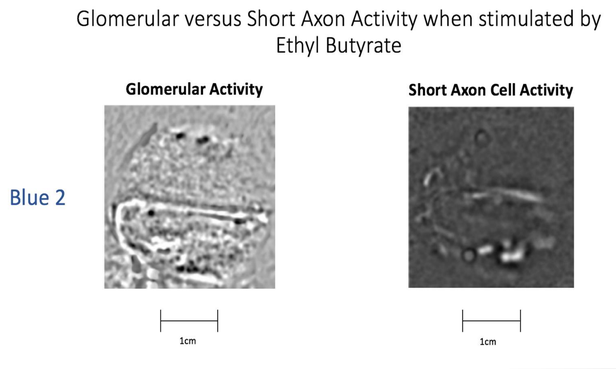

Figure 4: This figure represents a comparison of the activity of the short axon cells versus the activity of olfactory glomeruli when stimulated by ethyl butyrate. Glomerular activity was recorded with intrinsic imaging while short axon cell activity was recorded through fluorescent imaging. (n=1)

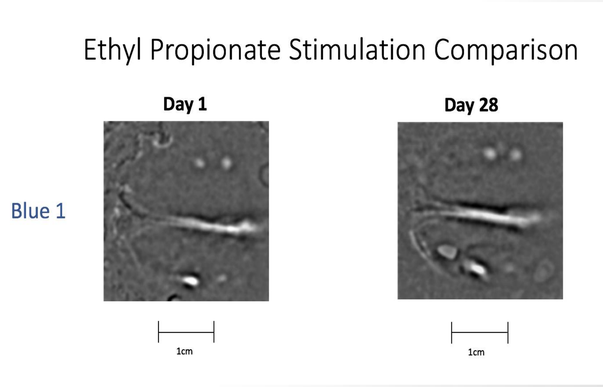

Figure 5: This figure represents a temporal comparison of the activity of the short axon cells of the olfactory bulb when exposed to ethyl propionate. The image on the left represents the short axon cell response on day 1 while the image on the right represents the short axon cell response on day 28. (n=1)

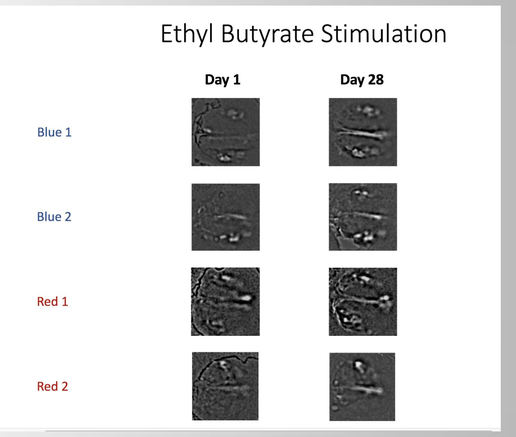

Figure 6: This figure represents a temporal comparison of short cell activity when stimulated by ethyl butyrate. The images on the left represent the short axon cell response on day 1 while the images on the right represent the short axon cell response on day 28 (N=4).

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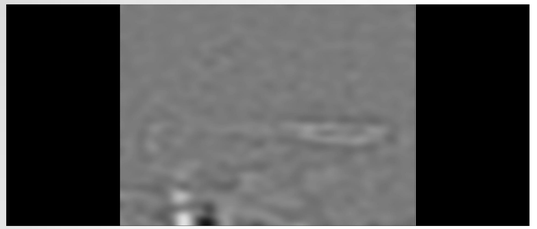
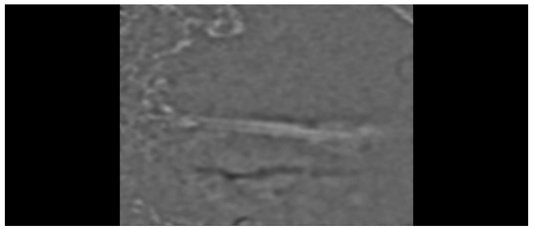
Data Analysis

- Odor responses were calculated by dividing the average fluorescence during odor presentation by the average fluorescence preceding odor presentation.
- The signals were further denoised by calculating a z score average image and that image was used to draw ROIs of the olfactory glomeruli using image J.
- The first purpose of drawing ROIs was to identify and match corresponding glomeruli between the intrinsic and fluorescent imaging sessions in order to compare glomerular and short axon cell activity (see figure 7).
- The second purpose of drawing ROIs was to match the glomeruli between the fluorescent imaging sessions of Day 1 and Day 28 in order to compare the short axon cell activity between the two sessions.



Movie I: This movie represents an intrinsic imaging session capturing glomerular activity in an awake DAT-Cre mouse when stimulated by an odor (ethyl butyrate).



Movie II: This movie represents a fluorescent imaging session capturing the short axon cell activity in an awake DAT-Cre mouse when stimulated by an odor (ethyl butyrate).

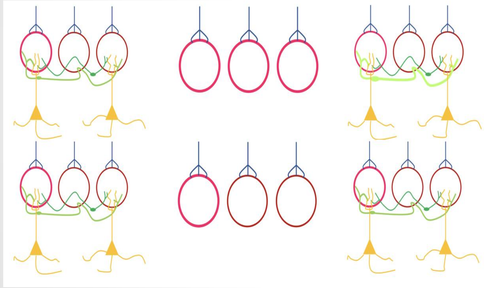


Figure 10: This cartoon represents the short axon cell activity of a target odor when paired with a strong odor (top) and when paired with a weak odor (bottom). In this figure, the glomeruli are represented by the red circles while the short axon cells are represented by the green lines; the strength of activation of the glomeruli and short axon cells are denoted by the brightness and width of the correspond shapes. When a target odor is paired with a weak odor, the short axon cells show no difference in the strength of activation on day 28 when compared to Day 1. However, when a target odor is paired with a strong odor, the activity of the short axon cells increases on day 28 when compared to the activity on day 1.

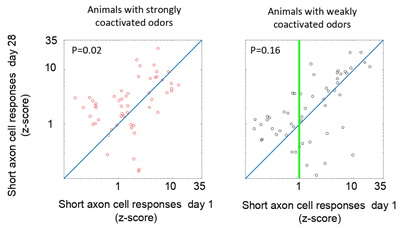


Figure 9: This figure represents the effects of short axon cell activity when pairing a target odor with a strong odor and when pairing a target odor with a weak odor. The results indicate that the pairing of a weak odor with a target odor had no effect on the magnitude of short axon cell activity. This figure also demonstrates that when a target odor is paired with a strong odor and presented to the mouse that the short axon cell activity increases on day 28, if the glomerular and short axon cell were originally weakly activated ($Z < 1$) ($p < .05$).

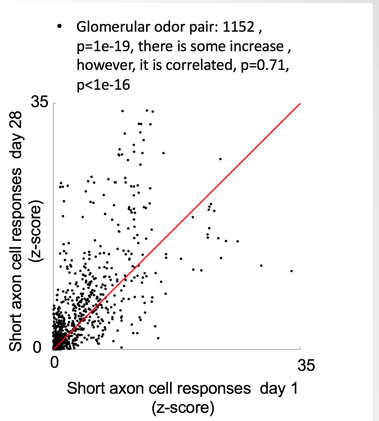


Figure 8: This figure demonstrates a comparison of the strength of short axon cell activity on Day 1 (x axis) and Day 28 (y axis) of the experiment. The correlation between short axon cell activity from the two imaging sessions was strong ($R=.71$, $p > 10^{-16}$). This correlation suggests that the short axon cell network exhibits general temporal stability. This figure also suggests that short axon cells that exhibited a strong activation pattern on Day 1 also exhibited a strong activation pattern on day 28. However, some short axon cells that produced a weaker signal on the first day, generated a stronger signal on day 28, which may be explained by the results of figure 9.

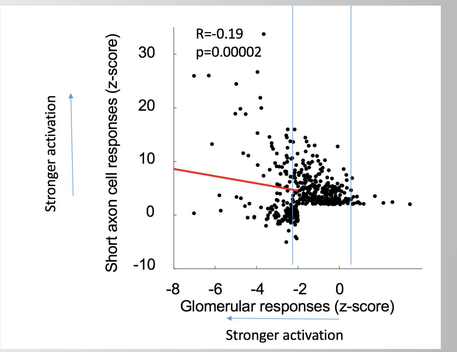


Figure 7: This figure represents the relationship between glomerular and short axon cell activity. This figure indicates that glomerular stimulation, via the olfactory sensory pathway, is necessary for short axon cells to become activated and exhibit their inhibitory activity on mitral and tufted cells.

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Discussion

The results of the experiment indicate that short axon cells require glomerular stimulation in order to become activated. The study also demonstrated that the short axon cells express temporal stability when odors are either presented alone, or when paired with a weak odor such as limonene. Lastly the experiment revealed that the strength of the short axon cells may be potentiated if an odor is paired with a strong odor and continuously stimulated. This potentiation may be mediated by either the short axon cells themselves, by the extra input from external tufted (ET cells) or by cortical feedback that regulates the short axon cells. We believe that the increase in short axon cell activity weakens the output of other glomeruli through inhibiting the activity of the mitral cells that synapse with other glomeruli. One potential role for this plasticity is that the short axon cells that synapse to the glomeruli sensing the target odor become stronger so they can indirectly downregulate the activity of the glomeruli sensing the strong background odors via the inhibition of the mitral and tufted cells. Hence, we believe this inhibition is vital for the central nervous system to be able to recognize a weaker target odor in the presence of stronger background odors and to facilitate the discrimination between them. Deficits in this circuit may explain the olfactory sensory gating deficiencies that individuals with ASD experience, specifically with odor discrimination (Sinclair et al).

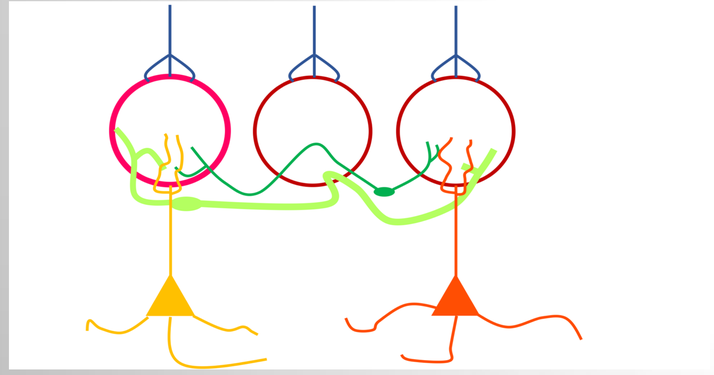

Figure 11: This figure shows the potential effect of short axon cell potentiation. In this figure the increased activity of the short axon cell can potentially mitigate the signal carried by the mitral cell (synapsing to a different glomerulus) which may play a role in recognizing a weaker odor in the presence of a stronger one and facilitate in their discrimination.

Future Directions

One prospective series of future experiment would be to study the mechanism behind the potentiation of the short axon cell activity when an odor is paired with a strong odor. One potential method to study the effect would be to use optogenetics to shut down the piriform cortex and ultimately inhibit cortical feedback to the olfactory bulb in order to determine whether the short axon cell potentiation is mediated by cortical feedback.

Another possible experiment would be to study short axon cell activity in phenotypic mouse models of autism and schizophrenia and determine whether their short axon cells exhibit similar patterns of stability and plasticity to the DAT-Cre/Ai95 mouse model. This project would allow us to gain further insight into the biological basis of the previously mentioned disorders and may prove to be a vital step in developing treatments for autism and schizophrenia.

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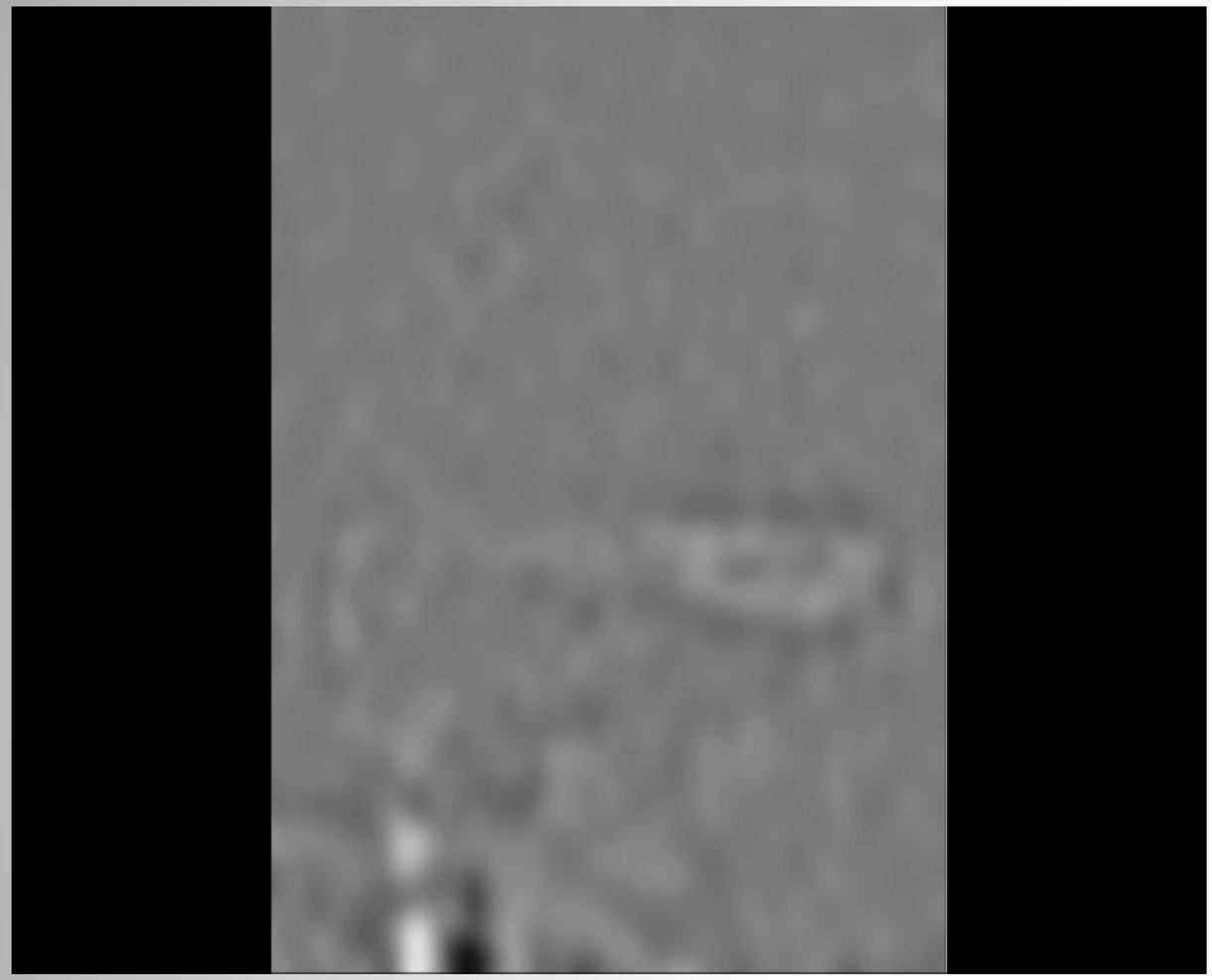

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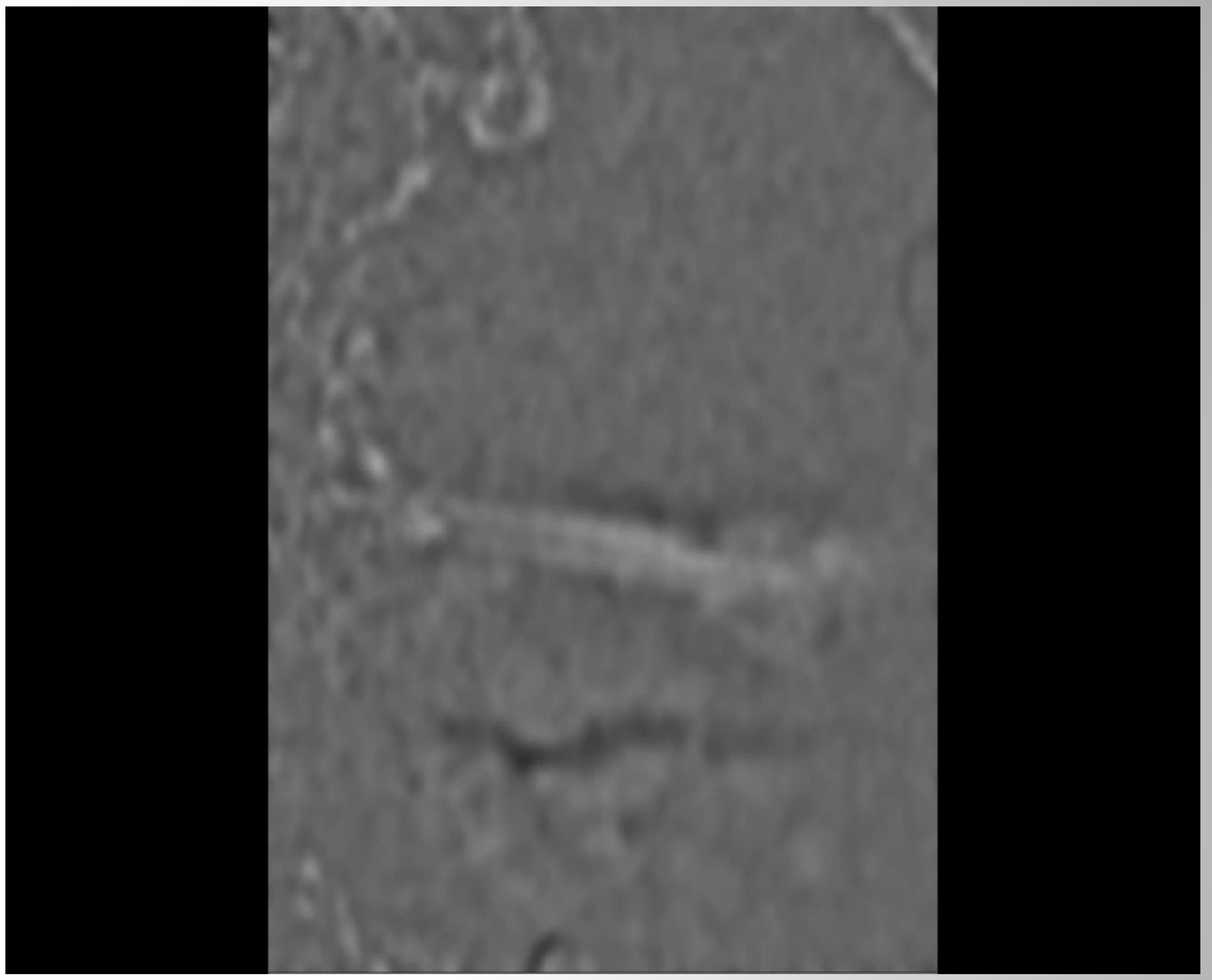
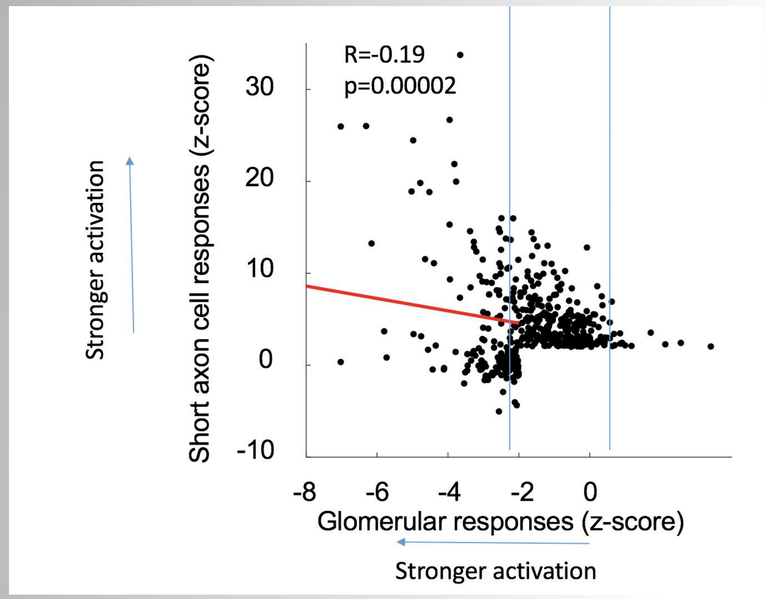


Movie II: This movie represents a fluorescent imaging session capturing the short axon cell activity in an awake DAT-Cre mouse when stimulated by an odor (ethyl butyrate)



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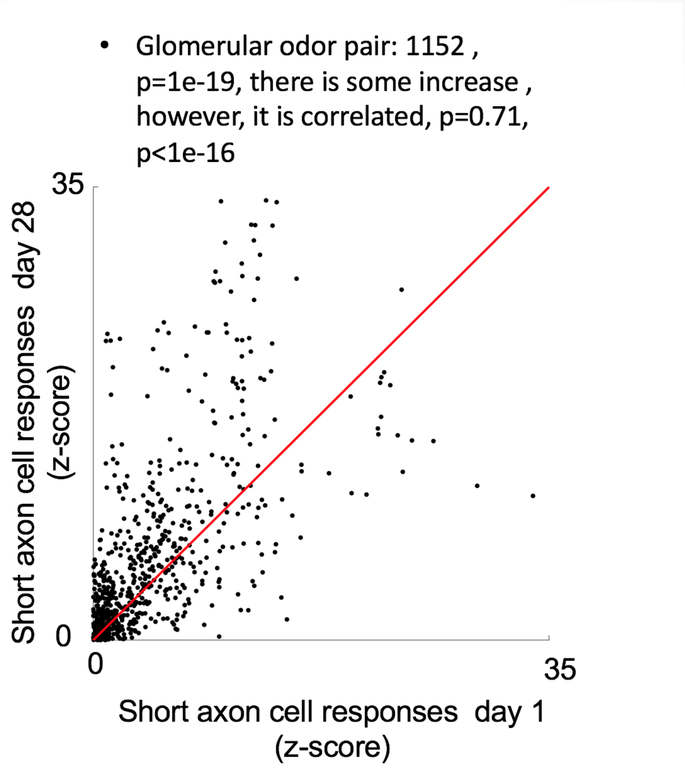

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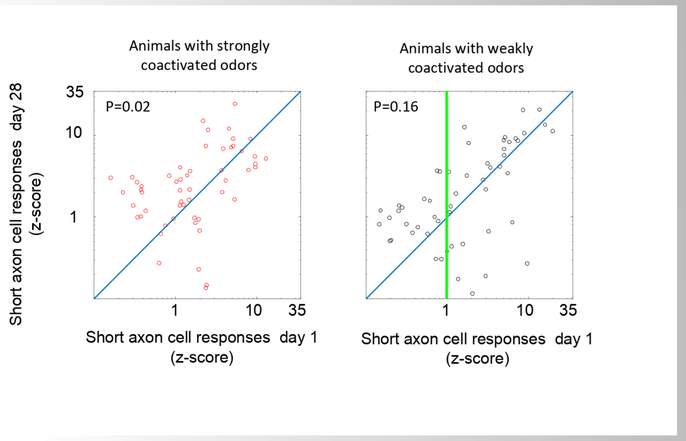

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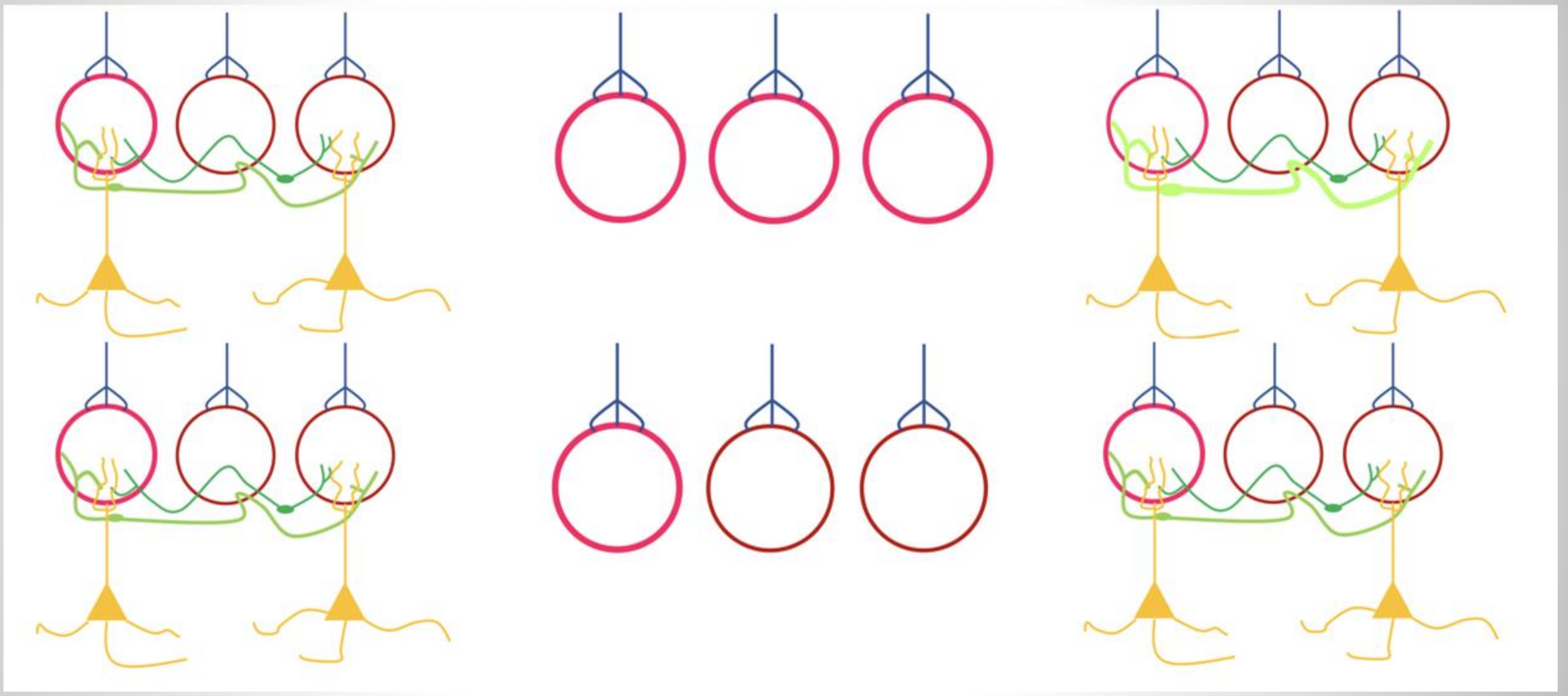

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