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Circuit Mechanism of D1/D2 Cells in the Temporal Association Cortex for Auditory Perceptual Decision-Making

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Abstract: The temporal association cortex (TeA) is a higher-order node in the auditory system, involved in processing complex sound and steering behavior. Although dopamine transmission, specifically mediated by D1 and D2 receptors, is well understood to influence decision-making in subcortical structures such as the VTA, its role within the TeA is generally unclear. The goal of the present study was to examine the requirements for D1/D2 receptor-mediated pathways within the TeA for a task of auditory discrimination. We conditioned mice on a task wherein they had to decide depending on frequency in sound. We then utilized a mix of pharmacological intervention with ketamine, in vivo fiber photometry to assess dopamine dynamics, and post-mortem c-Fos immunofluorescence to investigate neural activation. Our results revealed three major findings. First, behavioral analysis showed that ketamine administration severely disrupted the ability of the mice to discriminate as evidenced by a flattening of the psychometric performance curve. Second, immunofluorescence imaging showed that both the TeA and the linked Ventral Tegmental Area (VTA) were strongly activated when the task was performed. Finally, real-time recordings showed that ketamine displaced the temporal profile of dopamine release in the TeA upon the sound stimulus to render a clear, stimulus-locked peak into a flattened and disorganized, multi-peaked profile. All together, these findings demonstrate that the normal functioning of dopamine circuits within the TeA is not merely associated with, but a necessity for, accurate auditory-guided decision-making. Interference with these signals is definitive proof that the coordinated, balanced activity through D1 and D2 receptors within the TeA is a necessity for this process.

1. Introduction

Sensitive sensory perception is a fundamental prerequisite for survival, enabling animals to rapidly detect environmental changes, assess the significance of sensory cues, and make adaptive decisions. Through continuous monitoring of the external world, sensory systems allow organisms to avoid predators, locate food, and navigate complex social interactions essential for reproduction and survival. However, perceiving sensory stimuli is only the first step. Transforming these inputs

into context-appropriate behavioral responses requires the integration of perception with memory, emotion, and executive control, engaging distributed neural networks across cortical and subcortical regions.

In the auditory system, the TeA plays a pivotal role in the higher-order processing of sound information. The auditory pathway begins in the cochlea, where sound waves are transduced into neural signals by mechanosensory hair cells. These signals travel via the auditory nerve to brainstem nuclei, then ascend to the inferior colliculus and subsequently to the medial geniculate nucleus (MGN) of the thalamus. From the MGN, information is relayed to the primary auditory cortex (A1), which encodes basic acoustic parameters such as frequency, intensity, and temporal structure. While A1 processing is indispensable, it does not, on its own, produce meaningful behavioral responses. For that, incoming auditory information must be integrated with past experiences, contextual cues, and motivational states—processes in which the TeA is critically involved.

The TeA functions as a hub for auditory perception–decision transformation. It compares current sound inputs with previously stored auditory representations, evaluates their salience and emotional valence, and assigns them behavioral relevance. For example, a sound previously associated with danger may trigger defensive behaviors, whereas the same sound in a safe context may be ignored. This evaluative process is essential for perceptual decision-making, where the brain must weigh sensory evidence against internal goals and decide on appropriate action. The TeA accomplishes this by interacting with memory-related regions such as the hippocampus, emotion-related regions such as the amygdala, and executive areas such as the prefrontal cortex, thereby bridging sensory analysis and behavioral execution.

Dopamine is a crucial neuromodulator influencing both subcortical and cortical processing. It exerts its effects primarily via D1-type and D2-type dopamine receptors, which are expressed by distinct neuronal populations[1]. In the basal ganglia, D1-expressing neurons form the direct pathway, projecting monosynaptically to the output nuclei of the basal ganglia (GPi/SNr). When activated, these neurons inhibit GPi/SNr neurons, thereby disinhibiting the thalamus and facilitating movement initiation and cognitive engagement. In contrast, D2-expressing neurons form the indirect pathway, which projects polysynaptically through the external segment of the globus pallidus and the subthalamic nucleus before reaching GPi/SNr[2]. Activation of D2 neurons increases GPi/SNr activity, enhancing thalamic inhibition and suppressing action initiation. These opposing roles create a push–pull mechanism for fine-tuning motor output, cognitive flexibility, and decision thresholds. While the basal ganglia circuitry of D1 and D2 neurons is well established, their function in associative cortical regions such as the TeA is largely unknown. Evidence from prefrontal cortex studies suggests that D1 and D2 cortical neurons may also participate in distinct microcircuits, differentially regulating excitation–inhibition (E/I) balance and influencing local information flow. In the TeA, D1/D2-expressing neurons could, in theory, serve analogous but context-specific roles—modulating the integration of auditory evidence, adjusting decision thresholds, and biasing choice behavior under conditions of sensory uncertainty. However, the circuit-level mechanisms underlying such modulation remain uncharacterized.

One plausible hypothesis is that TeA D1 neurons facilitate the selection of behaviorally relevant auditory cues by enhancing cortico-cortical and cortico-subcortical signaling toward motor and executive regions, thereby promoting decisive action when sensory evidence strongly supports a choice. Conversely, TeA D2 neurons may serve to suppress premature or irrelevant responses by strengthening inhibitory control, either locally within TeA microcircuits or through long-range projections to inhibitory hubs. The dynamic interplay between these populations could provide the neural substrate for flexible, context-dependent auditory decision-making. Investigating these mechanisms requires mapping the input–output connectivity of D1 and D2 neurons in the TeA,

monitoring their activity patterns during auditory discrimination tasks, and testing the causal impact of selective activation or inhibition on behavioral performance. Modern tools such as cell-type-specific viral tracing, optogenetics, and in vivo calcium imaging now make it possible to dissect these microcircuits with unprecedented precision. For example, retrograde viral tracers can identify whether TeA D1 and D2 neurons preferentially receive input from auditory, limbic, or dopaminergic sources, while optogenetic manipulations can test their causal influence on choice accuracy and reaction times.

The present study is designed to address this knowledge gap by systematically characterizing the circuit mechanisms of TeA D1/D2 neurons in auditory perceptual decision-making. Specifically, we aim to identify the anatomical projections and synaptic partners of TeA D1 and D2 neurons; record their functional responses during behaviorally relevant auditory tasks; and determine how selective modulation of each cell type alters decision outcomes. Understanding these pathways will shed light on how dopaminergic signaling shapes higher-order sensory processing and may reveal novel principles of cortical circuit organization relevant to both healthy perception and disorders of auditory cognition, such as hallucinations in schizophrenia[3].

2. Methods

2.1 Experimental design

In this study, mouse were behaviorally trained and able to perform correct actions corresponding to auditory stimuli. To investigate the circuit mechanism of D1 and D2 neurons in the temporal association cortex, the experiment was separated into two steps. Firstly, we inhibited the d1 and d2 neurons of the mouse that had normal auditory ability to observe the changes of dopamine levels. The behavioral changes after the prohibition were observed. Then we inhibited d1 and d2 separately, the changes and effects were observed. After establishing a baseline in normal mouse, we start to inhibit the d1 and d2 neurons in mouse that had hallucination-like behavior. Inhibit d1 and d2 simultaneously and then separately, observe the effects and whether the hallucinatory-like behaviors was improved.

2.2 Mouse behavioral training

The auditory decision tasks of the mouse were performed in a custom-designed transparent box which was put within a sound-attenuating enclosure. Three ports were positioned on the side walls of the chamber with infrared sensors and detectors. A water tube positioned in the centre of the box was used to deliver water as rewards when the mouse performed correctly. The auditory stimuli were delivered through the speakers (ES1,Tucker-Davis Technologies) positioned inside the enclosure. An infrared camera was positioned at the top of the enclosure for monitoring the behavior of the mouse. The experiment was controlled by Bpod system, an open source behavioral control system base on MATLAB. Before the behavioral training, water was prohibited for mouse for 24 hours. The experiment started with the illumination of the light in the centre port for cuing the mouse to touch the port. Then the speaker could play a pure sound of a low of high frequency randomly after a 50ms delay after the mouse touched the port. Mouse were trained to associate low-frequency tones (<10khz) with the left port and high-frequency tones (>10khz) with the right port. If mouse perform correctly by touching the corresponding port, 2.5μm of water would be delivered through the tube as a reward. An incorrect choice would result in a mild punishment of white noise.

Generally, mouse were able to master the behavioral training within four weeks. The training protocol consisted of three main stages. Stage 1: Two frequencies, 5000 Hz and 20000

Hz, were presented to mice. Wrong choices were not punished. Stage 2: White noise was applied as punishment for wrong choices in order to facilitate learning. Stage 3: The complexity was increased by adding three low-frequency tones, three high-frequency tones, and a boundary tone of 10000 Hz. Mice were programmed to poke at the left port for low tones and the right port for high tones. During the tone 10000 Hz presentation, mice chose the right port with about 50% probability.

2.3 Stereotactic Injection

Stereotactic Injection in Mouse BrainGlass micropipettes (inner diameter: 0.3 or 0.5 mm) were pulled using a laser-based micropipette puller to achieve a tip diameter of approximately 10-13 μm and a neck length of 5-10 cm. The micropipette was mounted onto the arm of a stereotaxic apparatus, and a sufficient volume of viral solution was aspirated for a single injection. Mice were induced with 5% isoflurane anesthesia prior to surgery and subsequently mounted on the stereotaxic apparatus, where anesthesia was maintained with 0.5% isoflurane during surgery. Erythromycin ophthalmic ointment was placed on the eyes to prevent drying of the cornea. The scalp was prepared with an iodine solution and incised along the midline for access to the skull.The target of injection was the Temporal Association Cortex (TeA), which was located at the following coordinates: Anteroposterior (AP): -3.4 mm from bregma; Mediolateral (ML): ±4.7 mm from the midline; and Dorsoventral (DV): -1.15 mm from the surface of the cortex. A craniotomy was performed over the targeted region to expose the dura mater. The virus was then injected into the TeA at a very controlled rate of 1 nL/s through a micro-syringe pump.[4] At the end of injection, the micropipette was left in place for 20 minutes to facilitate adequate diffusion of the virus before slow withdrawal. Mice were maintained on a heating pad after surgery to maintain body temperature until anesthetic recovery. Animals were singly housed and closely monitored for a minimum two-week recovery period to allow sufficient viral expression for subsequent experiments.

2.4 Mouse Brain Frozen Sectioning

After finishing our experiments, we had to scan the brain of the mouse to see if the drugs that we had injected and the optical fibers that we had implanted were correctly positioned. We also had to cut the brain into extremely thin slices so we could monitor the spread of some of these unique substances throughout the brain. To start, we anesthetized the mouse. Then, in order to preserve the brain tissue, we "rinsed" its circulation system through its heart. We started by flushing through with a saline solution (PBS) all of the blood, and then with a liquid called PFA, which fixes the brain tissue and supports its shape, so it is a delight to work with. After we pulled out the brain, we stuck it in the fridge to soak up even more of this fixative solution for a day to ensure it was hard all over. Then there was the anti-freeze part. We put the brain in a very sweet sucrose sugar water solution until it had absorbed enough liquid and sunk to the bottom. This prevents ice crystals from shattering the brain when we freeze it. And the final step was slicing. We placed the brain into a special gel (OCT), froze it solid into a block, and then into a super-freezing slicing machine (imagine a deli slicer in a deep freezer). We sliced it through to convert the brain into very, very thin slices, only 45 micrometers thick (thinner than human hair). We then stacked these slices in three batches for storage purposes for future study.

2.5 Immunofluorescence

For immunofluorescence examination, brain sections were first brought to room temperature and washed three times in Phosphate-Buffered Saline (PBS) for 5 minutes for each washing to remove the Optimal Cutting Temperature (OCT) compound. For tissue permeabilization, sections were

maintained in PBS with 0.5% Triton X-100 (PBST) for 30 minutes. Non-specific binding was prevented by incubating the sections in PBST with 5% Bovine Serum Albumin (BSA) for 1 hour at room temperature. Subsequently, the sections were incubated overnight at 4℃ using primary antibodies diluted accordingly in PBST with 1% BSA. The primary antibodies applied here were: rabbit anti-c-Fos (1:5000), rabbit anti-CaMKII (1:400), rabbit anti-Parvalbumin (PV, 1:500), and rabbit anti-Somatostatin (SOM, 1:500)[5]. Sections were washed three times in PBST on the following day and incubated in the respective Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (dilution 1:1000) for 2 hours at room temperature under protected light. Sections were counterstained using DAPI (4′,6-diamidino-2-phenylindole, dilution 1:5000) for 10 minutes for staining the nucleus. Antibodies were washed three times with PBS and mounted on glass slides and coverslipped in an anti-fade mounting medium. Slides were stored in the dark at 4℃ until imaging following preparation.

3. Results

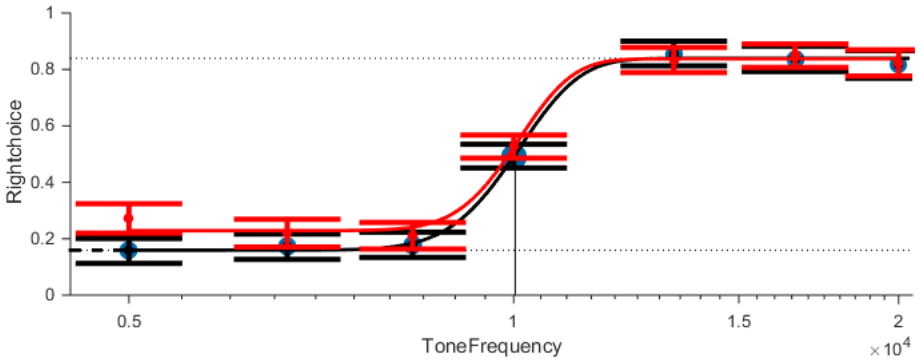

Figure 1 Mouse behavioral training result

Figure 1 illustrates the effect of ketamine on auditory discrimination performance in a single mouse. The test was conducted under two conditions so that performance could be compared. The x-axis indicates the frequency of the auditory tone in Hertz (Hz). The y-axis indicates the probability of the mouse making a 'right choice'. In the control session (black curve), where the mouse received a saline injection, the animal performed accurately. Performance was a steep, sigmoidal function, indicating that there was a sharp boundary between frequencies with a well-defined decision point around 10 kHz. After ketamine was administered to the same mouse (red curve), its discriminative ability was severely disrupted. The psychometric curve was considerably shallower, and the distinction between the low-and high-frequency plateaus was less pronounced. This comparison makes it quite evident that ketamine administration significantly interferes with the sensory discrimination ability of this mouse, flattening its psychometric response curve.

Figure 2 is a representative micrograph of a coronal section of the mouse brain that was subjected to auditory discrimination training. The section had been immunofluorescence treated to mark areas of the brain functionally engaged in the task. In the image, neuronal activation is indicated by c-Fos protein expression (green fluorescence), with all the cell nuclei counterstained using DAPI (blue). There is a dense concentration of c-Fos-positive cells prominently visible in the Temporal Association Area and adjacent auditory cortices, as indicated by the intense green color of the cortices. Intense expression indicates that the TeA was strongly activated over the course of the behavioral task, especially compared to basal activity levels within the other cortical areas. Added to this strong cortical activation, there is also a strong, but less intense, cluster of c-Fos

expression located in the VTA. This pattern of co-activation clearly suggests that a functional cortico-midbrain loop, consisting of both the TeA and VTA, was active and employed while performing this learned, choice behavior.

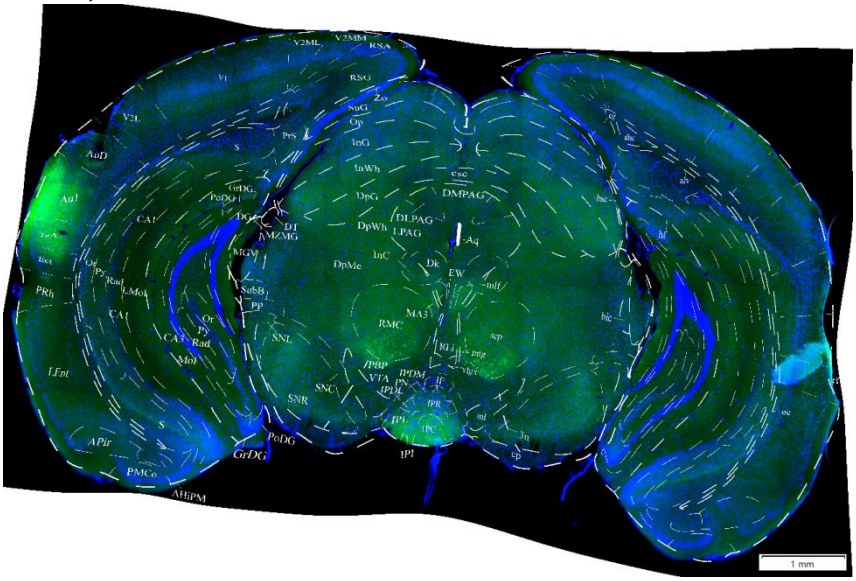

Figure 2 The immunofluorescence micrograph of TeA

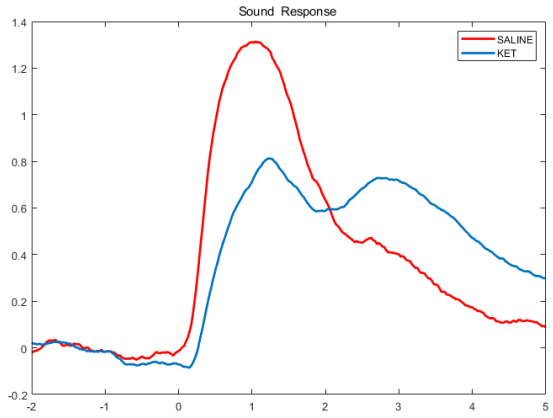

Figure 3 Dopamine release level of saline injection and ketamine injection

Figure 3 indicates the effect of an auditory stimulus on the calcium signal, a proxy for neural activity, in the Temporal Association Area (TeA). The test was conducted in one mouse under two conditions. The x-axis is time in seconds with an auditory stimulus from t=0 to t=1. The y-axis is calcium signal intensity.

Under the control condition (red line), where the mouse received a saline injection, the sound stimulus elicited a strong, steep increase in neural activity. This reached a sharp peak at t=1.1 seconds and then fell gradually. After the same mouse received ketamine (blue line), the neural response to the sound was substantially dampened. The calcium signal had a significantly lower amplitude and wider peak after t=2 seconds.

The controlled condition(red line) directly shows the correlation between the temporal association cortex and d1,d2 receptors. The same mouse received ketamine(blue line), shows the direct correlation between the temporal association cortex(TeA) with the d1, d2 receptors.

4. Conclusion

The dopaminergic receptors d1,d2 in TeA is working cooperatively in the auditory pathway. In my research, we investigate the function of neurons containing d1 and d2 cells in the temporal association area through comparing the effect of ketamine injection which influences auditory-decision ability. The experimental group was mice had been injected ketamine, while the control group had only injected saline. Figure 1 shows the mice were able to finish the behavioral training correctly in normal condition and the abnormal behavior with the injection of ketamine. This result indicates that ketamine will influence auditory discrimination and auditory decision. Figure 2 is the brain slice highlighting the area engage in the task during the behavioral training. The direct result of the image is the TeA is the most actively involved area, and the final result of the auditory pathway is related to the TeA. Then in Figure 3, the result of the controlled condition (red line) shows the direct reaction of dopamine secretion of TeA to the auditory stimuli for auditory decisions. The ketamine injection condition(blue line) shows the influence to the dopamine secretion indicates in the same pattern with the behavior disorder. We prove that there must be a relationship between TeA and d1,d2 receptors when there is auditory stimuli through proving the necessary relevance through destroying the ability of auditory decision by using ketamine. We notice the same pattern and the correlation between dopamine secretion and behavior under the effect of ketamine and we get the conclusion. Because of the time limit and other factors, we are not able to find out the specific role the d1,d2 play separately. This will be the next topic of further study.

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