The serotonergic psychedelic DOI impairs deviance detection in the auditory cortex

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Abstract

Psychedelics are known to induce profound perceptual distortions, yet the neural mechanisms underlying these effects, particularly within the auditory system, remain poorly understood. In this study, we investigated the effects of the psychedelic compound 2,5-Dimethoxy-4-iodoamphetamine (DOI), a serotonin 2A receptor agonist, on the activity of neurons in the auditory cortex of awake mice. We examined whether DOI administration alters sound-frequency tuning, variability in neural responses, and deviance detection (a neural process reflecting the balance between top-down and bottom-up processing). Our results show that while DOI does not alter the frequency selectivity of auditory cortical neurons in a consistent manner, it increases trial-by-trial variability in responses and consistently diminishes the neural distinction between expected (standard) and unexpected (oddball) stimuli. This reduction in deviance detection was primarily driven by a decrease in the response to oddball sounds, suggesting that DOI dampens the auditory cortex's sensitivity to unexpected events. These findings provide insights into how psychedelics disrupt sensory processing and shed light on the neural mechanisms underlying the altered perception of auditory stimuli observed in the psychedelic state.

Introduction

Psychedelics, a subclass of hallucinogenic psychoactive compounds, have long fascinated humanity by offering profound and often transformative experiences that alter perception, cognition, and mood (Shulgin and Shulgin, 1991, 1997). Additionally, there has been a recent resurgence in the use of psychedelics as therapeutic agents for the treatment of conditions such as depression, anxiety, and trauma-related disorders (Reiff et al., 2020; Mitchell and Anderson, 2024). These factors underscore the importance of understanding the neural mechanisms underlying the perceptual changes induced by these substances.

Serotonergic psychedelics primarily exert their effects through the serotonin 2A (5-HT2A) receptor (Nichols, 2016), leading to various perceptual distortions across sensory systems. For classical psychedelics, such as LSD and psilocybin, perceptual distortions are dominated by visual phenomena, such as vividly colored, constantly changing geometric figures that can evolve into complex images and scenes at medium to high doses. True hallucinations, defined as perceptions that feel real despite the absence of external stimuli, are less common than perceptual distortions but can occur at higher doses. While effects in auditory perception during the psychedelic state are generally reported to be less dominant than their visual counterparts, psychedelics can produce strong distortions in the passing of time and in the perception of sounds (Preller and Vollenweider, 2018; Aday et al., 2021; Wittmann et al., 2007), suggesting direct effects onto the auditory system.

Studies using human subjects provide invaluable information about the psychological effects of different classes of psychoactive compounds, yet a detailed understanding of the neural mechanisms underlying perceptual distortions requires the level of experimental access that animal models provide. Studies in animals have provided key insights into the molecular mechanisms of action of psychedelics (Kwan et al., 2022), however, our understanding of how psychedelics influence neuronal activity in the auditory system to produce these perceptual distortions remains limited. Several processes have been proposed for the distortions observed during the psychedelic state, including disruption of thalamocortical connectivity, altered cortical network dynamics, and impacts on the default-mode network (Vollenweider and Geyer, 2001; Carhart-Harris et al., 2014; Preller et al., 2019). A prevailing theory, compatible with the aforementioned mechanisms, suggests that psychedelics induce perceptual changes by altering the balance between top-down and bottom-up processing in the brain. For instance, researchers have proposed that psychedelics result in a relax of top-down control, leading to enhanced bottom-up sensory processing and novel perceptual experiences (Carhart-Harris and Friston, 2019).

Here, we use systemic administration of the psychedelic 2,5-Dimethoxy-4-iodoamphetamine (DOI) to evaluate the effects of the psychedelic state on the representation of sounds by auditory cortical neurons. We first test whether DOI affects the overall activity and/or selectivity to sound frequency of auditory cortical neurons. We then characterize the effects of DOI on a neural phenomenon that depends on the balance between bottom-up and top-down processes, namely that of deviance detection (Nelken, 2014). Our findings reveal that while DOI administration results in no net effect in frequency-tuning across the population of auditory cortical neurons, it consistently diminishes the response difference between expected and unexpected stimuli in most of these neurons.

Materials and methods

Animals

Four adult C57BL/6J mice (JAX 000664), two of each sex, were used in this study. At the time of electrophysiological recordings, three mice were 20 weeks old and one mouse was 28 weeks old. All procedures followed the National Institutes of Health animal care standards and were approved by the University of Oregon Animal Care and Use Committee.

Sound presentation

Experiments were performed inside a single-walled sound-isolation box (IAC Acoustics, North Aurora, IL). Auditory stimuli were presented in an open-field configuration from a speaker (MF1, Tucker-

Davis Technologies, Alachua, FL) contralateral to the side of electrophysiological recordings. Speakers were calibrated using an ultrasonic microphone (ANL-940-1, Med Associates, Inc., Fairfax, VT) to obtain the desired sound intensity level for frequencies between 1 kHz and 40 kHz. Stimuli were generated using Python software developed in-house (https://taskontrol.readthedocs.io/). During sound presentation, mice were awake and head-fixed on top of a freely-moving wheel, leaving them free to move their limbs while their heads remained stationary.

Three different ensembles of stimuli were used in our experiments: one for evaluating frequency tuning, and two different ensembles for evaluating deviance detection. To evaluate frequency tuning, we used pure-tone pips (100 ms duration) at 16 frequencies logarithmically-spaced between 2 kHz and 40 kHz at 70 dB SPL (with a 2 ms amplitude ramp up and ramp down) presented in random order. A session consisted of at least 20 repetitions per frequency with interstimulus intervals randomized in the range 1–1.4 seconds. For the first type of oddball paradigm, we used chords composed of 12 simultaneous pure tones (50 dB SPL each) logarithmically spaced in the range $f/1.2$ to $f \times 1.2$, for a given center frequency f. Two center frequencies, 8 kHz and 13 kHz were used. The sequence consisted of sounds 50 ms long spaced by 500 ms of silence, where one frequency (the standard) was repeated nine to eleven times followed by a single presentation of the other frequency (the oddball) followed by another sequence of standards and so on. After the presentation of at least 50 oddball stimuli, the sequence was repeated, but exchanging which stimulus served as the standard. For the second type of oddball paradigm, we used frequency modulated (FM) sounds consisting of either an up-sweep between 8 and 13 kHz or a down-sweep between 13 and 8 kHz, at an intensity of 70 dB SPL. Sounds in the sequence were 100 ms long spaced by 500 ms silence, and followed a repetition rate similar to the one for chords (nine to eleven standards for each oddball). After the presentation of at least 50 oddball stimuli, the sequence was repeated, but exchanging which stimulus served as the standard.

Surgical procedure

Animals were anesthetized with isoflurane administered via a nose-cone while positioned on a stereotaxic apparatus. Mice were surgically implanted with a head-bar and a Neuropixels 1.0 probe in the auditory cortex (two mice on the left hemisphere and two mice on the right hemisphere), using a design based on van Daal et al. (2021). The craniotomy was centered 2.65 mm posterior to Bregma and 4.4 mm lateral to the midline. The probe was lowered 3 mm from the brain surface, and it spanned multiple subregions of the auditory cortex. All animals were monitored after surgery and recovered fully before electrophysiological experiments.

Electrophysiological recordings and administration of DOI

Electrical signals were collected using Neuropixels 1.0 probes (IMEC) via an NI PXIe-8381 acquisition module and the OpenEphys software (www.open-ephys.org). During the experiment, mice were head-fixed and allowed to run on top of a wheel. We performed electrophysiological recordings on five different days for each mouse. Each recording day consisted of multiple sessions before and after injections of reagents and while presenting the stimulus ensembles described above. We began with electrophysiological recordings before administering any reagents, during which we presented three stimulus ensembles to evaluate frequency tuning and deviance detection. We then injected a volume of 5 ml/kg of sterile saline (0.9% concentration) subcutaneously and waited 15 minutes before another set of recordings during the presentation of all three stimulus ensembles. Finally, we injected 5 ml/kg of 2,5-Dimethoxy-4-iodoamphetamine, (±)-DOI hydrochloride (Sigma-Aldrich, D101) at a concentration of 10 mg/kg, and waited another 15 minutes before the last set of recordings during the presentation of all three stimulus ensembles.

Estimation of locomotion state

The locomotion state of the animal (running *vs.* not-running) was estimated from video recordings during each session. Videos were collected with a USB infrared camera at 30 frames per second and processed using the software package FaceMap v0.2 (Syeda et al., 2024) to extract the average change in pixel intensity in the area containing the animal's paws. The trace of pixel intensity change was smoothed using a 10-frame square window, and the value at stimulus onset was thresholded to determine whether the animal was in a running or not-running state.

Analysis of neural data

Spiking activity of single units was isolated using the spike sorting package Kilosort v2.5 (Pachitariu et al., 2024) and manually curated using the software Phy (Rossant et al., 2016). The resulting data were analyzed using in-house software developed in Python (www.python.org). To ensure that differences in neural responses between conditions were not merely due to the passage of time in the rig, we focused all analysis on neurons for which the cell's firing, spontaneous or evoked, did not change more than 30% between the pre-injection and the saline conditions. Responsiveness to sounds was evaluated by calculating whether there was a statistically significant difference between evoked firing (throughout the duration of each sound) and baseline activity (-200 ms to 0 ms from sound onset), for any of the sounds presented, using a Wilcoxon signed-rank test with Bonferroni correction for multiple-comparisons. Evoked firing was evaluated in the period from 15 ms after stimulus onset to

15 ms after stimulus offset to account for the signal transmission delay from the ear to the auditory cortex.

To evaluate the pure-tone frequency selectivity of a neuron, a Gaussian function was fit to the spike counts in response to each frequency (using a logarithmic space for frequency). The best frequency (BF) of a neuron was defined as the peak of this Gaussian function. The width of tuning was defined as the full width at half maximum of the normalized Gaussian, estimated as 2.355σ, where σ is the standard deviation of the Gaussian. The maximum change in firing (Max Δ firing) was estimated as the difference between the Gaussian peak and the baseline firing rate.

To estimate differences between the responses to oddball and standard sounds, we defined an Oddball Enhancement Index, $\text{OEI} = (O-S)/(O+S)$, where O is the average response to the oddball and S is the average response to the standard. To quantify these effects of a specific reagent on the responses to either oddball or standard sounds, we defined a modulation index $\text{MI} = (\text{DOI} - \text{Sal})/(\text{DOI} + \text{Sal})$, where DOI and Sal are the average responses under DOI and Saline, respectively.

To estimate the trial-by-trial variability in neural responses, we calculated the Fano Factor for the responses to each pure tone for each neuron: $\text{FF} = \sigma^2/\mu$, where σ^2 is the variance of the evoked firing rate across trials and μ is the mean. We then calculated the average FF across stimuli for each neuron before comparing these values between saline and DOI conditions. To obtain reliable values of the FF when analyzing running and stationary trials separately, we included in the FF average only those stimuli with at least four trials in each running condition.

Statistical analysis

Non-parametric tests were used throughout the study to evaluate differences in neural responses under different conditions, as indicated in the main text. We used a Wilcoxon signed-rank test to determine whether neurons were responsive to a particular sound, treating the spike counts during baseline and during the stimulus as matched samples for each trial. We used a Wilcoxon signed-rank test to determine differences between Saline and DOI conditions, treating the average response of a neuron under each condition as matched samples.

Results

DOI decreased spontaneous and evoked firing in AC

To evaluate the effects of the hallucinogen 2,5-Dimethoxy-4-iodoamphetamine (DOI) on the representation of sounds by auditory cortical neurons, we recorded neural activity from awake head-fixed mice using Neuropixels probes while animals were presented with auditory stimuli before and after

subcutaneous injection of saline (as control) or 10 mg/kg of DOI. Each experimental session consisted of three periods: one before saline injection, one after saline injection but before DOI injection, and one after DOI injection (Fig. 1A). During each period, we presented pure tones and frequency-modulated sounds to evaluate the overall responsiveness, frequency tuning, and deviance detection for each recorded neuron. We recorded five experimental sessions from each of the four mice. For each animal, we confirmed that the dose used resulted in the head twitches expected from administration of psychedelics in rodents (Canal and Morgan, 2012).

We first tested the effect of DOI on the overall firing rate of each recorded neuron. To ensure any results would not be simply due to the passage of time during the experimental session, we focused our analysis on neurons that passed two criteria: (1) the cell's firing, spontaneous or evoked, did not change by more than 30% between the pre-injection and the saline conditions, and (2) the cell was responsive to pure tones during all three periods, with an evoked firing rate above five spikes per second.

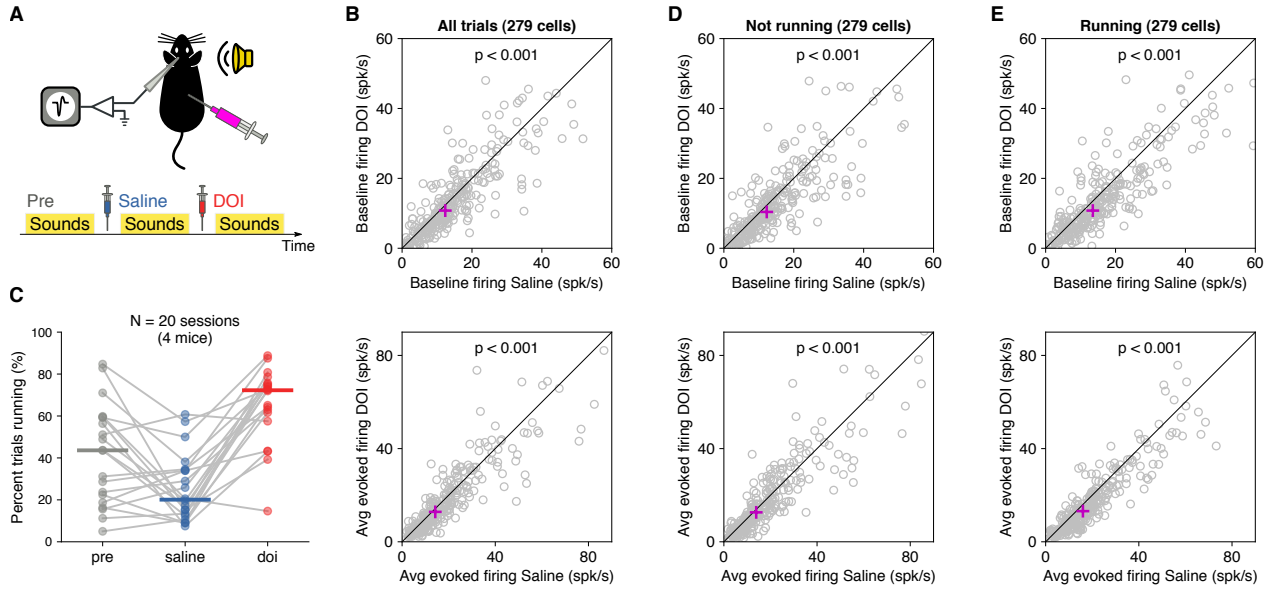

Figure 1: DOI decreased spontaneous and sound-evoked firing in the auditory cortex. (A) We recorded neural activity from the auditory cortex of awake, head-fixed mice during the presentation of auditory stimuli. Each experimental session consisted of three periods of sound presentation: one before saline injection, one after saline injection, and one after DOI injection. **(B)** Average spontaneous (top) and sound-evoked (bottom) neural activity decreased after DOI. Each dot represents one neuron. The magenta cross indicates the median value across neurons. **(C)** The percentage of trials animals spent running increased after DOI injection. Each dot represents one period for one animal, with periods from the same session connected by lines. **(D)** Same as (B) but including only trials where the animal was stationary. The decrease in spontaneous and sound-evoked neural activity remains apparent. **(E)** Same as (B) but including only trials where the animal was running. The decrease in spontaneous and sound-evoked neural activity remains apparent.

These strict criteria ensured that only cells that were stable during our recordings were included in the analysis. From the 1,590 isolated single neurons during our recordings, 279 passed these criteria. We found that, on average, the population of neurons showed a decrease in both baseline spontaneous firing (Fig. 1B top) and sound-evoked firing (Fig. 1B bottom) after DOI injection compared to the saline control period ($p < 10^{-5}$ for both spontaneous and sound-evoked conditions, Wilcoxon signed-rank test).

Because psychedelics like DOI can cause changes in locomotion (Halberstadt et al., 2009), and locomotion can alter sound-evoked responses in auditory cortical neurons (Schneider et al., 2014), we measured the effect of DOI on running behavior in the head-fixed animals. We found that after DOI injection, animals were more likely to run on the wheel, with median percentages of trials (across sessions) of 44% before injections, 20% after saline, and 72% after DOI. However, we found that the effects of DOI on auditory cortical neurons could not be simply explained by the increase in running during DOI trials, since similar results were obtained from analyses of both stationary (Fig. 1D) and running (Fig. 1E) trials ($p < 10^{-4}$ for all conditions tested, Wilcoxon signed-rank test).

DOI increased the trial-to-trial variability in neural responses

Sensory neurons typically exhibit some variability in their responses to repeated presentations of the same stimulus. To assess whether DOI influenced this inherent trial-to-trial variability, we measured the Fano Factor (FF) for responses to each pure tone presented before and after DOI administration. For each cell, we estimated an average FF across tone frequencies and compared this measure of response variability between the saline and DOI conditions. We found that the median FF increased from 0.81 under saline to 0.87 under DOI ($p < 10^{-6}$, Wilcoxon signed-rank test), with 65.2% of neurons exhibiting a higher FF under DOI compared to saline.

We next tested whether this increase in response variability was present during both running and stationary trials. Because animals occasionally had very few trials in a specific running condition during some sessions, we limited our analysis to stimuli with at least four trials in both running conditions. We found that the difference in response variability was clearly apparent during running trials, with the median FF increasing from 0.66 under saline to 0.79 under DOI ($p < 10^{6}$, Wilcoxon signed-rank test) and 65.9% of cells showing larger values under DOI compared to saline during running trials. In contrast, we found little difference in response variability during trials when animals were stationary, with median FF of 0.72 for both saline and DOI ($p = 0.54$, Wilcoxon signed-rank test) and about 47.7% of cells showing larger values under DOI compared to saline during not-running trials. These results suggest that while DOI increases the overall trial-by-trial variability of responses, these effects may vary depending on the state of the animal.

DOI did not change the frequency tuning of AC neurons in a consistent manner

We next tested whether DOI changed the frequency tuning of neurons in the auditory cortex. For each neuron, we estimated the responses to randomly presented pure tones (100 ms) of frequencies ranging from 2 kHz to 40 kHz. Consistent with the overall changes in firing quantified above, we found neurons that showed a decrease in evoked response to pure tones after DOI (Fig. 2A), neurons that had no change in firing across frequencies (Fig. 2B), and neurons that showed an increase in evoked responses after DOI (Fig. 2C). However, overall changes in frequency tuning curves seemed minimal. To quantify potential changes in frequency tuning, we fit a Gaussian curve to the evoked responses of each neuron and measured 3 parameters of this curve: (1) the best frequency (BF), (2) the width of tuning, and (3) the maximum change in firing evoked by the presentation of pure tones (Max Δ firing) (Fig. 2D). For this analysis, we focused on neurons that showed a positive responses to pure tones, and had a reasonably good Gaussian fit ($R^2 > 0.05$). A total of 197 neurons passed these criteria.

We found that while the estimated best frequency changed for several neurons after DOI, these effects did not appear to follow a consistent direction, resulting in both increases and decreases in BF

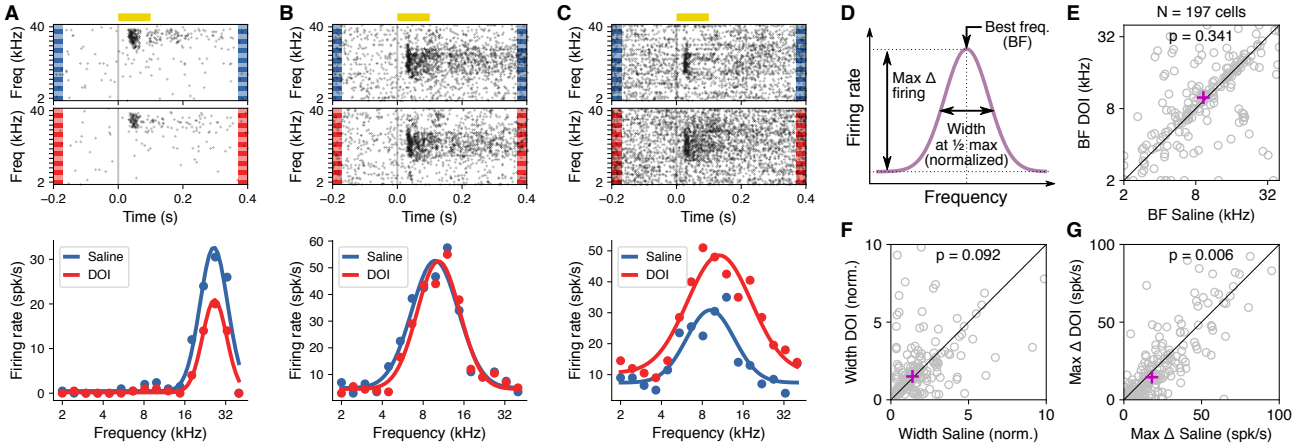

Figure 2: DOI did not change the frequency tuning of AC neurons in a consistent manner. (A) Frequency tuning for an example neuron under saline or DOI. Sound-evoked responses decrease under DOI, without a clear change in frequency tuning. **(B)** Example neuron showing no change in responsiveness or frequency tuning under DOI. **(C)** Example neuron showing an increase in sound-evoked responses under DOI, and minimal change in frequency tuning. **(D)** Illustration of the parameters of frequency tuning quantified from a Gaussian fit for each neuron: best frequency (BF), width of frequency tuning at half maximum normalized by Gaussian amplitude (Width), and maximum change in firing (Max Δ firing). **(E)** Best frequency for each neuron under DOI *vs.* saline. Magenta cross shows the median. There is no consistent change in best frequency. **(F)** Tuning width for each neuron under DOI *vs.* saline. There is no consistent change in tuning width. **(G)** Maximum change in firing evoked by sounds for each neuron under DOI *vs.* saline. There is a consistent decrease in the maximum firing after DOI.

across the population (Fig. 2E) with no change in the median across neurons ($p = 0.341$, Wilcoxon signed-rank test). Similarly, we found no difference in the median values of frequency-tuning width (Fig. 2F) between saline and DOI conditions ($p = 0.092$, Wilcoxon signed-rank test). Moreover, we found no correlation between the BF or tuning width for a neuron during saline and the change in evoked response magnitude after DOI ($r = 0.11, p = 0.13$ for BF; $r = 0.037, p = 0.61$ for tuning width, Pearson correlation). The only consistent change we observed when quantifying responses to pure tones was a decrease in the magnitude of the evoked responses after DOI, measured as the height of the Gaussian fit (Fig. 2G). The median change across neurons was statistically significant ($p = 0.006$, Wilcoxon signed-rank test), aligned with the reduction in sound-evoked firing rates described earlier.

DOI reduced sensitivity to oddball sounds

A robust and widespread phenomenon observed in sensory systems is an increased neural response to an infrequently occurring stimulus compared to the response to the same stimulus when it appears repeatedly (Ulanovsky et al., 2003; Nelken, 2014). Here, we tested whether DOI affects this deviance detection phenomenon by evaluating the difference in responses to a sound appearing frequently in a sequence *vs.* the same sound occurring rarely in another sequence (Fig. 3).

We first used narrowband chords (50 ms duration) centered at two different frequencies (8 and 13 kHz) to create a sequence of sounds where one of the chords (the standard) appears repeatedly, while the other chord (the oddball) appears once every 9 to 11 presentations. In one recording session, the high-frequency chord was presented as the standard, while in a subsequent session, it was presented as the oddball (Fig. 3A). As expected for the saline control condition, we found that neural responses to a sound appearing as oddball were on average stronger than responses to the same sound appearing as standard (Fig. 3B). After DOI injection, however, this difference in responses between oddball and standard was greatly reduced (Fig. 3C).

To quantify this effect across the population of recorded neurons, we calculated for each neuron an Oddball Enhancement Index (OEI) defined as $(O-S)/(O+S)$, where O is the average response to the oddball and S is the average response to the standard. For this analysis, we included only cells that met two criteria: (1) they were responsive to the evaluated stimulus, and (2) their OEI varied by less than 30% between the pre-injection and the saline conditions. We found that the median OEI across the population of neurons was significantly lower under DOI compared to saline (Fig. 3D). This effect was observed for both the high-frequency chord ($N = 128$ cells, $p < 10^{-5}$, Wilcoxon signed-rank test) and the low-frequency chord ($N = 79$ cells, $p = 0.034$, Wilcoxon signed-rank test).

Next, we aimed to test the effects of DOI on deviance detection phenomena using stimuli that are more likely to engage higher-level processing in the auditory cortex. To this end, we created sequences of standards and oddballs with two frequency-modulated (FM) sounds, one up-sweep and one down-

sweep, where the frequency range (8–13 kHz) and duration (100 ms) were identical. Similar to the results obtained with sequences of chords, we found a reliably positive OEI under saline (Fig. 3F) that decreased under DOI (Fig. 3G). Consistent with the results for chords, the median OEI across the population of neurons was diminished for down-sweeps ($N = 65$ cells, $p = 0.016$, Wilcoxon signed-rank test) and for up-sweeps ($N = 95$ cells, $p = 0.006$, Wilcoxon signed-rank test) (Fig. 3H).

The difference in OEI observed between saline and DOI conditions could result from either a reduction in responses to oddballs or an enhancement of responses to standards. Fig. 4A shows the data for the same example cell presented in Fig. 3F and Fig. 3G, but with responses to standard stimuli (top) and oddball stimuli (bottom) shown separately. This example suggests that the observed changes in OEI after DOI were primarily due to a decrease in responses to the oddball, rather than an increase

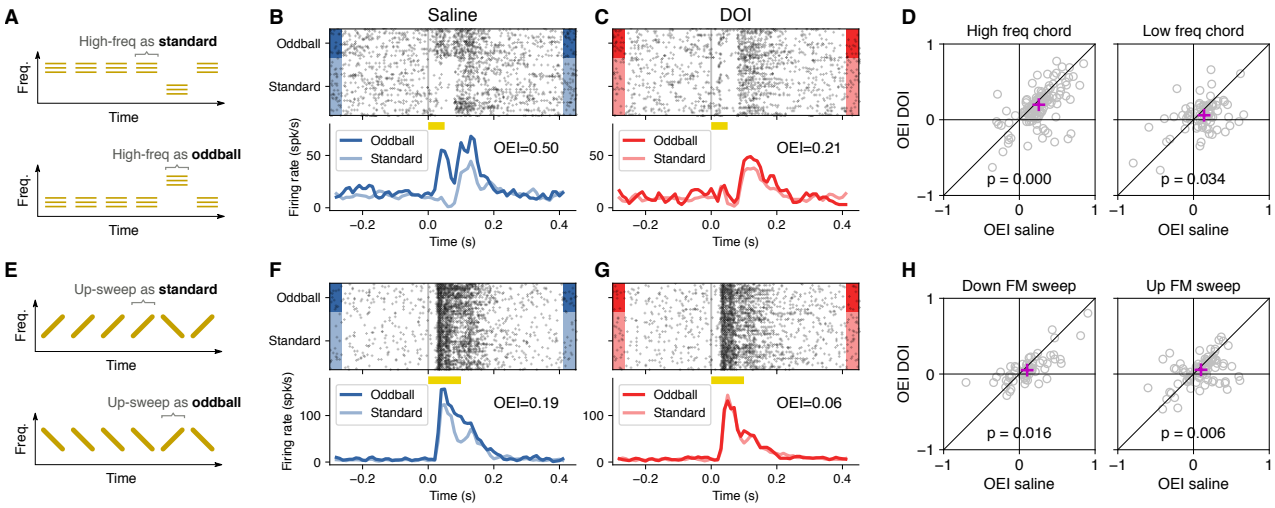

Figure 3: DOI reduced the difference between responses to oddballs and to standards. **(A)** Sequences of chords. The high-frequency chord appeared either as the standard (top) or as the oddball (bottom). **(B)** Responses to the high-frequency chord from an example AC neuron under the saline condition. The response to the sound was larger when it appeared as oddball. **(C)** Responses to the high-frequency chord from the example neuron in (B) after DOI injection. The difference between responses to oddball and standard was greatly reduced. **(D)** The oddball enhancement index (OEI) was smaller after DOI injection compared to the saline condition for both high- and low-frequency chords. Each dot represents one neuron. The magenta cross indicates the median value across neurons. **(E)** Sequences of frequency modulated (FM) sounds. The up-sweep sound appeared either as the standard (top) or as the oddball (bottom). **(F)** Responses to the up-sweep FM sound from an example neuron under the saline condition. The response to the sound was larger when it appeared as oddball. **(G)** Responses to the up-sweep FM sound from the example neuron in (F) after DOI injection. The difference between responses to oddball and standard was greatly reduced. **(H)** The oddball enhancement index (OEI) was smaller after DOI injection compared to the saline condition for both down- and up-sweep sounds. Each dot represents one neuron. The magenta cross indicates the median value across neurons.

in responses to the standard. To quantify these effects across the population of neurons, we calculated a modulation index, $\mathrm{MI} = (\mathrm{DOI} - \mathrm{Sal})/(\mathrm{DOI} + \mathrm{Sal})$, separately for standards and for oddballs. Across the population, we found that the median MI was negative for both oddball and standards (consistent with the overall reduction in responsiveness described above), but that the change in response to the oddball was larger than the change in responses to the standard. This pattern held true for chords (p$< 10^{-5}$ for high-freq and $p = 0.039$ for low-freq, Wilcoxon signed-rank test)(Fig. 4B) and for FM sounds (p=0.015 for down-sweeps and $p = 0.006$ for up-sweeps, Wilcoxon signed-rank test)(Fig. 4C).

Finally, to determine whether the reduction in responses to oddball sounds could be attributed to general response adaptation over the session, rather than the specific effects of DOI, we compared changes in responses from saline to DOI with changes from pre-injection to saline conditions. For this analysis, we used all cells that responded to the stimulus (regardless of whether their OEI was stable between pre-injection and saline). We found that there was no statistically significant change in the response to the oddball between the periods before and after saline for any stimuli, but a statistically significant change between saline and DOI for all but one stimulus tested (high-freq, $N = 523$, Pre-Saline: $p = 0.49$, Saline-DOI: $p = 0.004$; low-freq, $N = 510$, Pre-Saline: $p = 0.58$, Saline-DOI: $p < 0.001$; down-sweep, $N = 469$, Pre-Saline: $p = 0.13$, Saline-DOI: $p = 0.16$; up-sweep, $N = 514$, Pre-Saline: $p = 0.82$, Saline-DOI: $p < 10^{-6}$, Wilcoxon signed-rank test). We also found that the change in oddball response between saline and DOI was more negative than the change between pre-injection and saline conditions (high-freq: MI(Sal-DOI) $= -0.065$ *vs.* MI(Pre-Sal) $= -0.054$, $p = 0.18$, low-freq: MI(Sal-DOI) $= -0.078$ *vs.* MI(Pre-Sal) $= -0.030$, $p = 0.001$, down-sweep:

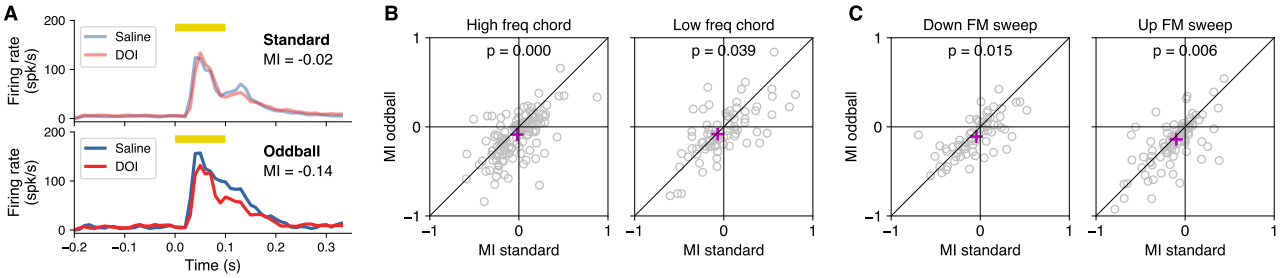

Figure 4: DOI reduced sensitivity to oddball sounds. **(A)** Responses of the neuron from Fig. 3F to the up-sweep FM sound when presented as the standard. Responses were almost identical under saline or DOI. **(B)** Responses of the same neuron to the up-sweep FM sound when presented as the oddball. The response was smaller under DOI compared to saline. **(C)** There was a larger reduction in responses to oddballs compared to standards after DOI, for both high- and low-frequency chords. Each dot represents the modulation index (MI) from saline to DOI for each neuron. The magenta cross indicates the median value across neurons. **(D)** There was a larger reduction in responses to oddballs compared to standards after DOI, for both down- and up-sweep FM sound. Data presented as in (C).

MI(Sal-DOI) $= -0.072$ *vs.* MI(Pre-Sal) $= -0.034$, $p = 0.006$, up-sweep: MI(Sal-DOI) $= -0.106$ *vs.* MI(Pre-Sal) $= -0.039$, $p < 10^{-6}$, Wilcoxon signed-rank test). Together, these results indicate that the observed reduction in sensitivity to oddballs is primarily due to the effects of DOI and not merely the result of stimulus adaptation.

Discussion

In this study, we evaluated changes in the activity of auditory cortical neurons when an animal is under the influence of the psychedelic 2,5-Dimethoxy-4-iodoamphetamine (DOI), a serotonergic hallucinogen. During the presentation of sounds, we found that the average firing rate of auditory cortical neurons decreased under DOI influence. This effect included a reduction in spontaneous firing, in the absolute sound-evoked firing rates, and in the relative evoked response with respect to baseline firing (Fig. 2G). We also found that in the presence of DOI, the trial-by-trial response variability increased but the frequency tuning of auditory neurons was mostly preserved across the population. When evaluating changes in sound-evoked responses during an oddball paradigm (where sequences of sounds include expected and unexpected stimuli), we found that the classically observed difference in neuronal responses between oddball and standard sounds was reduced, and that this reduction was primarily driven by a decrease in responses to oddball stimuli, suggesting a reduction in deviance detection in these neurons.

Changes in neural activity in the auditory system after manipulation of the serotonergic system have been observed across several brain structures along the auditory pathway (Hurley and Hall, 2011). A suppression of neural activity after either application of serotonin or serotonin receptor agonists has been reported in the cochlear nucleus (Ebert and Ostwald, 1992), inferior collicullus (Hurley and Pollak, 1999), medial geniculate nucleus of the thalamus (Monckton and McCormick, 2002) and the auditory cortex (Ji and Suga, 2007). While activity suppression is more commonly observed, enhancement of activity was also seen for many cells in most of these studies. This suggests that characterizing the effects of psychedelics simply as a one-directional change on overall firing rates or a decrease in responsiveness is an insufficient model for the effects of psychodelics on sound processing. Moreover, a recent study in mice found that a systemic dose of psilocybin, a serotonergic psychedelic, resulted in an initial increase in movement and neural responses in the auditory cortex followed by a decrease in responses about 30 minutes later (Brockett and Francis, 2024), suggesting that the dynamical nature of the psychedelic effect may also need to be integrated when modeling their effects on sound processing. The timeline of our measurements matches the late stage of that study and yields a similar decrease in responses. Importantly, our study demonstrates that the effects on neural activity in the auditory cortex are not fully explained by changes in behaviors such as running,

since the overall decreases in activity were observed in running as well as not-running trials.

A potential mechanism for the perceptual auditory distortions that occur with psychedelics is a consistent sharpening or broadening of frequency tuning. Our data, however, indicates that on average there is no consistent direction for changes in tuning. This result is consistent with observations that psilocybin does not significantly change pure-tone frequency selectivity of auditory cortical neurons in mice (Brockett and Francis, 2024). Similarly, experiments evaluating the effects of systemic DOI in the visual in mice found that the orientation selectivity was preserved after DOI (Michaiel et al., 2019). Together, these results suggest that the mechanisms behind psychedelic-induced perceptual distortions go beyond changes in basic features of sensory processing such as stimulus tuning.

Hallucination resulting from schizophrenia and other mental disorders are hypothesized to arise from a change in the balance between external (bottom-up) and internal (top-down) signals in the brain (Grossberg, 2000). This mechanism has also been proposed as a key feature of psychedelic action (Carhart-Harris and Friston, 2019). In the context of our experiments, a reduction in overall firing and evoked response magnitude suggests a dampening of bottom-up sensory input. This could be interpreted as a decreased ability of the auditory cortex to faithfully represent incoming auditory stimuli. However, our data also suggests alterations in top-down modulation. For instance, the reduced difference between oddball and standard responses, particularly the greater reduction in oddball responses, implies a disruption in deviance detection which relies on top-down predictive processes.

In humans, the oddball paradigm is commonly used to estimate prediction error signaling by measuring the mismatch negativity (MMN) of event-related potentials or fields, an enhancement in the negative deflection of the waveform in deviant *vs.* standard stimuli (Näätänen et al., 1982; Garrido et al., 2009). Manipulating the serotonergic system in humans via acute tryptophan depletion (ATD), which reduces the brain synthesis of serotonin, resulted in an increased MMN (Kähkönen et al., 2005), consistent with a role of the serotonergic system in deviance detection as observed in our study. Moreover, a study using lysergic acid diethylamide (LSD), a serotonergic hallucinogen, found a decrease in the MMN effect after administration of this compound (Timmermann et al., 2018). That study found a decrease in the response to deviant stimuli under LSD, consistent with our results for DOI, although they also found an enhanced response to the standard stimuli, something not present in our data. Studies using other serotonergic hallucinogens have also found significant decreases in evoked responses in humans, but only minimal effects on the MMN when using psilocybin (Umbricht et al., 2003; Schmidt et al., 2012; Bravermanová et al., 2018) or DMT (Heekeren et al., 2008). These various results suggest that the effects of psychedelics on deviance detection may not be the same for all compounds or may depend on specific experimental conditions.

There are at least two mechanisms that can explain the enhanced responses to oddball sounds compared to standard sounds under normal conditions: an adaptation to standard sounds and/or the

neural representation of prediction errors. The first mechanism is based on the principle of neural adaptation or habituation, such that when a sound is repeatedly presented as a standard, neurons responding to that sound may decrease their firing rate over time (due to either short-term synaptic depression, activation of local inhibitory circuits, or depletion of readily releasable neurotransmitter pools). Previous studies suggest that this adaptation does not fully account for the effects observed in oddball paradigms (Nelken, 2014). A second mechanism is that of prediction error representation, based on predictive coding theories of brain function. The brain constantly generates predictions about incoming sensory input based on recent history and context, and neurons in the auditory cortex may encode the difference between the predicted input and the actual input (*i.e.*, the prediction error). In this framework, responses to standard sounds are reduced because they are well-predicted, and oddball sounds elicit larger responses because they violate the prediction, generating a large prediction error. This mechanism involves both bottom-up sensory input and top-down predictive signals. Considering these mechanisms, we can interpret the effects of DOI on oddball responses in several ways: (1) if DOI primarily affects adaptation mechanisms, it might interfere with the normal habituation to standard sounds, reducing the contrast between standard and oddball responses; (2) if DOI impacts predictive coding processes, it could disrupt the generation or propagation of prediction errors, leading to a reduced differentiation between expected and unexpected stimuli; (3) DOI might affect both mechanisms, potentially through its action on serotonin receptors, which are known to modulate both local circuit dynamics and long-range connections involved in predictive processing.

In the context of our experiments, if DOI primarily affected adaptation, we would expect a reduction in the difference between oddball and standard responses, as observed in our data. However, we might expect this to occur primarily through an increase in the response to standards (due to reduced adaptation) rather than a decrease in the response to oddballs. The overall reduction in responses and the greater effect on oddballs suggest that something more complex is occurring. If DOI primarily affected prediction error signaling, we would expect a reduction in the difference between oddball and standard responses, but also a more pronounced effect on oddball responses, as these represent larger prediction errors. This aligns with our observation of a greater reduction in oddball responses. Moreover, the overall reduction in responsiveness is consistent with a general dampening of prediction error signaling. Therefore, we conclude that our results align more closely to the prediction error mechanisms being affected by DOI. This of course does not rule out effects on adaptation, but rather, it suggests that the impact on prediction error signaling may be more prominent in our experimental paradigm.

References

Aday JS, Wood JR, Bloesch EK, Davoli CC (2021) Psychedelic drugs and perception: A narrative review of the first era of research. *Reviews in the Neurosciences* 32:559–571.

Bravermanová A, Viktorinová M, Tylš F, Novák T, Androvičová R, Korčák J, Horáček J, Balíková M, Griškova-Bulanova I, Danielová D, Vlček P, Mohr P, Brunovský M, Koudelka V, Páleníček T (2018) Psilocybin disrupts sensory and higher order cognitive processing but not pre-attentive cognitive processing—study on P300 and mismatch negativity in healthy volunteers. *Psychopharmacology* 235:491–503.

Brockett AT, Francis NA (2024) Psilocybin decreases neural responsiveness and increases functional connectivity while preserving pure-tone frequency selectivity in mouse auditory cortex. *Journal of Neurophysiology* 132:45–53.

Canal CE, Morgan D (2012) Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: A comprehensive history, a re-evaluation of mechanisms, and its utility as a model. *Drug testing and analysis* 4:556–576.

Carhart-Harris RL, Friston KJ (2019) REBUS and the Anarchic Brain: Toward a Unified Model of the Brain Action of Psychedelics. *Pharmacological Reviews* 71:316–344.

Carhart-Harris RL, Leech R, Hellyer PJ, Shanahan M, Feilding A, Tagliazucchi E, Chialvo DR, Nutt D (2014) The entropic brain: A theory of conscious states informed by neuroimaging research with psychedelic drugs. *Frontiers in Human Neuroscience* 8.

Ebert U, Ostwald J (1992) Serotonin modulates auditory information processing in the cochlear nucleus of the rat. *Neuroscience Letters* 145:51–54.

Garrido MI, Kilner JM, Stephan KE, Friston KJ (2009) The mismatch negativity: A review of underlying mechanisms. *Clinical Neurophysiology* 120:453–463.

Grossberg S (2000) How hallucinations may arise from brain mechanisms of learning, attention, and volition. *Journal of the International Neuropsychological Society* 6:583–592.

Halberstadt AL, van der Heijden I, Ruderman MA, Risbrough VB, Gingrich JA, Geyer MA, Powell SB (2009) 5-HT2A and 5-HT2C Receptors Exert Opposing Effects on Locomotor Activity in Mice. *Neuropsychopharmacology* 34:1958–1967.

Heekeren K, Daumann J, Neukirch A, Stock C, Kawohl W, Norra C, Waberski TD, Gouzoulis-Mayfrank E (2008) Mismatch negativity generation in the human 5HT2A agonist and NMDA antagonist model of psychosis. *Psychopharmacology* 199:77–88.

Hurley LM, Hall IC (2011) Context-dependent modulation of auditory processing by serotonin. *Hearing Research* 279:74–84.

Hurley LM, Pollak GD (1999) Serotonin Differentially Modulates Responses to Tones and Frequency-Modulated Sweeps in the Inferior Colliculus. *Journal of Neuroscience* 19:8071–8082.

Ji W, Suga N (2007) Serotonergic Modulation of Plasticity of the Auditory Cortex Elicited by Fear Conditioning. *Journal of Neuroscience* 27:4910–4918.

Kähkönen S, Mäkinen V, Jääskeläinen IP, Pennanen S, Liesivuori J, Ahveninen J (2005) Serotonergic modulation of mismatch negativity. *Psychiatry Research: Neuroimaging* 138:61–74.

Kwan AC, Olson DE, Preller KH, Roth BL (2022) The neural basis of psychedelic action. *Nature Neuroscience* 25:1407–1419.

Michaiel AM, Parker PRL, Niell CM (2019) A Hallucinogenic Serotonin-2A Receptor Agonist Reduces Visual Response Gain and Alters Temporal Dynamics in Mouse V1. *Cell Reports* 26:3475–3483.e4.

Mitchell JM, Anderson BT (2024) Psychedelic therapies reconsidered: Compounds, clinical indications, and cautious optimism. *Neuropsychopharmacology* 49:96–103.

Monckton JE, McCormick DA (2002) Neuromodulatory Role of Serotonin in the Ferret Thalamus. *Journal of Neurophysiology* 87:2124–2136.

Näätänen R, Simpson M, Loveless NE (1982) Stimulus deviance and evoked potentials. *Biological Psychology* 14:53–98.

Nelken I (2014) Stimulus-specific adaptation and deviance detection in the auditory system: Experiments and models. *Biological Cybernetics* 108:655–663.

Nichols DE (2016) Psychedelics. *Pharmacological Reviews* 68:264–355.

Pachitariu M, Sridhar S, Pennington J, Stringer C (2024) Spike sorting with Kilosort4. *Nature Methods* 21:914–921.

Preller KH, Razi A, Zeidman P, Stämpfli P, Friston KJ, Vollenweider FX (2019) Effective connectivity changes in LSD-induced altered states of consciousness in humans. *Proceedings of the National Academy of Sciences* 116:2743–2748.

Preller KH, Vollenweider FX (2018) Phenomenology, Structure, and Dynamic of Psychedelic States In Halberstadt AL, Vollenweider FX, Nichols DE, editors, *Behavioral Neurobiology of Psychedelic Drugs*, pp. 221–256. Springer, Berlin, Heidelberg.

Reiff CM, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM, and the Work Group on Biomarkers and Novel Treatments, a Division of the American Psychiatric Association Council of Research (2020) Psychedelics and Psychedelic-Assisted Psychotherapy. *American Journal of Psychiatry* 177:391–410.

Rossant C, Kadir SN, Goodman DFM, Schulman J, Hunter MLD, Saleem AB, Grosmark A, Belluscio M, Denfield GH, Ecker AS, Tolias AS, Solomon S, Buzsáki G, Carandini M, Harris KD (2016) Spike sorting for large, dense electrode arrays. *Nature Neuroscience* 19:634–641.

Schmidt A, Bachmann R, Kometer M, Csomor PA, Stephan KE, Seifritz E, Vollenweider FX (2012) Mismatch Negativity Encoding of Prediction Errors Predicts S-ketamine-Induced Cognitive Impairments. *Neuropsychopharmacology* 37:865–875.

Schneider DM, Nelson A, Mooney R (2014) A synaptic and circuit basis for corollary discharge in the auditory cortex. *Nature* 513:189–194.

Shulgin AT, Shulgin A (1991) *Pihkal: A Chemical Love Story* Transform Press, Berkeley, CA.

Shulgin AT, Shulgin A (1997) *Tihkal: The Continuation* Transform Press, Berkeley, CA.

Syeda A, Zhong L, Tung R, Long W, Pachitariu M, Stringer C (2024) Facemap: A framework for modeling neural activity based on orofacial tracking. *Nature Neuroscience* 27:187–195.

Timmermann C, Spriggs MJ, Kaelen M, Leech R, Nutt DJ, Moran RJ, Carhart-Harris RL, Muthukumaraswamy SD (2018) LSD modulates effective connectivity and neural adaptation mechanisms in an auditory oddball paradigm. *Neuropharmacology* 142:251–262.

Ulanovsky N, Las L, Nelken I (2003) Processing of low-probability sounds by cortical neurons. *Nature Neuroscience* 6:391–398.

Umbricht D, Vollenweider FX, Schmid L, Grübel C, Skrabo A, Huber T, Koller R (2003) Effects of the 5-HT2A Agonist Psilocybin on Mismatch Negativity Generation and AX-Continuous Performance Task: Implications for the Neuropharmacology of Cognitive Deficits in Schizophrenia. *Neuropsychopharmacology* 28:170–181.

van Daal RJJ, Aydin Ç, Michon F, Aarts AAA, Kraft M, Kloosterman F, Haesler S (2021) Implantation of Neuropixels probes for chronic recording of neuronal activity in freely behaving mice and rats. *Nature Protocols* 16:3322–3347.

Vollenweider FX, Geyer MA (2001) A systems model of altered consciousness: Integrating natural and drug-induced psychoses. *Brain Research Bulletin* 56:495–507.

Wittmann M, Carter O, Hasler F, Cahn BR, Grimberg U, Spring P, Hell D, Flohr H, Vollenweider FX (2007) Effects of psilocybin on time perception and temporal control of behaviour in humans. *Journal of Psychopharmacology* 21:50–64.