Top-down modulation of sensory processing and mismatch in the mouse posterior parietal cortex

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ABSTRACT

An important function of the neocortex is to compare sensory feedback stimuli with internal predictions of the outside world and evoke mismatch responses to deviations, thus allowing expectations to be updated. The mechanisms behind sensory feedback mismatch and prediction formation however remain unclear. Here we created a learned association of an auditory-tactile stimulus sequence in awake head-fixed mice, where sound predicted an up-coming whisker stimulus, and introduced mismatches by omitting or altering the whisker stimulus intensity. We show that layer 2/3 posterior parietal cortex (PPC) neurons can report stimulus sequence mismatches, as well as display neural correlates of expectation which can be prolonged temporally. Inhibition of PPC-projecting secondary motor cortex M2 neurons suppressed these correlates, along with population mismatch responses, while conversely enhancing sensory processing. Hence, M2 can influence sensory processing in the PPC and potentially provide the prediction of sensory feedback from learned relationships within sequences of sensory stimuli.

INTRODUCTION

The experience of different sensory stimuli enables us to make associations between them, especially when presented as part of a sensory sequence. For example, we learn to anticipate the sound of thunder after a flash of lightning. We would be surprised in the

absence of the expected stimulus, because there was a mismatch between what we anticipated and what we actually experienced. The predictive processing framework postulates that the brain constantly predicts sensory feedback based on an internal representation of the outside world built upon prior sensory experience[1,2]. Mismatch responses, or prediction errors, that are generated during deviations between what was predicted and the actual sensory input are then used for updating stimulus expectations[3]. This is an essential feature at both the physiological and behavioral level as it facilitates the detection of unexpected, potentially dangerous events[4], and is thus crucial to survival. Sensory feedback mismatch responses have been described in the primary visual cortex (V1) in mice[5,6], as well as in the primate primary auditory cortex[7] and the primary auditory pallium of songbirds[8]. Local cortical circuits implicated in generating mismatch responses have been investigated in mice, mainly during locomotion in a virtual reality setting. There, the predicted sensory feedback from self-generated motion was compared against the actual sensory flow, when coupled with closed-loop visual, auditory or tactile stimuli[9-11]. Conversely it was recently shown that neurons within the posterior parietal cortex (PPC) of awake head-fixed mice can report mismatches in sensory sequences in the absence of locomotion/motor-output-related sensory feedback predictions, such as during the omission of a previously experienced passive touch to the whiskers[12]. The PPC, a sensory associative area, receives bottom-up sensory input from the primary sensory cortices[13-15], and has reciprocal connections with the higher order cortical areas (secondary motor cortex M2, cingulate cortex, and orbitofrontal cortex) that can provide top-down feedback information[16-18]. It could therefore compare incoming sensory stimuli with the expectations of these stimuli constructed from prior experience, and report mismatches between the two, analogous to what has been described in hierarchical visual processing in mouse V1[19]. However, little is known about how such learned associations formed from experiencing sensory sequences, where one stimulus predicts another, can influence sensory processing as well as the generation of mismatch responses.

Here, we presented awake head-fixed mice with an auditory stimulus via a loudspeaker followed by tactile stimuli through whisker deflection with a magnetic coil. Hence, we

created a sensory stimulus sequence where the sound predicts the up-coming whisker stimulus. We could then introduce a mismatch in this sequence by varying the intensity of the whisker stimuli, or omitting its presentation, while recording neuronal activity of layer 2/3 PPC neurons at single cell resolution with *in-vivo* 2-photon calcium imaging. We demonstrate that the PPC can make associations of sequences of sensory stimuli and reliably generate mismatch responses to deviations in these sequences. We further reveal that this dynamic representation of sensory stimuli is modulated by top-down feedback from M2, and can be interpreted within the framework of predictive processing.

RESULTS

Creating a sensory association at the PPC and reporting mismatch

In order to create an association between two sensory stimuli, we first presented awake head-fixed mice with an auditory tone ("sound" session), either looming or non-looming in intensity (Fig. 1a). This was followed by trials with only a whisker stimulus ("whisker" session). We then presented the whisker stimulus immediately after the auditory stimulus ("pairing" session), in this case the looming sound, so that the sound would predict the up-coming whisker stimulus. In subsequent trials ("interleaved" session), we created a mismatch (mismatch trials) in the experienced auditory-tactile sequence by randomly omitting the whisker stimulus in 20% of the trials. Hence the interleaved session comprised of randomized paired (sound followed by whisker stimulus) and mismatch (sound followed by whisker stimulus omission) trials (Fig. 1a). Neuronal activity within the PPC was measured using 2-photon calcium imaging at single cell resolution in layer 2/3 neurons of mice expressing the genetically encoded calcium indicator (GECI) RCaMP1.07[20], with a chronic cranial window in place. Expression of this viral construct was previously shown to be mainly in pyramidal neurons[11]. Using intrinsic optical signal (IOS) imaging (see Methods and Supplementary Fig. 1) we localized area A of the PPC via exclusion, where mismatch responses to the omission of tactile stimuli were previously reported[12].

We identified 3745 neurons (n = 6 mice, imaged across 6 sessions) that showed a response to one of the presented stimuli based on their average trace. Among these,

1080 neurons were classified as significantly responsive when compared to the shuffled data (see Methods). We subsequently based all our analysis on these shuffle-corrected neurons and followed their activity through the different sessions described below. Here, we present the mean $\Delta F/F$ computed during a 1s window from stimulus onset minus the baseline mean $\Delta F/F$ 1s prior to stimulus. During sound presentation, 8.5% of neurons (92 of 1080 neurons, 31.3% response probability) were responsive to the looming tone (Fig. 1c, d, see Supplementary Fig. 2 for non-looming sound responses). We also identified neurons (88 of 1080, or 8.2%, 19.1% response probability) that were responsive at the end of the sound or sound offset-responsive. During whisker stimulation ("whisker" session) 27.0% of PPC neurons (292 of 1080 neurons, 30.4% response probability) were responsive to whisker deflection. The pairing of auditory and whisker stimuli ("pairing" session) recruited a new population of neurons that were previously weakly or non-responsive to either presented modality (Fig. 1d). Whisker stimulus evoked responses were comparable in both the whisker and pairing sessions ($p = 0.92$, $n = 292$ whisker-responsive neurons, $n = 161$ pairing-responsive neurons, with 19 overlapping neurons or 4.4%, Wilcoxon-Mann-Whitney test) (Fig. 1e). On the other hand, the auditory responses in the pairing session were smaller than those evoked during the sound session ($p = 0.042$, $n = 92$ looming-responsive neurons, $n = 123$ pairing sound-responsive neurons, with 3 overlapping neurons or 1.4%, Wilcoxon-Mann-Whitney test) (Fig. 1e). The suppression of the auditory stimulus in this sensory sequence that predicts an up-coming whisker stimulus suggests top-down mediated inhibition as the relationship between the stimuli is learnt through experience[19].

In the subsequent interleaved session, we found that 6.3% of neurons within the PPC (68 of 1080 neurons, 21.5% response probability) could report the omission of the previously experienced whisker stimulus. These mismatch responses were observed from the very first mismatch trial and were overall stable over time (Fig. 1f). Among the mismatch-responsive neurons, only one neuron was previously identified as looming offset-responsive in the sound session. This confirms that the mismatch neurons represent a newly recruited group of cells that report whisker stimulus omission and are not merely sound offset-responsive neurons. The mismatch response was also not associated with whisker movements (Fig. 1b, d, interleaved mismatch), consistent with

previous studies[12]. We also observed mismatch responses of comparable size when the non-looming sound was paired with the whisker stimulus (Supplementary Fig. 2). Compared to the whisker stimulus response in the prior whisker and pairing sessions, the mismatch response was also smaller in size (Fig. 1g). In the interleaved session, we found that a larger fraction of neurons (280 of 1080, 26.0%, with 17.0% response probability) were responsive to the interleaved paired trials, but with a significantly smaller response compared to the mismatch response ($p = 0.015$, $n = 68$ mismatch-responsive neurons, $n = 280$ interleaved paired-responsive neurons, Wilcoxon-Mann-Whitney test). Hence, the PPC can associate sensory stimuli in a stimulus sequence and report mismatches in the experienced sequence, with mismatch responses being larger than that of the predicted stimulus.

Dynamic representation of whisker stimuli in PPC compared to S1

While the PPC can report auditory and tactile stimuli, the presentation of sensory sequences along with the introduction of mismatch trials appeared to recruit different groups of neurons that were selectively responsive in some sessions and not others (Fig. 1d). To determine if this was specific to the PPC, we recorded neuronal responses from the neighbouring primary somatosensory cortex S1 and focused on the neuronal responses to the whisker stimulus. Hence, we repeated these experiments in Thy-1GCaMP6f mice that constitutively express GCaMP6f in excitatory neurons across the neocortex[21] and imaged layer 2/3 excitatory neurons in the PPC and S1, in separate sessions (Fig. 2). Excitatory neurons in S1 (4 mice, across 6 sessions, 799 stimulus-responsive neurons, of which 158 were shuffle-corrected and identified as significantly whisker stimulus responsive, see Methods) were able to report whisker stimuli in the whisker and pairing sessions (whisker-responsive and pairing-responsive neurons respectively in Fig. 2a, b), with some neurons being responsive in both sessions (Fig. 2c; 22.4%, 28/125). The introduction of mismatch trials in the interleaved session led to a decrease in the whisker stimulus response of both whisker- and pairing-responsive neurons, while the interleaved paired-responsive neurons remained overall stable in their response (Fig. 2a, b). Nonetheless, the response reliability was lower in the interleaved paired trials compared to the pairing trials (Fig. 2e). In contrast to S1, we found that excitatory neurons in the PPC (4 mice, across 6 sessions, 682 stimulus

responsive neurons, of which 131 were shuffle-corrected and identified as significantly whisker stimulus responsive, see Methods) responded primarily to the whisker stimuli in their respective sessions (whisker-responsive and pairing-responsive neurons in Fig. 2f, see Supplementary Fig. 3 for corresponding analysis of data in Fig. 1) with little overlap between both sessions (Fig. 2h; 2.3%, 2/88). We further observed that the interleaved paired-responsive neurons increased their response to the whisker stimulus, compared to their prior response in the pairing session (Fig. 2f, g). This was matched with an increase in the mean $\Delta F/F$ 0.5 s before whisker stimulus onset (shaded window of interleaved paired-responsive neurons in Fig. 2f), which was absent during the pairing session (Fig. 2i) and was not observed in S1 (Fig. 2d). Despite these differences, the response to the whisker stimulus of interleaved paired-responsive neurons in the interleaved session was diminished compared to that of the pairing-responsive neurons in the pairing session, in both S1 and the PPC (Fig. 2b, g; $p = 5.0 \times 10^{-4}$, $p = 7.6 \times 10^{-4}$, respectively, two-sided Wilcoxon-Mann-Whitney test). We also saw a corresponding decrease in response probability in the interleaved paired compared to pairing session (Fig. 2e, j). These findings suggest the presence of functional groups of neurons within the PPC that are differentially recruited during exposure to a sensory stimulus sequence with mismatch trials.

Neural correlates of expectation in the PPC

We then asked if the pre-stimulus increase in the mean $\Delta F/F$ during the predicting sound window (Fig. 2i) could represent a neural correlate of expectation. In a separate set of interleaved sessions we introduced a 1 s delay between the looming sound and the onset of the whisker stimulus, to test if this pre-stimulus increase in neural response could be prolonged and/or enhanced. We observed that in the interleaved paired trials, the pre-stimulus response was indeed prolonged as well as enhanced with the delay, along with an increase in the post-stimulus whisker response (Fig. 3a, b). In the mismatch trials, there was also a corresponding prolongation and enhancement of the pre-stimulus response, while the mismatch response itself remained comparable in size (Fig. 3c, d). Hence the pre-stimulus response could potentially represent a top-down

driven neural correlate of expectation in the PPC, as mismatch trials are interleaved with the paired trials.

PPC can report different types of sensory mismatch

So far, we employed the omission of the whisker stimulus as the mismatch in the interleaved trials. Can the PPC also report mismatches in stimulus intensity? We designed two different types of interleaved sessions that were presented separately to the mice (Fig. 3a). In one session, we interleaved the paired trials with mismatch trials that had a smaller whisker stimulus intensity. These decreased-intensity trials would then signal a negative mismatch. In contrast, to signal a positive mismatch in another session, we interleaved the paired trials with mismatch trials of a higher whisker stimulus intensity (increased-intensity trials). This would allow for the identification of potential negative and positive mismatch neurons as described in the canonical microcircuit for predictive processing[22]. Effectively the PPC was able to report mismatches in stimulus intensity in both directions, where the mismatch response was larger than the corresponding interleaved paired response (Fig. 3f, g). The mismatch responses were also encoded by largely separate groups of neurons (Fig. 3h and Supplemental Fig. 4), indicating the presence of distinct negative and positive mismatch neurons. We further observed that the pairing-responsive neurons showed a response in the increased-intensity mismatch trials, and conversely the increased-intensity mismatch neurons were responsive in the prior pairing trials. Both groups were largely non-responsive in the interleaved session with the omission-mismatch trials (Supplementary Fig. 4c, d). This suggests that pairing-responsive neurons can potentially function as positive mismatch neurons, and vice versa (see Discussion). Thus, the PPC can report positive and negative mismatches with distinct groups of neurons whose response dynamics can match the canonical microcircuit for predictive processing.

M2 modulates sensory processing at the PPC

Next, we investigated the source of the top-down feedback to the PPC that could drive the pre-stimulus response/expectation. In addition to the primary sensory cortices, the

PPC has strong reciprocal connections with higher order cortical areas, including M2[12,18]. These cortices have been shown to exchange sensory and motor information, respectively[23], with the PPC. To test the potential contribution of M2 as the source of top-down feedback, we expressed the inhibitory DREADD hM4Di in ipsilateral PPC-projecting M2 neurons using a retro-Cre based viral expression strategy in mice that expressed RCaMP1.07 in PPC neurons (3 mice, 6 sessions with and without CNO) (see Methods). We then administered CNO i.p. to the mice to silence the PPC-projecting M2 neurons and presented the auditory-tactile sequence as described in Fig. 1a. We observed that in the absence of top-down feedback from ipsilateral M2, the whisker stimulus responses were enhanced in all sessions ($p = 1.63 \times 10^{-13}$, $p = 1.70 \times 10^{-3}$ and $p = 3.50 \times 10^{-5}$ for whisker, pairing, and interleaved paired sessions, respectively, comparing with and without CNO, Wilcoxon-Mann-Whitney test); moreover, some neurons were responsive across sessions. This contrasted with sensory processing with intact top-down feedback (Fig.4b, c vs g, h). Indeed, the overlap of responsive neurons between the whisker and pairing session was higher (12.4%, 27/218) in the absence of top-down feedback, compared to control conditions (4.3%, 12/277), and analogous to what we observed between S1 and the PPC (Fig. 2c, h). Furthermore, the block of M2 inputs to the PPC completely abolished the pre-stimulus response in interleaved paired-responsive neurons, as well as that observed in the pairing-responsive neurons (Fig. 4b, e vs g, j). For the interleaved mismatch trials, we found that neurons could still report the omission mismatch, with a response size comparable to control experiments in the absence of M2 blockade (Fig. 5a). There were, however, less neurons recruited to report the mismatch when M2 was inhibited (21.8%, 41/188 vs 35.1%, 98/279 where 188 and 279 are the sum of the mismatch and paired-responsive neurons, with and without CNO, respectively). When we analyzed the impact of M2 inhibition in the interleaved session at the population level (combined average of interleaved paired- and mismatch-responsive neurons, for paired and mismatch trials), we observed that the mismatch response was suppressed while that of the paired trials was enhanced (Fig. 5b). Taken together, these findings show that M2 can modulate sensory processing at the PPC, selectively suppressing bottom-up inputs while driving the pre-stimulus response/expectancy in different groups of neurons. Furthermore, M2 also

contributes to the top-down prediction to the PPC that drives the omission mismatch neurons (Supplementary Fig. 5).

DISCUSSION

Using an auditory tactile stimulus sequence, we were able to follow the formation of a sensory association in mice, along with the updating of stimulus expectations while we introduced mismatches in the sequence, as represented by the neural activity of layer 2/3 neurons in the PPC. We have shown that the PPC is able to dynamically represent sensory sequences with different groups of neurons, in contrast to S1. One of the features of learned sensory associations is the inhibition of the expected bottom-up input [24,25]. This became evident in the size of the whisker stimulus response, which was significantly reduced in the transition from the pairing to interleaved trials. Indeed, both the whisker- and pairing-responsive neurons were suppressed outside of their respective sessions. This was in part modulated by top-down feedback from M2, which has been shown to suppress[26], as well as enhance[27-29] sensory processing in primary sensory cortices, as part of a widespread cortical circuit motif[27,30]. Effectively, the paired-responsive neurons increased their response to the whisker stimulus in the interleaved session, indicating that local microcircuits can be modulated differentially by top-down feedback at the same time. As both parvalbumin (PV) and vasoactive intestinal peptide (VIP) expressing inhibitory neurons can be directly activated by top-down inputs[26,27], it would be important to determine their role in our findings.

In our experiments we used a sound presentation to predict an up-coming whisker stimulus, and subsequently show the appearance of a pre-stimulus response that could represent a neural correlate of expectation (Fig. 2f, i). The absence of a clear response in pairing-responsive neurons in Thy-1GCaMP6f mice compared to neurons in Fig. 1d and Supplementary Fig. 3a could be explained by the expression of the GECI in subsets of cortical excitatory cells but not the entire population[21]. Nonetheless, the pre-stimulus response was present in the interleaved paired-responsive neurons and could be prolonged and enhanced temporally upon introducing a delay between the predicting sound and the expected whisker stimulus (Fig. 3a, b). We were able to suppress the

pre-stimulus response with the inhibition of M2 feedback to the PPC, demonstrating that M2 can provide the top-down expectation/prediction to the PPC, which could be interpreted as an attentional signal as well[27]. Neurons in the PPC can then compare this prediction with the actual bottom-up sensory information and report mismatches.

We have demonstrated that the PPC can report mismatches in the form of omission as well as deviations from the predicted strength of the whisker stimulus. Based on the canonical microcircuit for predictive coding[10,22], these mismatch neurons could be categorized into negative and positive error neurons that would report mismatches that are less (omission and decreased intensity mismatch) or more (increased intensity mismatch) than what is expected. In our experimental paradigm the pairing-responsive neurons could function as positive error neurons, as they become suppressed by top-down feedback from M2 (Supplementary Fig. 5), as mismatch trials are introduced in the interleaved session. Indeed, when the bottom-up input exceeded this prediction, the pairing-responsive neurons could report this positive mismatch (Supplementary Fig.4b). Conversely, the increased-intensity mismatch-responsive neurons were responsive in the prior pairing session. The paired-responsive neurons on the other hand showed mixed responses, integrating both top-down predictions and bottom-up stimulus information, in the form of the pre- and post-stimulus response. Finally, the omission and decreased-intensity mismatch-responsive neurons could function as negative error neurons as they are driven largely by the top-down prediction. Notably, we observed little overlap between the omission and decreased-intensity mismatch neurons, with mismatch responses of comparable size. Differences in the degree of bottom-up driven inhibition for the two mismatch types could potentially level out eventual expected differences in prediction error magnitude.

While the silencing of ipsilateral M2 feedback to the PPC reduced the number of mismatch neurons, it did not affect the size of single neuron mismatch responses. This suggests that multiple higher order areas, in addition to ipsilateral M2, could contribute to the top-down prediction. Nonetheless, the mismatch response was suppressed at the

net population average which combined the mismatch- and paired-responsive neurons in the interleaved trials. This demonstrates a strong effect of M2 on the population code.

Based on these findings, we propose that during the interleaved paired trials, top-down inputs predicting the whisker stimulus are matched by bottom-up whisker stimuli that recruit neighboring inhibitory neurons, such as the somatostatin (SST) expressing inhibitory neurons. These neurons are strongly driven by local excitation and could inhibit the dendrites of layer 2/3 neurons in the PPC [10,22]. In the interleaved mismatch trials, the top-down prediction can directly activate the dendrites of the PPC neurons in the absence of this locally recruited inhibition to drive the mismatch response. This would be in accordance with the framework of predictive coding during sensorimotor transformations and brings our study in line with the hierarchical visual processing described in mouse V1[10,19].

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All experimental procedures followed the guidelines of the Veterinary Office of Switzerland and were approved by the Cantonal Veterinary Office in Bern. The data was collected from wild-type C57/BL6 mice (n = 12) and Thy-1GCaMP6f mice (n = 4), and both males and females were used. All mice were at least 8 weeks old at the time of viral injection/head-post implantation.

METHOD DETAILS

Experimental design

This study did not involve randomization or blinding. No data or mice were excluded from the analysis.

Surgery

Mice were anesthetized with isoflurane (3%) and subcutaneously injected with carprofen (5 mg/kg) prior to surgery for viral injections. For calcium imaging, the genetically encoded calcium indicator (GECI) RCaMP1.07 was injected into the PPC at

1.7 mm lateral and 2.0 mm posterior of bregma, to target layer 2/3 (~250 μm below the pial surface). For the silencing of M2 neurons, the DREADD hMD4i was injected into M2 at 0.5 mm lateral and 0.2 mm anterior of bregma, to target layers 2/3 and 5 (~250 and 500 μm below the pial surface). For long-term in vivo calcium imaging, a cranial window was implanted 24 hours to 1 week after virus injections over the PPC as described previously[31]. Mice were anesthetized with isoflurane and subcutaneously injected with buprenorphine (0.1 mg/kg) prior to surgery for window implantation. Briefly, a craniotomy was performed at the injection site. A cover glass (4 mm diameter) was placed directly over the exposed dura mater and sealed to the skull with dental acrylic. A metal post was fixed to the skull with dental acrylic, posterior to the cranial window, to allow for subsequent head fixation. One week after chronic window implantation, mice were handled daily for another week, and gradually habituated to head fixation. Intrinsic optical signal (IOS) imaging was performed on the mice to identify the location of PPC by exclusion as previously described[12]. In brief, to avoid the activation of surrounding whiskers, all whiskers except the γ-barrel-column whisker on the right whisker pad of the mice were trimmed and their locations were mapped using IOS on the exposed skull during whisker stimulation (rostrocaudal deflections at 10 Hz). The location of the primary visual cortex (V1) was similarly mapped using full-field stimulation with a green LED placed 5 mm in front of the contralateral eye. The non-activated region between these two identified sites was delineated as PPC for subsequent imaging sessions. For calcium imaging experiments in S1 of Thy1-GCamp6f mice, the area surrounding the activated region upon γ-barrel-column whisker stimulation was used.

Viral constructs

For calcium imaging, AAV1/2-hEF1α-RCaMP1.07-WPRE-hGHp(A) (300 nL, ~5.0×10^{12} μg/mL) was injected into the PPC of wild-type mice targeting layer 2/3 to induce expression of the GECI RCaMP1.07 in neurons. For the silencing of M2 neurons, AAVretro2-hSyn-EBFP-iCre was injected in layer 2/3 of the PPC, along with RCaMP1.07 (for calcium imaging), and AAV2-hSyn-dlox-HA-hM4D-Gi-mCitrine was injected in M2 to selectively target PPC projecting M2 neurons.

DREADD inhibition experiments

The inhibitory DREADD hM4Di was used to chemogenetically silence M2 neurons projecting to the PPC. An intraperitoneal (i.p.) injection of clozapine N-oxide (CNO dihydrochloride, 1 mg/kg, Tocris cat. no. 4936), the ligand that activates hM4Di, was done 30 minutes before presentation of the sensory stimulus sequence. We performed multiple imaging sessions with the presence of CNO first over several days, before repeating them in the absence of CNO at least 48 hours after the last CNO session.

Presentation of sound and tactile stimuli

To create a sensory association of the stimulus sequence, each mouse was presented with, in the following order, the sound session (approximately 25 randomly interleaved trials each of the looming and non-looming sound), the whisker session (approximately 50 whisker stimulus trials), the pairing session (approximately 20 trials with looming sound followed by the whisker stimulus), the interleaved session (approximately 120 paired trials of looming sound followed by the whisker stimulus, randomly interleaved with approximately 30 mismatch trials). The looming (increasing intensity) and non-looming (constant intensity) sounds were based on previously recorded soundtracks[12] and recreated in MATLAB, that consisted of a cloud of tones (0.1–8 kHz). Each sound was 1 s long and was delivered via a loudspeaker at 75 dB placed contralateral to the imaging site. Tactile stimuli were delivered as deflections (3 × 10 Hz, 1 ms pulse) to multiple whiskers. This was achieved by attaching small metal particles to the whiskers and subsequently moving them via a brief magnetic field generated by a coil placed beneath the head of the mouse[32]. The whisker stimulus intensity was set to 80% for all experiments except for those performed during the intensity mismatch experiments (Fig. 3e, f). Here, the interleaved paired stimulus was set to 60%, and the decreased and increased intensity mismatch stimuli were set to 40% and 80% respectively. Each trial began with a 2 s baseline, followed by a brief sound cue (50 ms, 85 dB) to signal trial start to the mouse. In the pairing and interleaved trials, the looming sound was played 1 s after the sound cue and was followed immediately by the whisker stimulus. In a subset of control experiments, the non-looming sound was played instead of the looming sound (see Supplementary Fig. 2). Each trial was 8 to 9 s long and trials were presented with

an inter-trial interval of 2 to 5 s, to render them irregular in presentation timing. White noise (65 dB) was played during the entire duration of the imaging session. Sound and whisker stimulus delivery was controlled by custom-written software in C.

Whisker tracking

The whisker field was illuminated with a 940-nm infrared LED light and movies were acquired at 100 Hz (500 × 500 pixels) using a pixy camera system coupled with an Arduino board to track the position of the whiskers in real time [33].

Two-photon calcium imaging

We used a custom-built 2-photon microscope controlled by ScanImage 2019 equipped with a fixed wavelength fiber laser at 1064 nm (Fidelity; Coherent) and a Ti:sapphire laser system (~100-fs laser pulses; Mai Tai BB; Newport Spectra Physics), a water-immersion objective (16×LWDPF, 0.8 NA; Nikon), resonant scan mirrors (model 6210; Cambridge Technology), and two Pockel's Cell (Conoptics) for laser intensity modulation. For calcium imaging, RCaMP1.07 was excited at 1064 nm with the Fidelity and GCaMP6f at 915 nm with the Mai Tai. Emitted fluorescence was collected with red (617/73 nm) and green (520/60 nm) emission filters for RCaMP and GCaMP fluorescence detection, respectively. Images were acquired at 30 Hz with 512 x 512-pixel resolution.

Histology

After the calcium imaging recordings, mice were deeply anaesthetized by intraperitoneal injection of 80/10 ketamine/xylazine mixture and transcardially perfused with 4% paraformaldehyde (PFA). Brains were removed and post-fixed in PFA for 24–48h at 4°C and were subsequently washed in phosphate-buffered saline and sliced at 100 µm. Brain slices were mounted using Mowiol® 4-88 prior to imaging on a LEICA m205 FCA fluorescence stereo microscope.

QUANTIFICATION AND STATISTICAL ANALYSIS

Two-photon calcium data processing

Somatic calcium signals were automatically detected using the Python-based CaImAn analysis pipeline, which performed motion correction, source extraction, spike deconvolution and component registration[34]. Calcium signals are presented here as $\Delta F/F=(F-F_0)/F_0$, where F_0 was calculated for each trial as the bottom 10th percentile of that trial. Detected neurons were considered responsive to a stimulus (sound, sound-offset, whisker, mismatch) if their mean $\Delta F/F$ calculated in a 1 s window from stimulus onset was significantly larger than its mean baseline, calculated in a 1 s window preceding the stimulus onset (30 frames before and after the stimulus onset, Wilcoxon-Mann-Whitney test). The significance of their response was then determined by comparing the distribution of their single trial responses (single trial mean $\Delta F/F$ calculated in a 1 s window from stimulus onset minus the mean baseline window 1 s before stimulus onset) against the distribution of 1000 randomly selected events (random baseline corrected mean $\Delta F/F$ calculated in a 1 s window as above). Significance was determined with a two-sided Mann-Whitney-U test ($p < 0.05$). We subsequently classified these shuffle-corrected neurons as looming-, non-looming-, looming offset-, non-looming offset-, whisker-, pairing sound-, pairing-, paired- or mismatch-responsive, based on the session in which they were significantly responsive. A neuron was considered mismatch-responsive if the mean $\Delta F/F$ of the response to the mismatch trials (determined 1 s from whisker stimulus/omission onset) was larger than its corresponding response to the interleaved paired trials. A neuron was considered paired-responsive in the interleaved trials if it met the same conditions as mismatch neurons, but for paired trials. Hence the mismatch- and interleaved paired-responsive populations were mutually exclusive. Response probabilities of neurons were calculated by dividing the number of responsive trials by the total number of trials presented for a particular stimulus in a session. A neuron was considered responsive in a trial when its $\Delta F/F$ trace in a 1 s window from stimulus onset was significantly larger than its baseline, calculated in a 1 s window preceding the stimulus onset different (30 frames before and after the stimulus onset, Wilcoxon-Mann-Whitney test). Population average responses presented here were determined from the averaged traces of each neuron that has been identified as being session responsive in its respective stimulus window. The

population average neuronal traces were baseline-corrected by subtracting the mean of the baseline (1 s prior to stimulus onset, corresponding to the sound presentation window) from the entire averaged trace. For the experiments in Fig. 3 with a 1 s delay, the sound presentation window was used as the baseline. We present the mean $\Delta F/F$ as a first measure of neuronal activity.

Statistical analysis

All data are presented as mean ± s.e.m. unless stated otherwise. The non-parametric Wilcoxon signed-rank paired test and Wilcoxon-Mann-Whitney test for paired and unpaired group comparisons were performed respectively. All tests were two-sided. We did not test for a normal distribution of the data.

ACKNOWLEDGEMENTS

We thank K. Wilmes and M. Aime for comments on an earlier version of the manuscript. We thank the members of the Nevian laboratory for comments on the manuscript. We thank C. Dellenbach, C. Käser, E. Scheuner, and J. Burkhalter for their excellent technical support in electronics and mechanics. We thank M. Känzig for his help in animal husbandry.

AUTHOR CONTRIBUTIONS

C.R., S.K., B.L and S.S. performed the experiments and analyzed the data. T.N. provided materials and resources. S.K. setup the IOS, whisker tracking, and data analysis pipeline. C.R. and S.S designed the experiments. S.S. built the microscope and secured the funding. C.R. and S.S. wrote the manuscript with comments from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure legends

Fig. 1 Creating a sensory association at the PPC. **a** Schematic of the sequence of stimulus presentation. All trials begin with a brief sound cue (green dot in **b**). Looming and non-looming sounds (horizontal black bars in **b**) are randomly interleaved in the sound trials. The whisker stimulus (orange dot in **b**) delivered by a magnetic coil is presented alone in the whisker session. The looming sound is paired with the whisker stimulus in pairing session. In the interleaved session, the paired trials (looming sound followed by whisker stimulus) are randomly interleaved with the mismatch trials where the whisker stimulus is omitted. **b** Example stimulus-responsive neurons classified as looming sound-, looming sound offset-, whisker-, pairing sound-, pairing whisker-, interleaved paired-, and interleaved mismatch-responsive neurons from layer 2/3 of the PPC, expressing RCaMP1.07. Average (black) and single trial (grey) $\Delta F/F$ calcium traces are shown, along with the average whisker position (green). **c** Heat maps representing the average stimulus responses of looming sound offset- (88), whisker- (292), pairing whisker- (161), interleaved paired- (280) and interleaved mismatch-responsive neurons (68) from 6 wild-type mice. Mean pre-stimulus activity (1 s) was subtracted from the calcium transients and neurons were sorted to their peak $\Delta F/F$ response times in the stimulus window. **d** Population averages (± s.e.m.) of $\Delta F/F$ traces of the neurons in (**c**), in addition to looming sound- (92) and pairing sound-responsive neurons (123), along with their corresponding population average for the other presented stimuli. The average whisker position from the above sessions is shown below in green. The number of neurons is indicated in brackets. **e** Average population responses of looming sound-, pairing sound- and tactile (whisker, pairing)-responsive neurons. **f** Average population responses of mismatch-responsive neurons over mismatch trials. **g** Same as **e** for stimulus versus mismatch-responsive neurons shown in **d**. Data represented as mean ± s.e.m. Statistical significance is indicated by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$, with two-sided Wilcoxon-Mann-Whitney test.

Fig. 2 Dynamic representation of whisker stimuli in the PPC compared to S1. **a** Schematic of the whisker stimulus presentation (same as **Fig. 1a**), with population

averages (± s.e.m.) of $\Delta F/F$ traces of whisker- (93), pairing- (60) and paired-responsive (67) layer 2/3 excitatory neurons in S1, along with their corresponding population average for the other sessions (4 Thy-1GCaMP6f mice across 4 field of view (fov)) The number of neurons is indicated in brackets. Traces are shown from 1.5 s prior to whisker stimulus onset. **b** Average population responses of the respective whisker-, pairing- and paired-responsive neurons in **a** across the sessions. **c** Venn diagram of session-responsive neurons in **a**, with their respective overlaps between sessions. **d** Average population pre-stimulus response (corresponds to shaded area in **f**), of pairing- and paired-responsive neurons, across the pairing and paired sessions. **e** Average response probability of whisker-, pairing- and paired-responsive neurons in **a**, in their respective session. **f**–**j** Same as **a**–**e**, but for excitatory layer 2/3 neurons in the PPC (whisker n = 47 neurons; pairing n = 43 neurons; paired n = 60 neurons, 4 thy-1GCaMP6f mice across 6 fovs). Data represented as mean ± s.e.m. Statistical significance is indicated by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$, with two-sided Wilcoxon signed-rank paired test (**b**,**d**,**g**,**i**) and two-sided Wilcoxon-Mann-Whitney test (**e**,**j**).

Fig. 3 PPC can reliably update the mismatch response and report different types of mismatch. **a** Heat maps and corresponding population averages (± s.e.m.) of $\Delta F/F$ traces of interleaved paired-responsive neurons with no delay (0 ms) and a 1000 ms delay between the looming sound and whisker stimulus presentation (n = 281 neurons without delay, n = 344 neurons with delay, 6 mice). The average whisker position from the above sessions is shown below in green. **b** Average population pre-stimulus and post-stimulus responses of interleaved paired-responsive neurons, without and with delay. **c**–**d** same as **a**–**b**, but for mismatch-responsive neurons (without delay n =84 neurons, with delay n = 105 neurons). **e** Schematic of the presentation of different mismatch trial types in the form of whisker stimulus omission, decreased and increased whisker stimulus intensity. **f** Population averages (± s.e.m.) of $\Delta F/F$ traces of interleaved mismatch-responsive neurons and paired-responsive neurons, for the interleaved sessions with omission (mismatch neurons n = 137; paired neurons n = 438), decreased (mismatch neurons n = 147; paired neurons n = 388) and increased

(mismatch neurons n = 145; paired neurons n = 378) whisker stimulus mismatches, with their corresponding paired (red) and mismatch (blue) trial averages. **g** Average population responses of mismatch-responsive and paired-responsive neurons, for the interleaved sessions with omission, decreased and increased whisker stimulus mismatches. **h** Venn diagram of the different mismatch-responsive neurons in **f**, their respective overlaps between mismatch sessions. Data represented as mean ± s.e.m. Statistical significance is indicated by ** for $p < 0.01$, and *** for $p < 0.001$, with two-sided Wilcoxon-Mann-Whitney test.

Fig. 4 M2 modulates sensory processing in the PPC. **a** Injection scheme to express the inhibitory DREADD hM4Di in M2 neurons projecting to the PPC (sagittal view), along with a coronal section showing their expression in M2. **b** Schematic of the of whisker stimuli presentation (same as **Fig. 2a**), to these mice. Population averages (± s.e.m.) of $\Delta F/F$ traces of whisker (174), pairing- (71) and paired-responsive (139) layer 2/3 neurons in the PPC with silencing of M2 by systemic application of CNO, along with their corresponding population average for the other sessions (3 mice, across 6 fovs). The number of neurons is indicated in brackets. **c** Average population responses of the respective whisker, pairing- and paired-responsive neurons in **b** across the sessions. **d** Venn diagram of session-responsive neurons in **b**, with their respective overlaps between sessions. **e** Average population pre-stimulus response (corresponds to shaded area in **g**), of pairing- and paired-responsive neurons, across the pairing and paired sessions. **f** Average response probability of whisker-, pairing- and paired-responsive neurons in **c**, in their respective session. **g**–**k** same as **b**–**f**, but for layer 2/3 neurons in the PPC without the silencing of M2 (whisker n = 203 neurons; pairing n = 86 neurons; paired n = 181 neurons, same mice as in **b**, 6 fovs). Data represented as mean ± s.e.m. Statistical significance is indicated by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$, with two-sided Wilcoxon signed-rank paired test (**c**,**e**,**h**,**j**) and two-sided Wilcoxon-Mann-Whitney test (**f**,**k**).

Fig. 5 M2 contributes to the top-down prediction to the PPC. **a** Population averages (± s.e.m.) of $\Delta F/F$ traces of interleaved mismatch-responsive neurons in layer 2/3 neurons

in the PPC with (n = 41 neurons) and without (n = 98 neurons) silencing of M2 by systemic application of CNO (same mice as in **Fig. 4**), with their corresponding average population response. **b** Population averages (± s.e.m.) of $\Delta F/F$ traces of interleaved paired-responsive and mismatch-responsive neurons (averaged together), with (n = 180 neurons) and without (n = 279 neurons) silencing of M2 by systemic application of CNO, with their corresponding average population responses. Data represented as mean ± s.e.m. Statistical significance is indicated by * for $p < 0.05$ and *** for $p < 0.001$, with two-sided Wilcoxon-Mann-Whitney test.

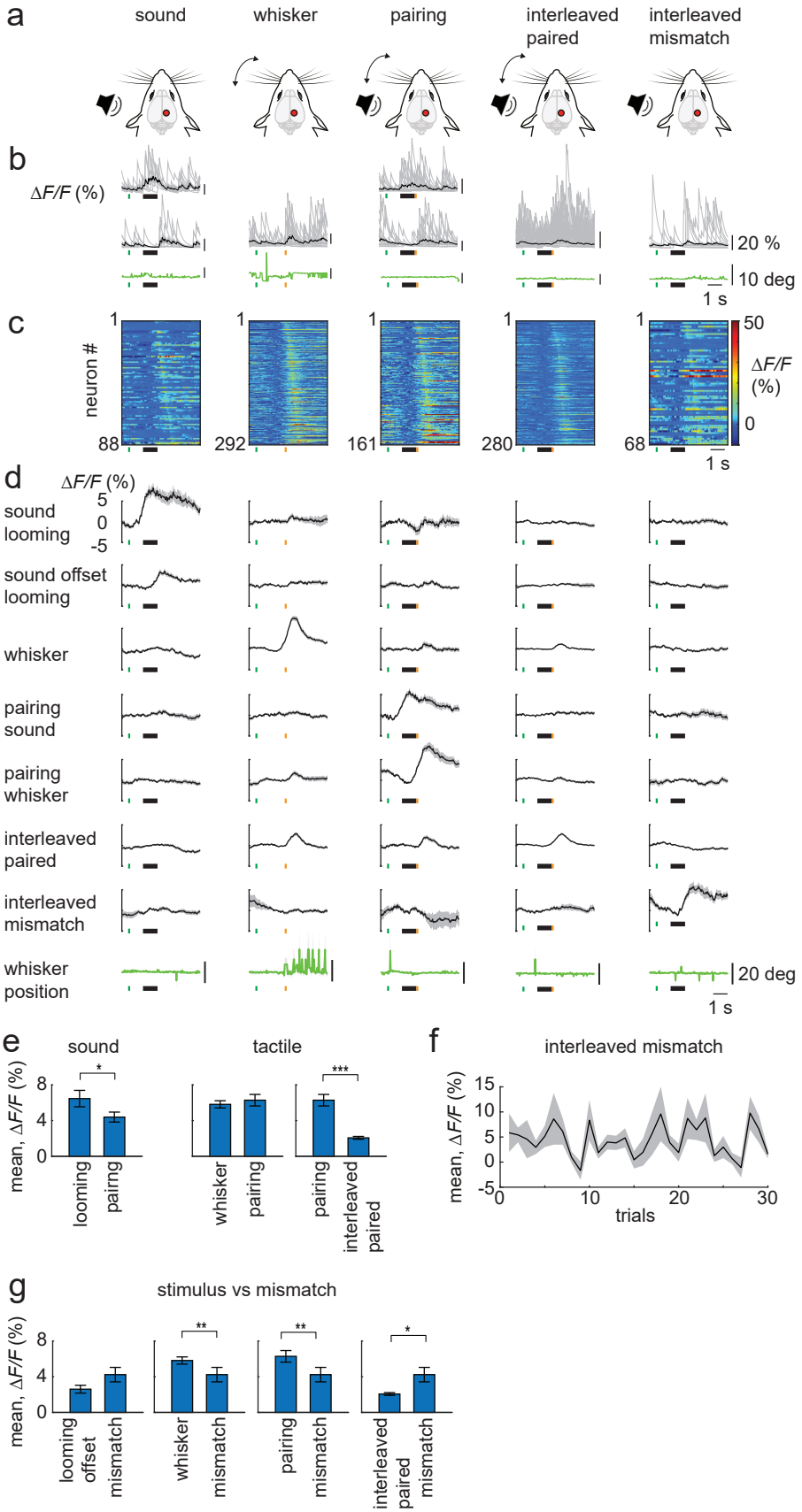

Figure 1
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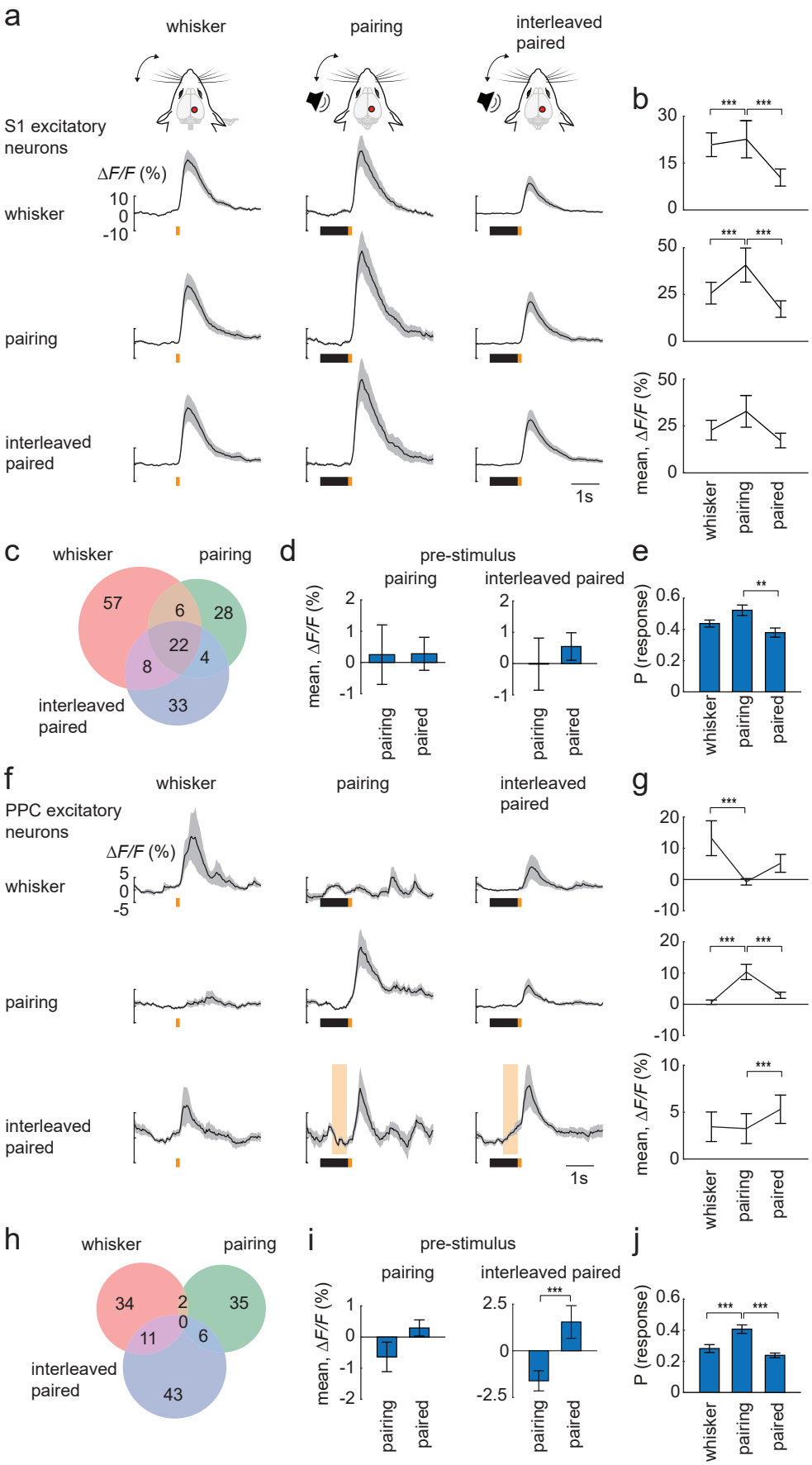

Figure 2
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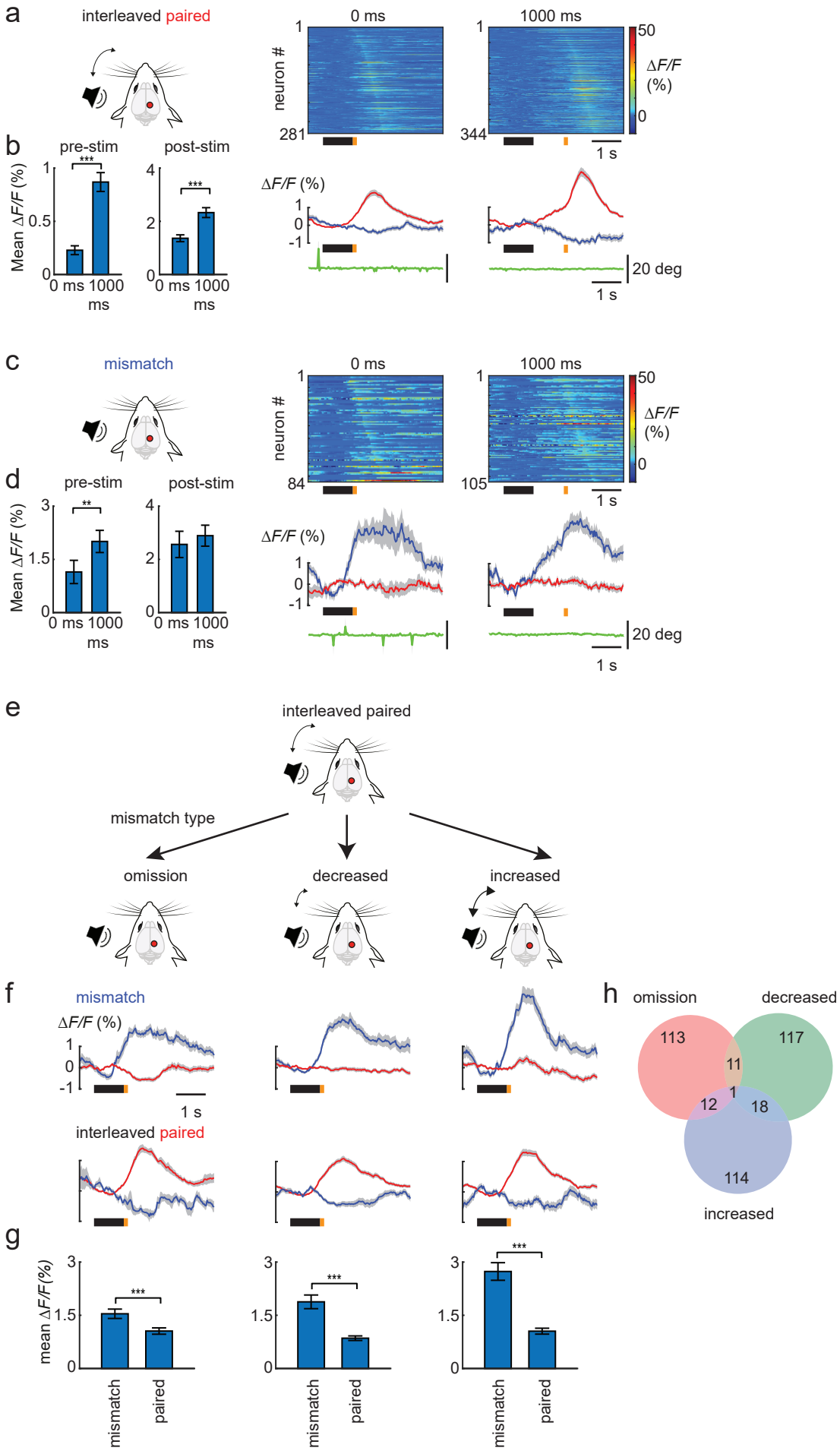

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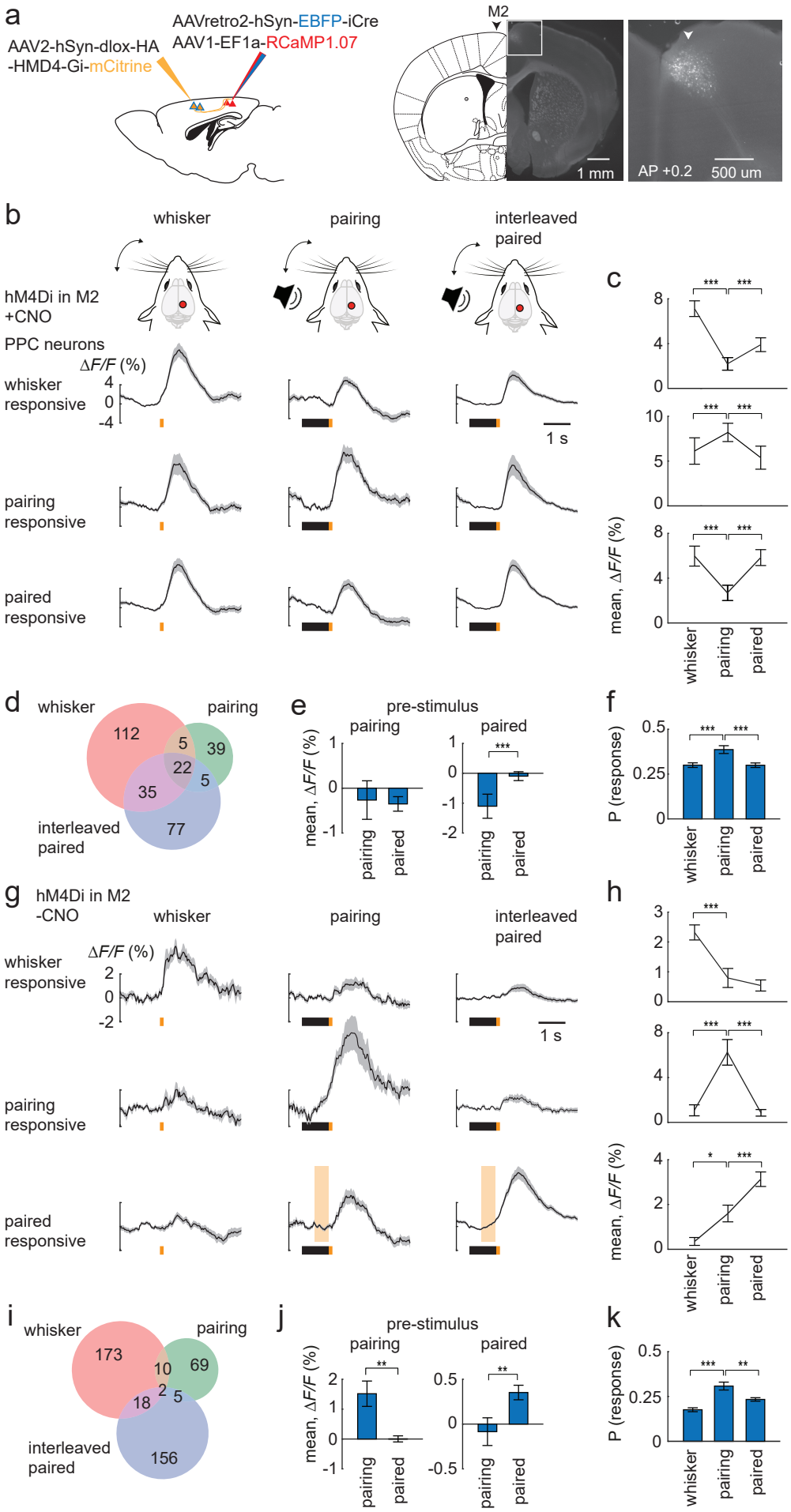

Figure 4
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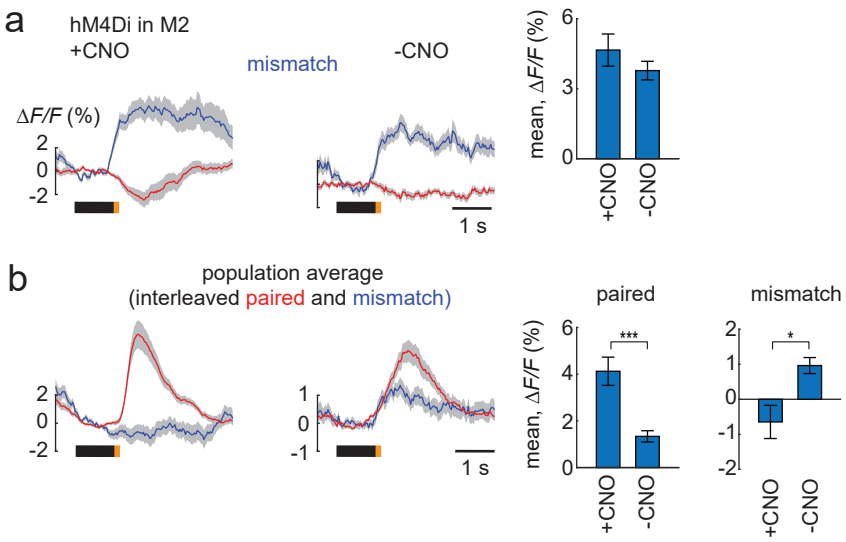

Figure 5
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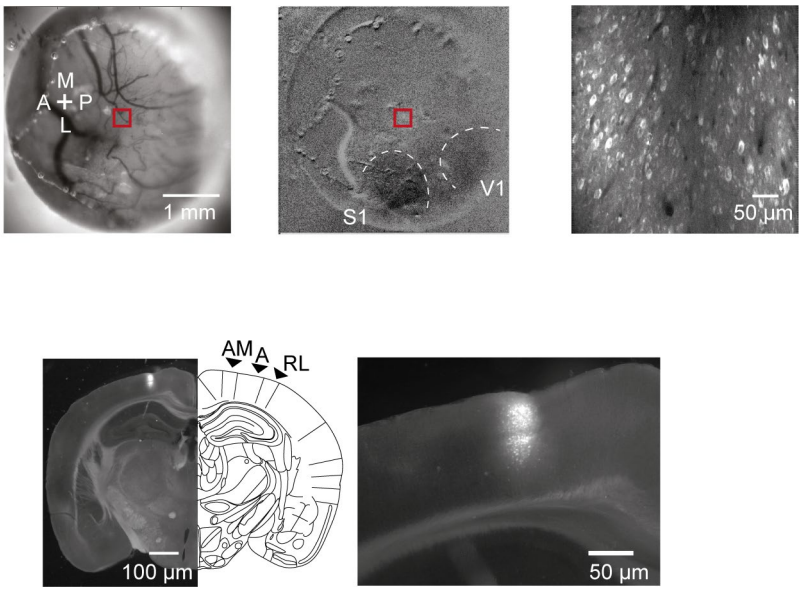
a

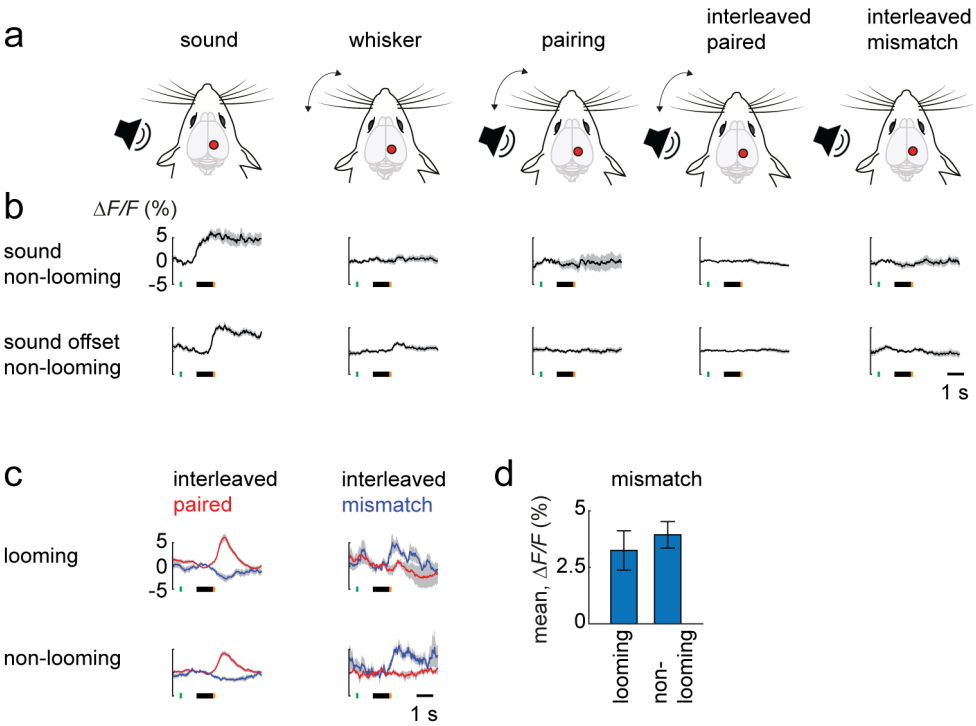
b



Supplementary Figure 1. Locating area A of the PPC through intrinsic optical signal imaging (IOS).

a Left, view of a chronically implanted cranial window over the PPC, with the imaging site outlined in red. Centre, location of the γ and δ -whisker barrel in S1 and of V1, as determined with IOS seen through a 4-mm cranial window. Right, view of the imaged field of view (red box) of layer 2/3 neurons expressing the GECI RCaMP1.07, as acquired with the 2-photon microscope.

b Left, transverse section of a brain slice with RCaMP1.07 expression in layers 2/3 of the PPC. Right, a close-up of the GECI expression site.



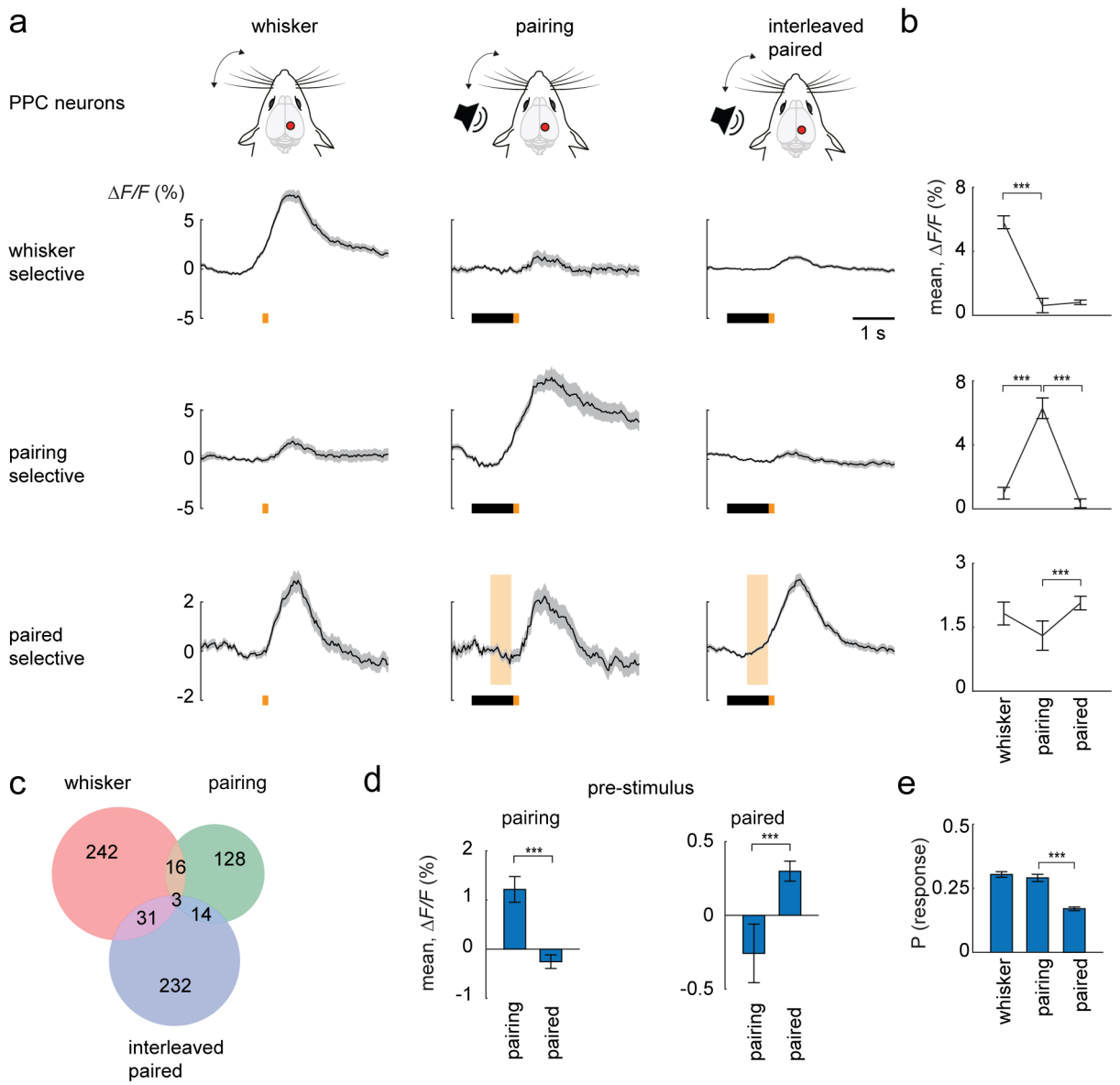
Supplementary Figure 2. Creating a sensory association with a non-looming sound.

a Schematic of the sequence of stimulus presentation (same as **Fig. 1a**).

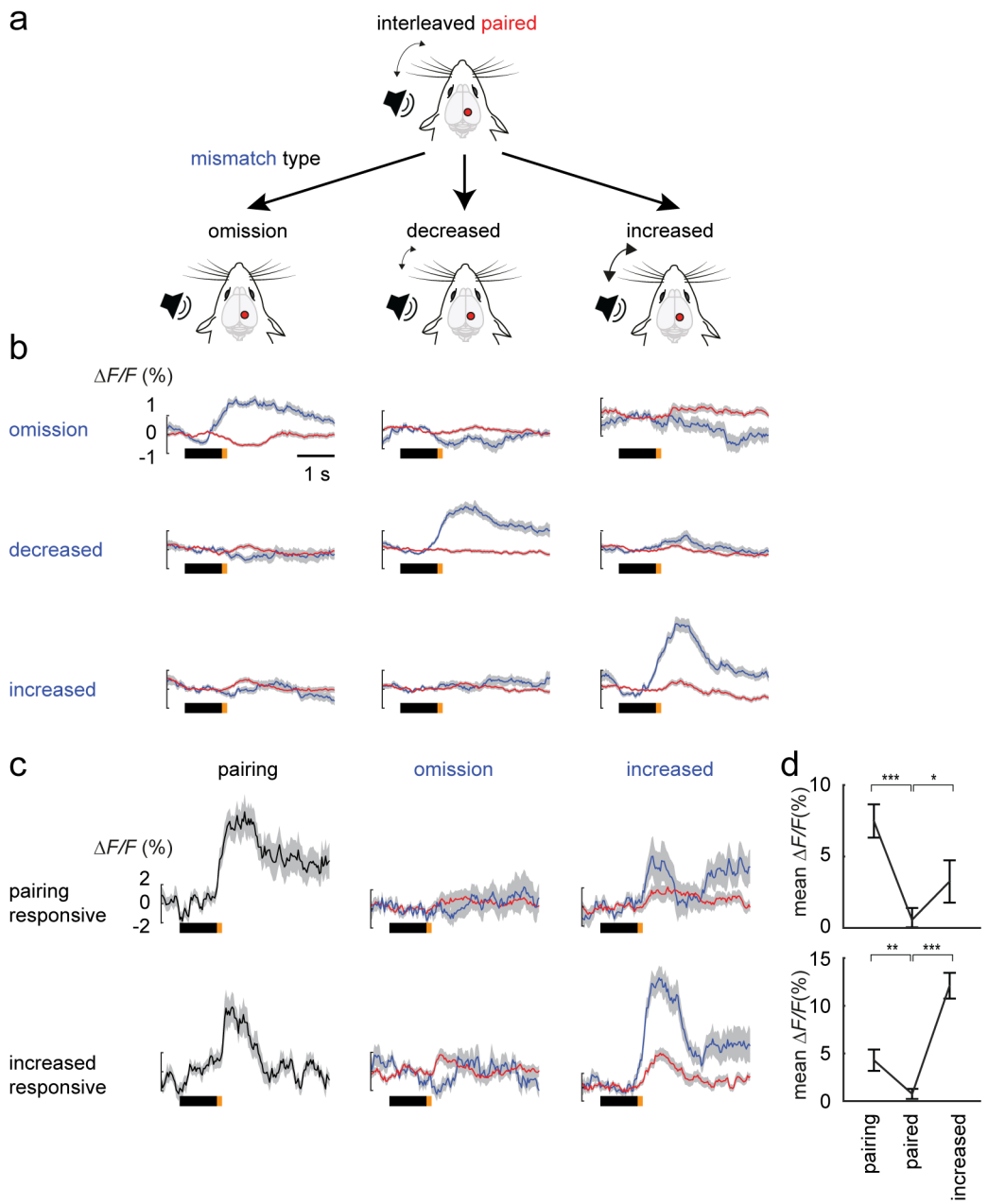
b Same as **Fig. 1c**, for non-looming sound (77 neurons) and non-looming sound offset-responsive neurons (132 neurons).

c Population averages (± s.e.m.) of ΔF/F traces of interleaved paired and mismatch-responsive neurons, when the whisker stimulus is associated with a looming and non-looming sound respectively.

d Average population responses of mismatch-responsive neurons, with a looming (n = 9 neurons) and non-looming (n = 16 neurons) sound. Data represented as mean ± s.e.m.



Supplementary Figure 3. Dynamic representation of whisker stimuli in the PPC. **a-e** Same as **Fig. 2**, for the neurons in **Fig. 1**. Data represented as mean ± s.e.m. Statistical significance is indicated by *** for $p < 0.001$, with two-sided Wilcoxon signed-rank paired test (**b**,**d**) and two-sided Wilcoxon-Mann-Whitney test (**e**).

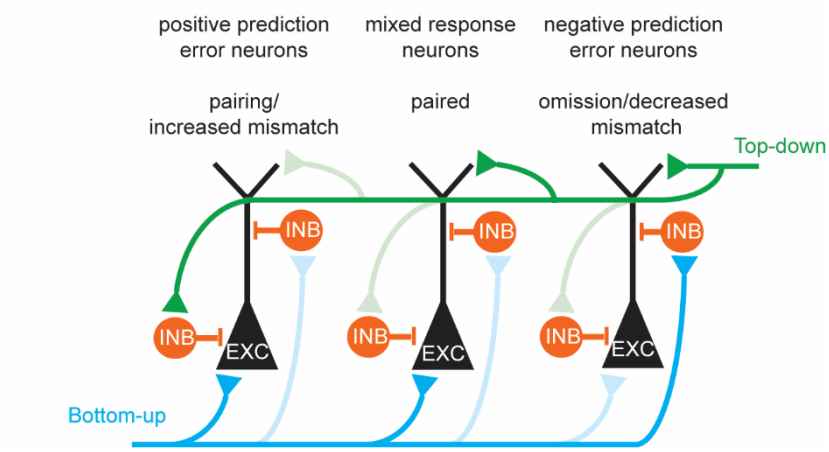


Supplementary Figure 4. PPC neurons can report different types of mismatch.

a-b Same as **Fig. 3e-f**, with corresponding population average traces across the different mismatch trials for each mismatch selective population.

c Population averages (± s.e.m.) of ΔF/F traces of pairing (41 neurons) and increased-responsive (mismatch) neurons (57 neurons), across the pairing, omission-mismatch and increased-mismatch sessions.

d Average population responses of the neurons in **c** across the sessions. Data represented as mean ± s.e.m. Statistical significance is indicated by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$, with two-sided Wilcoxon signed-rank paired test.



Supplementary Figure 5. Microcircuit for predictive processing in the PPC.

We have adapted the proposed canonical microcircuit for predictive processing to include the respective session-responsive neurons that we have identified, in this simplified model restricted to layer 2/3 neurons. In this model, the positive prediction error neurons receive bottom-up input in the form of the whisker stimulus, which is balanced by top-down inhibition. The pairing-responsive neurons could represent this class of positive prediction error neurons. They are suppressed in transition from the pairing to interleaved session, as the mismatch trials are introduced. These pairing-responsive neurons can than report the increased-mismatch (**Supplementary Fig. 4c-d**, where the whisker stimulus is stronger than expected, compared to the interleaved paired trials (positive prediction error). The paired-responsive neurons increase their response to the whisker stimulus in transition from the pairing to the interleaved session, as they now receive more top-down feedback (matched with an increased pre-stimulus response in **Fig. 2i**). Lastly the negative prediction error neurons receive top-down feedback that is balanced by bottom-up inhibition. Both the omission and decreased intensity mismatch-responsive neurons could represent these negative prediction error neurons as they are recruited during the omission of the whisker stimulus and the presentation of a decreased whisker stimulus intensity respectively.