Stimulation of the ventral tegmental area induces visual cortical plasticity at the neuronal level

Graphical abstract

Authors

Jerome Herpers, John T. Arsenault, Wim Vanduffel, Rufin Vogels

Correspondence

rufin.vogels@kuleuven.be

In brief

Herpers et al. show that pairing a visual orientation with electrical micro-stimulation of the ventral tegmental area (VTA-EM) during a demanding orthogonal task changes the response properties of posterior inferior temporal (PIT) cortical neurons in macaques.

Highlights

- Pairing a visual stimulus with VTA-EM biases behavioral stimulus preferences
- VTA-EM paired with an orientation increases the PIT firing rate for that feature
- VTA-EM-stimulus pairing during a demanding orthogonal task changes PIT firing rate
- VTA-EM-stimulus pairing is sufficient to induce plasticity at the spiking level

Article

Stimulation of the ventral tegmental area induces visual cortical plasticity at the neuronal level

Jerome Herpers,^{1,2} John T. Arsenault,^{1,2} Wim Vanduffel,^{1,2,3,4} and Rufin Vogels^{1,2,5,*}
¹Laboratory for Neuro- and Psychophysiology, Department of Neurosciences, KU Leuven Medical School, 3000 Leuven, Belgium

²Leuven Brain Institute, KU Leuven, 3000 Leuven, Belgium

³Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA 02129, USA

⁴Department of Radiology, Harvard Medical School, Boston, MA 02144, USA

⁵Lead contact

*Correspondence: rufin.vogels@kuleuven.be
<https://doi.org/10.1016/j.celrep.2021.109998>

SUMMARY

fMRI studies have shown that pairing a task-irrelevant visual feature with electrical micro-stimulation of the ventral tegmental area (VTA-EM) is sufficient to increase the sensory cortical representation of the paired feature and to improve perceptual performance. However, since fMRI provides an indirect measure of neural activity, the neural response changes underlying the fMRI activations are unknown. Here, we pair a task-irrelevant grating orientation with VTA-EM while attention is directed to a difficult orthogonal task. We examine the changes in neural response properties in macaques by recording spiking activity in the posterior inferior temporal cortex, the locus of fMRI-defined plasticity in previous studies. We observe a relative increase in mean spike rate and preference for the VTA-EM paired orientation compared to an unpaired orientation, which is unrelated to attention. These results demonstrate that VTA-EM-stimulus pairing is sufficient to induce sensory cortical plasticity at the spiking level in nonhuman primates.

INTRODUCTION

Constant adaptation to changing environments is paramount to survival. The brain facilitates behavioral adaptation by cortical plasticity (Huttenlocher, 2002; Kolb et al., 2003). Improvement in visual detection and discrimination as a result of training in visual detection and discrimination tasks, known as visual perceptual learning (VPL), correlates with plastic changes in visual cortical processing (for reviews, see Doshier and Lu, 2017; Li, 2016; Maniglia and Seitz, 2018; Sasaki et al., 2010; and Vogels, 2010). In visual cortical areas, bottom-up sensory information might be combined with extra-retinal regulatory signals to induce a change in synaptic connections during VPL. Regulatory signaling can come from top-down attentional modulation and/or from neuromodulatory reinforcement signals such as reward (Roelfsema et al., 2010; Watanabe and Sasaki, 2015).

Neuromodulatory signals, when paired with a visual target, are sufficient to induce VPL, even without directed attention to the stimulus. This has been demonstrated in seminal studies in which a task-irrelevant subliminal stimulus was paired with salient events, including rewards (Watanabe et al., 2001; Nishina et al., 2007; Seitz et al., 2009; Huang and Watanabe, 2012; Bruns and Watanabe, 2019). In such task-irrelevant VPL (TI-VPL) paradigms, the performance improvement was greatest when the saliency of the task-irrelevant stimulus was too weak to draw attention (Tsushima et al., 2008; Roelfsema et al., 2010).

One potential neuromodulatory system that is involved in TI-VPL, and VPL in general, is the dopaminergic system, originating from the ventral tegmental area (VTA; Bissonette and Roesch, 2016; Morales and Margolis, 2017). The VTA projects to the cortex, including the visual cortex (Berger et al., 1991; Zubair et al., 2021). VTA dopaminergic neurons generate a reward prediction error when there is a discrepancy between expected and experienced reward (Schultz, 2016). It has been demonstrated in rodents that pairing a suprathreshold auditory stimulus with electrical micro-stimulation of the VTA (VTA-EM) expands the cortical representation of the paired stimulus (Bao et al., 2001).

Recent studies in macaques showed increased activation to stimuli that were paired with VTA-EM (Arsenault and Vanduffel, 2019; Murris et al., 2021). In these studies, sub- or peri-threshold motion direction, grating orientation (Arsenault and Vanduffel, 2019), and faces and bodies (Murris et al., 2021) were paired with VTA-EM during an orthogonal color discrimination task, directing attention away from the VTA-EM paired stimuli. Under these conditions, pairing the task-irrelevant stimulus feature with VTA-EM enhanced both visual performance and fMRI activations for the paired feature (Arsenault and Vanduffel, 2019; Murris et al., 2021). Specifically, pairing VTA-EM with grating orientation or motion direction increased fMRI activation for the paired feature in posterior inferotemporal cortex (PIT; Arsenault and Vanduffel, 2019). The locus of the plastic changes for these simple stimulus features might be surprising because PIT is a higher-order visual area of the ventral pathway,

implicated in color and shape processing (Boussaoud et al., 1991; Zeki, 1996; Conway, 2018). However, a VPL single-unit study also showed changes in the orientation preference of PIT neurons following coarse orientation discrimination learning (Adab et al., 2014).

Arsenault and Vanduffel (2019) employed fMRI to demonstrate changes in PIT activation following VTA-EM-stimulus pairing. Since fMRI is an indirect measure of neural activity (Logothetis and Wandell, 2004), it remains to be tested whether spiking activity in PIT changes as a result of VTA-EM-stimulus pairing. To examine this, we recorded spiking activity in PIT of monkeys after pairing a task-irrelevant grating orientation with VTA-EM. We predicted greater neural responses and preference for the VTA-EM paired orientation compared to an orthogonal, unpaired orientation. To test this hypothesis, we first mapped with fMRI the region in PIT responsive to gratings at the visual field location employed in the subsequent VTA-EM-stimulus pairing. Then, we recorded multi-unit activity (MUA) in that PIT region after a period of daily VTA-EM-grating orientation pairings and assessed the change in neural response properties as a result of the VTA-EM-stimulus pairing.

RESULTS

We investigated in two macaques how pairing a low visibility task-irrelevant grating orientation with VTA-EM during an orthogonal color discrimination task affects the response properties of PIT neurons. The PIT region we recorded from was based on an fMRI localizer experiment that was performed before the VTA-EM and recording sessions in each monkey. Prior to the experiments in which we paired VTA-EM with a grating orientation, we verified that the implanted VTA electrodes affect free-viewing behavior in a stimulus-VTA-EM association paradigm.

Free viewing and VTA-EM-stimulus pairing

We chronically implanted microwire brush electrodes (Bondar et al., 2009) in the VTA of the right hemisphere (RH) of monkey M1 and the left hemisphere (LH) of monkey M2 (Figures 1A and 1B). Earlier, we implanted an electrode in the RH of M2, as well. However, MRI showed that this electrode was positioned slightly too deep, and therefore we do not describe the behavioral or recording data obtained with this electrode. It has been shown previously, with both operant and classical conditioning methods, that pairing a visual stimulus with VTA-EM produces a behavioral preference for the paired stimulus (Arsenault et al., 2014). We employed this behavioral effect to verify that VTA-EM with the implanted electrodes was effective. Specifically, we presented two colorful, geometrical fractal images during passive fixation in random order, and one of the two fractals was consistently paired with VTA-EM (about 60 pairing trials; current amplitude ranged from 300 to 900 μ A in M1 and was 1,000 μ A in M2; see STAR Methods for visual and EM parameters).

Before and after a VTA-EM pairing block, a free-viewing task was performed in which we presented the same two fractals measuring the preference of the monkey for one of the fractals. During the free-viewing task, the monkeys could look anywhere on the screen to receive a juice reward, while the two fractal im-

ages were presented left and right of the center, with their position interchanging randomly over trials. This procedure was repeated for different arbitrarily chosen fractal pairs in each session, with one of them paired with VTA-EM, across multiple daily sessions (16 and 11 fractal pairs for M1 and M2, respectively). A preference index (PI) was computed per free-viewing procedure by taking the time looked at the VTA-EM paired fractal minus the time looked at the non-paired fractal, divided by the sum of the two viewing times. The closer the PI value is to 1, the more time the monkey looked at the paired fractal image, while 0 indicates no preference, and -1 indicates a preference for the fractal not paired with VTA-EM. In Figures 1C and 1D we compare the PI of the pre- and post-VTA-EM free-viewing procedures by subtracting the PI of the pre-VTA-EM free-viewing procedure from the PI of the post-VTA-EM free-viewing procedure, where positive difference scores indicate longer viewing times post-stimulation for the VTA-EM paired fractal compared to the non-paired fractal.

We found that both monkeys spent relatively more time looking at the VTA-EM paired fractal post-stimulation compared to the non-VTA-EM paired fractal (Figures 1C and 1D, Wilcoxon signed-rank test: M1, $p = 0.005$; M2, $p = 0.001$). This demonstrates that the implanted VTA electrodes and our stimulation protocol were effective in changing the behavioral stimulus preference of the monkey, similar to Arsenault et al. (2014).

fMRI mapping of grating activations

Arsenault and Vanduffel (2019) showed that pairing a grating orientation in one visual field with VTA-EM enhances its representation in the PIT ventral area (PITv), but only by indirectly measuring neuronal activity using fMRI. Our aim of the present experiments was to reveal the changes in spiking activity that underlie the enhanced fMRI activation for the paired grating. First, we employed fMRI mapping to localize the PIT region that was activated by gratings using the same orientations, size, and visual field locations (5° eccentricity in the lower visual field) as employed in the subsequent VTA-EM pairing. Gratings (2° diameter) with orientations of 45° and 135° were presented in the lower left visual field (LVF) and lower right visual field (RVF) during fixation. To reveal the regions activated by the gratings, we contrasted the two stimulus presentation positions (LVF versus RVF), pooling the two orientations.

As expected, this resulted in activations in primary and retinotopic extrastriate visual cortex. Both monkeys showed activation to the grating in the posterior inferior temporal cortex. The location of activation in PITv was consistent among hemispheres and monkeys, mainly dorsal to the posterior middle temporal sulcus (PMTS) (Figures 1E and 1F). Based on the activations in PIT, recording chambers were positioned such that the activated area could be targeted.

PIT response properties after pairing a low-visibility grating with VTA-EM

An orthogonal color discrimination task (Adab et al., 2014; Arsenault and Vanduffel, 2019) was used to investigate whether pairing a low-visibility task-irrelevant grating (20% signal-to-noise ratio [SNR]) with VTA-EM induces changes in the neural response properties of PIT (Figures 2A and 2B). To direct attention away

Figure 1. VTA-EM and PIT recording locations

(A and B) Location of VTA microwire brush electrodes. Sagittal and horizontal planes of T1-weighted anatomical MRI image. The electrode tips (indicated by arrows and circles) are positioned in the midbrain posterior to the substantia nigra in the right hemisphere (RH) of M1 and the left hemisphere (LH) of M2.

(C and D) Effect of VTA-EM on free-viewing behavior after VTA-EM compared to before VTA-EM. The preference index (PI; [viewing time for the VTA-EM paired fractal – viewing time for the non-paired fractal]/total viewing time) in the free-viewing task was computed for each of n different stimulus-VTA-EM pairings. PI post – pre corresponds to the PI after pairing minus the PI before pairing. Median differences in PI post – pre are indicated ($\blacktriangledown$) on the distributions of PI post – pre for each monkey. The monkeys developed a greater preference for the fractal image paired with VTA-EM after stimulation (M1, $p = 0.005$; M2, $p = 0.001$).

(E and F) fMRI activations and estimated recording positions in the posterior inferior temporal cortex. fMRI activations (within white lines; grating in RVF versus grating in LVF; $t > 4.9$) in the lower visual field (5° eccentricity) are shown. The estimated recording sites are indicated in red for the hemispheres from which the data are presented.

from the task-irrelevant gratings, the monkeys performed a difficult color discrimination of a peripheral small square presented in the upper visual field, while the gratings were presented in the lower visual field. The difference between the color discriminanda was titrated daily so that the performance was $\sim 75\%$ – 85% , well below the ceiling level. The visibility and saliency of the grating was reduced by using two manipulations: the gratings were masked by white noise that varied across frames

and were embedded in a noise background that also changed each frame. Before starting the VTA-EM pairing with a grating orientation, we measured the color discrimination performance of each monkey for different combinations of stimulus duration (50–250 ms in steps of 50 ms) and SNR level (0%, 5%, 10%, 15%, 20%, and 50%). Based on these tests, we selected an SNR (20%) and duration (150 ms) of the grating that showed equal color discrimination performance compared to trials

Figure 2. Color discrimination paradigm and overview of VTA-EM conditions

(A and B) Color discrimination task.

(A) Trial without VTA-EM. After fixating for 500 ms, a colored square (cue) was shown in the upper visual field for 150 ms. Depending on the color, the monkey had to make a saccade to the left or the right target square after a delay period of 200 ms. The response targets were presented for 500 ms. During the presentation of the color cue, a grating (encircled by dashed lines) was shown in the lower left or lower right visual field at 20% SNR during the VTA-EM task and at 10%, 15%, 20%, or 40% SNR during the electrophysiological recordings. The cue, targets, and gratings are not shown to scale.

(B) VTA-EM trial. After presentation of the stimuli, VTA-EM was applied for 200 ms, after which the monkey had to respond with an eye movement to the left or right depending on the color of the square. Same conventions as in (A).

(C) Order of VTA-EM conditions. Recorded hemispheres after 15–49 VTA-EM-stimulus pairing blocks. The stimulated hemisphere (symbol: δ) and VTA-EM paired grating orientation (45° or 135°) are indicated. The current strength and frequency of the VTA-EM are presented to the right of the VTA-EM conditions. During the first VTA-EM condition of a hemisphere, the recording was alternated every 4 days between the hemisphere with the implanted VTA-EM electrodes (STIM) and the hemisphere without VTA-EM (NO STIM). The conditions for which the data are described are highlighted with a brighter background color.

when no grating was presented, thus for an invisible 0% SNR (full noise) stimulus (Figure S1A). Because the selected SNR and duration of the grating did not impair the color discrimination, we infer that the simultaneously presented grating did not elicit exogenous attention.

During a VTA-EM pairing block, the monkeys were performing the color discrimination task while one of two oriented gratings was presented in the lower LVF or RVF, at the same locations as in the prior fMRI mapping. The presentation of one orientation at the visual field location contralateral to the VTA electrode was followed by a 200-ms train of VTA-EM (see STAR Methods). Pseudo-randomly interleaved presentations of the orthogonal orientation at that location and of the two orientations at the ipsilateral location were not followed by VTA-EM. Recording sessions were preceded by 15–49 days of color discrimination + VTA-EM pairing sessions. Daily sessions starting with a VTA-EM block were followed by MUA recordings in PIT while presenting gratings during a passive fixation and the color discrimination task (Figure 2A; see STAR Methods for complete design).

First, we will present data of the main conditions in which a grating with an orientation of 45° was paired with VTA-EM (STIM45) followed by the condition in which an orientation of 135° was paired with VTA-EM (STIM135) in the RH of M1 and LH of M2 (Figure 2C; see STAR Methods for complete procedure). In between the STIM45 and STIM135 conditions of M1, we also recorded from PIT in the RH, yet without VTA-EM, to examine the effect of extinction on the neural responses. Also, we recorded from LH PIT in M2 before the LH VTA-EM pairing conditions. The data of these additional conditions without daily VTA-EM are described later.

Population response during fixation task

Based on the fMRI study by Arsenault and Vanduffel (2019), we predicted that the spiking activity for the orientation paired with VTA-EM would be on average greater than that for the orthogonal, unpaired orientation. To test this prediction, we measured MUA to single gratings (2° diameter, 100% Michelson contrast, two cycles/degree, phase randomized) of 80% SNR at eight orientations (0°–157.5° in steps of 22.5°), presented at the location that corresponded to that of the grating paired with VTA-EM (5° eccentricity in the lower visual field contralateral to the recorded hemisphere). The stimuli were presented during a passive fixation task immediately following the VTA-EM block. MUA recordings targeted PIT (Figures 1E and 1F) with 16-channel probes (NeuroNexus). Recording sites overlapped with the fMRI mapped activated region (see above) and mean receptive fields of recorded units overlapped with the grating location (Figure S2; see STAR Methods). For the fixation task, responsive units were selected with a split-plot ANOVA that compared the response to the stimulus with baseline across the eight orientations (see STAR Methods). This yielded 609 and 280 units for the STIM45 and STIM135 condition in M1, respectively, and 323 and 257 units for STIM45 and STIM135 in M2, respectively.

Figure 3B shows the averaged normalized net (baseline-subtracted) firing rate for all responsive units to each of the eight orientations for the STIM45 and STIM135 conditions of each monkey. The population responses differed significantly between the two stimulation conditions in each monkey (two-way ANOVA; interaction orientation and VTA-EM condition: M1, $p < 10^{-7}$; M2, $p = 0.003$). Each recording day, we made one penetration in PIT. In each penetration, the laminar probe was positioned at three and two nonoverlapping depths in M1 and M2,

Figure 3. PIT population response to high-visibility gratings during fixation

(A) Monkeys were presented with gratings (80% SNR) in eight orientations in the lower visual field contralateral to the recorded hemisphere. The difference in normalized net response to orientations 45° and 135° ($\Delta 45_{135}$) is shown for each VTA-EM condition and monkey. $\Delta 45_{135}$ is significantly greater for the STIM45 condition compared to the STIM135 condition in each monkey (***p < 0.001, ****p < 0.0001). Error bars indicate 95% CI. The inset shows an orientation that is parallel to a line radiating from the center of the visual field, i.e., congruent to the radial bias (RB) orientation, for the left visual field, represented in the RH. For the right visual field (LH), the same orientation is orthogonal to the radial bias orientation. Hence, for the RH of M1, 45° corresponds to the radial bias orientation while it is orthogonal to the radial bias orientation for the LH of M2. For the latter hemisphere and monkey, the radial bias orientation will be 135°.

(B) Mean normalized net response to the eight orientations for the two VTA-EM conditions (STIM45 and STIM135). The VTA-EM paired orientation (φ) is indicated for each condition by a color code, as is the orientation congruent to the radial bias orientation of that hemisphere (RB). An interaction effect between VTA-EM condition and orientation was found in both monkeys (M1, p < 10^{-7} , M2, p = 0.003). Number of units (n) are indicated for each condition. Error bars indicate standard errors of the mean.

(C) The contribution of VTA-EM (stimulation) and radial bias and their interaction to the normalized net responses for the 45° and 135° orientations was assessed with linear modeling. Error bars of the beta values correspond to 95% CI. The number of observations that entered the regression is indicated for each monkey. Note that for this analysis we equated the number of units of each condition.

(D) Regression coefficients resulting from a linear mixed-effects model in which the data of both monkeys were combined with monkey as a random variable. The numbers of units of each condition and monkey were equated. Same conventions as in (C).

respectively, and we recorded successively from these probe positions. Data from the different cortical depths recorded during the same condition showed similar patterns (data not shown). Since the orthogonal orientations of 45° and 135° were used in successive VTA-EM conditions, we compared the difference in response to the presentation of these orientations within VTA-EM conditions by subtracting the response of 135° from the response to 45° for each unit ($\Delta 45_{135}$). We expected that $\Delta 45_{135}$ would be greater for the STIM45 condition compared to the STIM135 condition, which was indeed the case in each monkey (Figure 3A; Mann-Whitney U test: M1, p = 10^{-11} ; M2, p = 0.0001). This held true when normalizing the net response of each unit by its maximal response across the eight orientations (Figure 3A), and also for unnormalized net responses (data not shown).

fMRI, two-photon Ca^{2+} imaging, and single-unit studies suggested that neurons in striate (Ju et al., 2021) and extrastriate cortex (Shen et al., 2014) show a preference for the orientation radiating from the center of the visual field (radial bias [RB]; Sasaki et al., 2006). To control for such radial bias effects, we used as the first VTA-EM condition the orientation congruent with the radial bias orientation in M1, and the orientation orthogonal to the radial bias orientation in M2. Since we recorded in the lower visual field representation of the RH of M1 and the LH of M2, the first VTA-EM condition was STIM45 for both monkeys.

Because of the counterbalanced radial bias orientation across monkeys, and the pairing of each of the two orthogonal orientations in successive stimulation conditions within each monkey, the consistently lower $\Delta 45_{135}$ for STIM135 compared to the STIM45 condition cannot be explained by the radial bias, but must be induced by VTA-EM-orientation pairing.

To assess the potential contributions of VTA-EM and radial bias to the responses, we combined the normalized responses to the 45° and 135° orientations of the two VTA-EM orientation pairing conditions in one linear model (LM) regression analysis. We employed two categorical predictors: (1) whether the presented orientation to the unit was paired with VTA-EM (STIM), and (2) whether it corresponded to the radial bias orientation. We also included an interaction term and fitted the normalized net response of the units using the predictors for each monkey separately (see STAR Methods). In both monkeys, the radial bias coefficient was significantly different from zero (95% confidence interval [CI] not overlapping zero) and positive (Figure 3C), indicating on average stronger responses for the radial bias orientation. Importantly, the STIM coefficient was positive in both monkeys, although it was only significant in M2. The interaction coefficient was significant in both monkeys, demonstrating an effect of VTA-EM that interacted with the radial bias orientation factor in each monkey. The sign of the interaction coefficient differed between animals: VTA-EM resulted in a larger difference between

Figure 4. Effect of VTA-EM orientation pairing on PIT response properties (high visibility gratings during fixation)

(A) Preferred orientation (PO). For both monkeys, the difference in percentage of units with preferred orientations 45° and 135° (Pc45_135°) is plotted for the STIM45 and the STIM135 conditions.

(B) Distribution of POs. The percentage of units with a preference for each of the eight orientations is shown for each monkey and VTA-EM condition. The VTA-EM paired orientation (ϕ) and the orientation congruent to the radial bias orientation (RB) are indicated. Same conventions as in Figure 3B.

(C) Fano factor. The difference in Fano factor between orientations 45° and 135° plotted for the VTA-EM conditions. Significant but opposite effects were found for both monkeys when comparing VTA-EM conditions.

(D) Noise correlations. The difference in noise correlations between orientations 45° and 135°. In accordance with the Fano factor, opposite effects were found over monkeys when comparing VTA-EM conditions. Error bars indicate 95% CI. **p < 0.01, ***p < 0.001, ****p < 0.0001.

the two VTA-EM conditions when the paired orientation coincided with the radial bias orientation than when the paired orientation was orthogonal to the radial bias orientation in M1 (i.e., a positive interaction coefficient) while the opposite was the case in M2 (Figure 3B). This difference between M1 and M2 in the sign of the interaction term could be due to an effect of the order of VTA-EM pairing, which differed between the monkeys, instead of radial bias per se. In M1, the orientation congruent with the radial bias orientation (45°) was the first one paired with VTA-EM in M1 (STIM45), while in M2, the radial bias orientation (135°) corresponded to the second VTA-EM condition in monkey M2 (STIM135). The sign of the interaction term in the regression analysis suggests a greater effect of VTA-EM for STIM45, i.e., the first VTA-EM pairing condition, compared to STIM135 in each monkey, which reconciles the results of the two monkeys. Whatever the reason for the difference in the sign of the interaction coefficient between monkeys, the significant interaction term demonstrates a genuine effect of VTA-EM on the responses in each monkey. Also, when combining the data of both animals and fitting a linear mixed-effects model with the monkey as a random variable (see STAR Methods) produced significant positive effects of radial bias and STIM, without a significant interaction coefficient (Figure 3D).

From these analyses, we conclude that VTA-EM-orientation pairing increases the response in PIT to the paired compared to the orthogonal, unpaired orientation.

Preferred orientation in fixation task

The increased population response to the VTA-EM paired orientation can result from more neurons preferring the paired compared

to the orthogonal orientation and/or stronger activity to the VTA-EM paired orientation. To assess the former hypothesis, we computed for each responsive unit its preferred orientation (PO) by taking the orientation with the largest net response. The distribution of the preferred orientation is shown in Figure 4B for each VTA-EM condition (STIM45 and STIM135) and monkey. We computed the percentage of units for each VTA-EM condition that preferred 45° and 135° and subtracted for each condition the percentage of units that preferred 135° from the percentage that preferred 45°. We hypothesized that this percentage difference (Pc45_135) would be larger for the STIM45 condition compared to the STIM135 condition, which was indeed the case (Figure 4A; randomization test (see STAR Methods): M1, p < 0.0001; M2, p = 0.004).

In principle, both the effect on preferred orientation and mean response might have resulted from a biased sampling of neurons with a particular preferred orientation, e.g., oversampling of an orientation column that happens to correspond to the orientation paired with VTA-EM. There are several arguments against this possibility. First, to the best of our knowledge, there is no evidence for orientation columns in PIT (e.g., Vanduffel et al., 2002). Second, even when orientation columns would exist in PIT, we avoided sampling from an orientation column by making penetrations that are mainly tangential to the cortex (Figures 1E and 1F), thus spanning many putative columns. Third, we assessed directly whether there was spatial clustering of preferred orientations in our laminar recordings. To do this, we computed the difference in preferred orientation for all channel pairs recorded in the same penetration and sorted these according to the distance between the channels of a pair. The mean difference in preferred orientation and 95% CI that is expected without spatial clustering was computed by permutation of the data (see STAR Methods). Evidence for clustering of preferred

orientation would manifest itself as an increase in the difference in preferred orientations as a function of distance. No such trend was apparent in either monkey, except for a somewhat lower difference in preferred orientations for neighboring channels. The mean differences in preferred orientation were close to those expected when there is no spatial clustering using the distribution of preferred orientations in the data (Figure S3).

Fano factor and noise correlations in fixation task

Previous studies reported a decrease in Fano factor (mean-normalized trial-to-trial variance) and/or noise correlations for learned stimuli after perceptual learning in visual discrimination tasks (Raiguel et al., 2006; Gu et al., 2011; Herrero et al., 2013; Adab et al., 2014; Yan et al., 2014; Ni et al., 2018; Sanayei et al., 2018). To examine whether VTA-EM-orientation pairing affects response variability and noise correlations for the paired orientation, we computed the Fano factor and noise correlations for 45° and 135° and compared these between VTA-EM pairing conditions.

We computed for each responsive unit the Fano factor by dividing for each orientation the trial-to-trial variance by the mean response of the same trials. Per VTA-EM condition, the Fano factor for orientation 135° was subtracted from the Fano factor for 45° for each unit. These differences in Fano factor were compared over VTA-EM conditions and revealed significant, but opposite, effects for both monkeys (Mann-Whitney U test: M1, $p = 0.004$; M2, $p = 0.003$; Figure 4C): a decrease of the Fano factor for the paired orientation in M1 but an increase in M2. These effects were not caused by differences in firing rate between the paired and unpaired orientation since spike rate mean-matched Fano factors (see STAR Methods) produced highly similar results.

Noise correlations, i.e., the correlation in a trial-by-trial response between simultaneously recorded units, for 135° were subtracted from noise correlations for 45° for each unit pair and subsequently compared over VTA-EM conditions. Similar to the Fano factor, the effects found for noise correlations were significant, but inconsistent, over monkeys (Figure 4D, Mann-Whitney U test: M1, $p = 0.001$; M2, $p = 0.003$): a reduction of noise correlations for the paired orientation in M1 and an increase in M2. Noise correlations were also computed as a function of the distance between the channels per penetration (Figure S4), showing as expected generally higher correlations for shorter distances between channels.

In sum, VTA-EM-orientation pairing produced correlated effects on noise correlations and the Fano factor, but the sign of the effects was opposite in the two animals, which renders their interpretation difficult.

Population response during the orthogonal color discrimination task

Thus far, the presented data were obtained during a passive fixation task using a high SNR grating. The VTA-EM-orientation pairing was performed while the animals were performing a demanding orthogonal color discrimination task during which a low SNR grating was task-irrelevant. To reduce the contribution of attention to the grating directly, we also measured MUA in PIT to task-irrelevant gratings (four orientations [0°–135° in steps of

45°] at low SNR levels of 10%, 15%, 20%, and 40%). As during the VTA-EM-orientation pairing, the difficulty of the color discrimination task was titrated to be around 80% correct, well below the ceiling level. To assess whether the VTA-EM pairing increased covert attention to the paired grating, we computed the difference in behavioral performance in the color discrimination task between the trials in which the paired and its orthogonal, unpaired orientation were presented. This was done for each recording session in which the color discrimination was performed, and the data were pooled over the VTA-EM conditions. Per monkey and SNR level, the median difference between the color discrimination performance for the VTA-EM paired orientation and the orthogonal orientation was (approximately) 0% (Figures S1C and S1D), suggesting that the VTA-EM pairing did not increase covert attention to the paired orientation. Thus, any difference in response between paired and orthogonal orientation we show below is unlikely to be attributed to attentional differences.

The number of units that were selected as responsive (split-plot ANOVA; see STAR Methods) in the color discrimination task were 696 (STIM45) and 255 (STIM135) for M1, and 272 (STIM45) and 217 (STIM135) for M2. Figure 5 shows the population peristimulus time histograms (PSTHs) for the orientations 45° and 135°, together with the difference in response between the two orientations. For comparison, we also show the PSTHs for the same orientations obtained in the passive fixation condition (80% SNR). As expected, the net normalized response increased with higher SNR. Importantly, the response difference between the 45° and 135° orientation was also observed in the color discrimination task, and Δ_{45_135} was greater for the STIM45 compared with the STIM135 condition, as in the passive fixation task. Note that the response differences between the paired and orthogonal orientations occurred at or close to response onset (~100 ms), suggesting that the increased response to the VTA-EM paired orientation is not due to feedback from downstream areas.

Figure 6 plots the Δ_{45_135} averaged in a 150-ms-long window (see STAR Methods) for each SNR and stimulation condition for each monkey, obtained during the color discrimination task. A split-plot ANOVA with as repeated factor SNR and between-unit factor stimulation condition showed a significant effect of the stimulation factor (M1, $p = 3e^{-61}$; M2, $p = 0.004$), in agreement with the results obtained in the passive fixation task.

Recordings without VTA-EM

To investigate whether the effect found after a period of VTA-EM would persist over time or whether it was dependent on daily stimulation blocks, we recorded after the STIM45 condition for 12 days in PIT of the RH of M1 without daily VTA-EM blocks (Figure 2C). MUA responses for gratings presented during the fixation ($n = 323$) and color discrimination task ($n = 360$) showed similar results to those obtained in the preceding STIM45 condition (Figure S5). The population response in the passive fixation task showed a clear peak at 45°, and the orientation of 135° elicited the smallest response of the eight orientations (Figure S5A). However, a significant interaction effect was found when these data were compared to the population response of the STIM45 condition (two-way ANOVA: interaction effect, $p = 0.0001$),

Figure 5. Population peristimulus time histograms (PSTHs)

Task-irrelevant gratings with a SNR of 10%, 15%, 20%, and 40% were presented during an orthogonal color discrimination task, and 80% SNR gratings were presented during fixation. Mean normalized net MUAs to gratings with orientations of 45° and 135° are plotted for each monkey and VTA-EM condition. ‡ indicates the orientation paired with VTA-EM. The differences in activity between the two orientations (Δ_{45-135}) are presented below each PSTH. Stimulus onset corresponds to 0. Shaded bands indicate standard errors of the mean. The numbers of units (n) are indicated for each condition.

suggesting a (small) reduction of the VTA-EM pairing effect. The distribution of the preferred orientation was similar to that obtained before in the STIM45 condition (Figure S5B). Responses to low SNR gratings during the orthogonal color discrimination task also showed a larger response for 45° than 135° for all SNRs (Figure S5C).

For M2, we also recorded in the LH before commencing VTA-EM in this hemisphere (NO STIM condition; Figure 1C). For the population responses and orientation preference obtained during the fixation task with high SNR gratings ($n = 405$), a radial bias effect can be observed, with responses and percentages of preferred orientations generally greater for the 90°–180° quadrant of the polar plot compared to the 0°–90° quadrant (Figures

S5D and S5E). For the population response, the NO STIM condition differed significantly from the later STIM45 condition (two-way ANOVA: interaction effect, $p = 0.04$). For the color discrimination task ($n = 325$), we observed that units had negative net normalized responses on average for the three lowest SNR levels and a response around 0 for an SNR level of 40% (Figure S5F). We hypothesized that the negative responses might be a result of spatial attention being drawn away excessively by the orthogonal color discrimination task in this subject. To investigate this, M2 performed a fixation task during which gratings were presented at the same SNR levels as in the color discrimination task (10%, 15%, 20%, and 40%). The observed mean normalized responses ($n = 92$) for the three lowest SNR

Figure 6. Difference in mean response to task-irrelevant gratings during color discrimination

The difference in mean net normalized response between orientation 45° and 135° (Δ_{45_135}) is plotted for both VTA-EM conditions. The Δ_{45_135} responses were greater for the STIM45 condition compared to the STIM135 condition in both monkeys (M1, $p = 3e^{-61}$; M2, $p = 0.004$). Error bars indicate 95% CI.

levels were hovering around 0 and around 0.4 for 40% SNR, suggesting that in this monkey there was indeed strong suppression of the response to the grating during the orthogonal task, before the VTA-EM-orientation pairing.

DISCUSSION

Pairing a task-irrelevant visual feature, an orientation, with VTA-EM, an area signaling reward prediction errors (Schultz, 2016), increased spiking activity in response to the paired feature in PIT. The orientation was paired with VTA-EM while the monkey was performing a demanding orthogonal color discrimination task, and behavioral measures showed that the presentation of the low SNR grating during this task did not capture attention. No evidence for increased covert attention for the paired orientation was present when the monkeys were performing the orthogonal color discrimination task during the PIT recordings after the pairing blocks. This suggests that the stronger response to the VTA-EM paired orientation observed in those recordings is not due to enhanced attention to the paired orientation. These electrophysiological data reveal the neural basis of the enhanced activations for orientations paired with VTA-EM observed with fMRI in macaque PIT (Arsenault and Vanduffel, 2019).

Radial bias and VTA-EM pairing

We demonstrated the effect of VTA-EM pairing by comparing neural responses within the same monkey to the same stimuli between consecutive periods in which different orthogonal orientations were paired with VTA-EM. This allowed us also to dissociate changes in response due to VTA-EM pairing and orientation biases of the population of PIT neurons. Some studies suggest that the orientation preferences of neurons in early visual cortical areas show a bias for radial orientations (Sasaki et al., 2006; Shen et al., 2014; Ju et al., 2021). The regression analysis suggested that our recorded PIT neurons show a similar bias for radial orientations. Importantly, this effect of radial bias interacted in each monkey with the orthogonally manipulated factor of VTA-EM pairing, demonstrating a significant effect of VTA-EM pairing on the spiking activity. The interaction between radial bias and VTA-EM pairing had a different sign in the two monkeys, but this may reflect the order in which the radial bias orientation was paired with VTA-EM, which differed between monkeys. This interpretation suggests that the relative effect of

VTA-EM pairing is greater for the first paired orientation compared to the second paired, orthogonal orientation. One explanation could be a long-term effect of the first orientation-VTA-EM pairing. This explanation agrees with our finding in M1 that after VTA-EM pairing of an orientation, the increased response and preference for the paired orientation compared to the orthogonal orientation was still present, even though that orientation was no longer paired with VTA-EM for 12 days. However, note that the paired orientation in that condition was the orientation congruent with the radial bias orientation, which might have caused this apparently slow extinction. Further studies are required to investigate sequential effects of stimulus-VTA-stimulation pairing and the timescale of its extinction.

Ruling out potential confounds

In addition to the radial bias in orientation preference, we can rule out other alternative explanations of the response changes that we observed in the VTA-EM pairing blocks. First, because the orientation paired with VTA-EM was presented as frequently as the unpaired, orthogonal orientation, the increased response to the paired orientation cannot be due to mere sensory exposure or familiarity. Second, oversampling of putative orientation columns in PIT is also unlikely to underlie the greater response to the paired orientation since no evidence for spatial clustering of orientation preference was present in our data samples. In fact, to our knowledge, there is no evidence for orientation columns in PIT (Vanduffel et al., 2002). Third, we did not observe a behavioral cost of the presentation of the paired orientation during the color discrimination task in either monkey, which suggests that attentional capture by the paired orientation is very unlikely a cause of the response enhancement for that orientation.

fMRI activations and behavioral changes following VTA-EM stimulus pairing

Arsenault and Vanduffel (2019) measured an increased fMRI response in visual areas after employing VTA-EM for task-irrelevant grating stimuli during a similar orthogonal color discrimination task. They observed the strongest effect of VTA-EM pairing in PIT, which motivated our choice of the target area for the recordings. The present results suggest that an increased spiking response to the VTA-EM paired orientation can underlie the increased fMRI activations.

VTA-EM-stimulus pairing can also enhance the behavioral discriminability of the paired stimulus features in macaques, even when these are subliminal and unattended (Arsenault and Vanduffel, 2019; Murris et al., 2021). The response increase to a task-irrelevant feature paired with VTA-EM that we demonstrated here may underlie this behavioral enhancement. A similar increased perceptual sensitivity has been observed in humans when a task-irrelevant stimulus is paired with a reward (Watanabe et al., 2001; Seitz et al., 2009). Thus, our findings may provide a plausible neural substrate of such task-irrelevant perceptual learning.

Mechanisms of plasticity following VTA-EM stimulus pairing

We show that prior to pairing a task-irrelevant grating with VTA-EM, neural responses to low SNR gratings can be suppressed, compared to baseline activity, while the monkey is performing the orthogonal task. Yet, after VTA-EM, the representation of the VTA-EM paired orientation is increased. This raises the question of how such weak, or even suppressed, responses are augmented by VTA-EM. It is very likely that VTA-EM increases the activity of the dopaminergic output neurons and thus enhances dopamine in its projection targets, which includes PIT (Zubair et al., 2021). *In vitro* studies suggest that dopamine can enhance synaptic plasticity through several mechanisms (Speranza et al., 2021), such as inhibition of GABAergic transmission (Bissière et al., 2003; Xu and Yao, 2010; Frémaux and Gerstner, 2016). Thus, a plausible scheme is that dopamine release enables the enhancement of the synaptic strength of the afferents that signal the paired stimulus by inhibiting GABAergic inhibition. However, it is plausible that other neuromodulators, which are co-activated by VTA stimulation, are involved as well. Conclusive evidence for the role of dopamine awaits experiments in which intracortical dopaminergic transmission is manipulated directly.

Task-relevant perceptual learning

In most perceptual learning paradigms, stimuli are associated with responses and correct responses are rewarded (at least in macaque perceptual learning studies). This begs the question to what extent reward (internal or external) is also driving plasticity in task-relevant perceptual learning (Watanabe and Sasaki, 2015). Task-relevant perceptual learning appears to produce changes in the tuning of neurons that are optimal to solve the trained task, e.g., fine orientation discrimination increases the sensitivity for the trained orientations in those visual cortical neurons of which the preferred orientation is offset from the trained orientation (Schoups et al., 2001; Raiguel et al., 2006), while this is not the case for coarse orientation discrimination (Adab and Vogels, 2011). It is unclear whether a global reinforcement signal, such as a dopaminergic reward prediction error signal, can produce such task-specific changes in tuning. A computational model that uses only reward prediction errors showed a reweighting of the sensory input to the decision stage (Law and Gold, 2009) but did not model changes at the sensory area level. However, models that simulated the changes in single-unit tuning following task-relevant perceptual learning involved either knowledge of the stimulus label (Wenliang and Seitz, 2018) or

combined a global reward prediction error and attentional feedback signal (Roelfsema et al., 2010). Indeed, selective attention to the trained stimulus is present when subjects are trained in task-relevant perceptual learning and can facilitate perceptual learning (Szpiro and Carrasco, 2015). However, it is unclear whether task-specific changes in neural tuning during perceptual learning occur, perhaps much slower and weaker, without selective attention mechanisms. Further studies are needed to assess the role of reward prediction error and/or dopaminergic activity in task-relevant perceptual learning and its ill-understood transfer to other features and tasks in some training protocols (Xiao et al., 2008).

Perceptual learning in orientation discrimination (Raiguel et al., 2006; Adab et al., 2014), contrast discrimination (Sanayei et al., 2018), and disparity discrimination (Gu et al., 2011) reduces the Fano factor in extrastriate visual areas. In addition, several studies reported decreased noise correlations with perceptual learning (Gu et al., 2011; Herrero et al., 2013; Yan et al., 2014; Ni et al., 2018; Sanayei et al., 2018), but their relationship to perceptual improvement still is unclear (Sanayei et al., 2018). We also found effects of VTA-EM-orientation pairing on noise correlations and response variability (Fano factor), but these differed between the monkeys. Nonetheless, these were consistent within each animal: the change across VTA-EM conditions in noise correlations mimicked a change in the same direction of the Fano factor. It is unclear what caused these differences between the monkeys. Since noise correlations depend on the performed task (Cohen and Newsome, 2008; Bondy et al., 2018), the implications of the (small) changes in noise correlation, measured outside the context of a discrimination task, for the performance in such tasks after task-irrelevant stimulus-reward pairing is a subject for further study.

Conclusions

We showed that pairing VTA-EM with a visual feature changed the directly measured neural response properties in PIT. These changes are unlikely to be a result of attentional effects and demonstrate a neural mechanism for the fMRI findings of Arsenault and Vanduffel (2019). These results demonstrate that VTA-EM-stimulus pairing is sufficient to induce sensory cortical plasticity at the spiking level in nonhuman primates.

Limitations of the study

In addition to changes in response strength, we observed a shift in orientation preference, with more PIT neurons preferring the paired compared to the orthogonal orientation. However, because we recorded MUA, it is difficult to dissociate a shift in orientation preference from an increased response to the paired orientation, in particular for PIT neurons, which have a broad orientation tuning (Adab et al., 2014). Interestingly, unlike for area V4 (Adab and Vogels, 2011), there is evidence that expertise in coarse orientation discrimination at low SNR levels increases the number of single PIT neurons that prefer the trained orientations (Adab et al., 2014). Thus, it is not unlikely that VTA-EM-orientation pairing shifts the orientation preference of PIT neurons.

Arsenault and Vanduffel (2019) found the strongest VTA-pairing effect in PIT, but early visual cortical areas, such as V4 and

V3, were activated as well. Therefore, it cannot be excluded that the response changes that we observed in PIT are partially (or completely) inherited from VTA-EM-induced plastic changes in earlier visual cortical areas. It is unlikely that the response differences related to VTA-EM pairing that we observed in PIT result from feedback from later areas, since the latency of these differences was close to the response onset of the PIT neurons.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
 - Lead contact
 - Materials availability
 - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
 - fMRI procedures
 - VTA-EM-stimulus association procedures
 - Main experimental procedures: PIT response properties after pairing a low-visibility grating with VTA-EM
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
 - fMRI data analysis
 - Behavioral analysis: Free viewing
 - Electrophysiology data analysis: Fixation task
 - Behavioral data analysis: Color discrimination
 - Electrophysiology data analysis: Color discrimination task
 - Electrophysiology data analysis: Receptive field mapping

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.celrep.2021.109998>.

ACKNOWLEDGMENTS

The authors thank M. Depaep, W. Depuydt, C. Fransen, A. Hermans, P. Kayenbergh, G. Meulemans, S. Murris, I. Puttemans, C. Ulens, M. Verbeeck, and S. Verstraeten for technical and administrative support. This work was supported by KU Leuven (grant C14/17/109), Fonds Wetenschappelijk Onderzoek-Vlaanderen (FWO-Flanders) (grants G0D5817N, G0B8617N, G090714N, G0E0520N, G088418N, and Odysseus G0007.12), and by the European Union's Horizon 2020 Framework Programme for Research and Innovation under grant agreement 945539 (Human Brain Project SGA3).

AUTHOR CONTRIBUTIONS

Conceptualization, R.V., J.T.A., W.V., and J.H.; methodology, R.V., J.T.A., W.V., and J.H.; investigation, J.H.; analysis, J.H. and J.T.A.; visualization, J.H.; writing – original draft: J.H., R.V., and W.V.; writing – review and editing, J.H., R.V., and W.V.; funding acquisition, J.T.A., R.V., and W.V.; resources and supervision, W.V. and R.V.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 23, 2021

Revised: September 20, 2021

Accepted: October 22, 2021

Published: November 9, 2021

REFERENCES

- Adab, H.Z., and Vogels, R. (2011). Practicing coarse orientation discrimination improves orientation signals in macaque cortical area v4. *Curr. Biol.* 21, 1661–1666.
- Adab, H.Z., Popivanov, I.D., Vanduffel, W., and Vogels, R. (2014). Perceptual learning of simple stimuli modifies stimulus representations in posterior inferior temporal cortex. *J. Cogn. Neurosci.* 26, 2187–2200.
- Arsenault, J.T., and Vanduffel, W. (2019). Ventral midbrain stimulation induces perceptual learning and cortical plasticity in primates. *Nat. Commun.* 10, 3591.
- Arsenault, J.T., Rima, S., Stemmann, H., and Vanduffel, W. (2014). Role of the primate ventral tegmental area in reinforcement and motivation. *Curr. Biol.* 24, 1347–1353.
- Avants, B.B., Tustison, N.J., Song, G., Cook, P.A., Klein, A., and Gee, J.C. (2011). A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage* 54, 2033–2044.
- Bao, S., Chan, V.T., and Merzenich, M.M. (2001). Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature* 412, 79–83.
- Berger, B., Gaspar, P., and Verney, C. (1991). Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. *Trends Neurosci.* 14, 21–27.
- Bissière, S., Humeau, Y., and Lüthi, A. (2003). Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat. Neurosci.* 6, 587–592.
- Bissonette, G.B., and Roesch, M.R. (2016). Development and function of the midbrain dopamine system: What we know and what we need to. *Genes Brain Behav.* 15, 62–73.
- Bondar, I.V., Leopold, D.A., Richmond, B.J., Victor, J.D., and Logothetis, N.K. (2009). Long-term stability of visual pattern selective responses of monkey temporal lobe neurons. *PLoS ONE* 4, e8222.
- Bondy, A.G., Haefner, R.M., and Cumming, B.G. (2018). Feedback determines the structure of correlated variability in primary visual cortex. *Nat. Neurosci.* 21, 598–606.
- Boussaoud, D., Desimone, R., and Ungerleider, L.G. (1991). Visual topography of area TEO in the macaque. *J. Comp. Neurol.* 306, 554–575.
- Bruns, P., and Watanabe, T. (2019). Perceptual learning of task-irrelevant features depends on the sensory context. *Sci. Rep.* 9, 1666.
- Churchland, M.M., Yu, B.M., Cunningham, J.P., Sugrue, L.P., Cohen, M.R., Corrado, G.S., Newsome, W.T., Clark, A.M., Hosseini, P., Scott, B.B., et al. (2010). Stimulus onset quenches neural variability: A widespread cortical phenomenon. *Nat. Neurosci.* 13, 369–378.
- Cohen, M.R., and Newsome, W.T. (2008). Context-dependent changes in functional circuitry in visual area MT. *Neuron* 60, 162–173.
- Conway, B.R. (2018). The organization and operation of inferior temporal cortex. *Annu. Rev. Vis. Sci.* 4, 381–402.
- Cox, R.W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res.* 29, 162–173.
- Doshier, B., and Lu, Z.L. (2017). Visual perceptual learning and models. *Annu. Rev. Vis. Sci.* 3, 343–363.
- Frémaux, N., and Gerstner, W. (2016). Neuromodulated spike-timing-dependent plasticity, and theory of three-factor learning rules. *Front. Neural Circuits* 9, 85.
- Gu, Y., Liu, S., Fetsch, C.R., Yang, Y., Fok, S., Sunkara, A., DeAngelis, G.C., and Angelaki, D.E. (2011). Perceptual learning reduces interneuronal correlations in macaque visual cortex. *Neuron* 71, 750–761.
- Herrero, J.L., Gieselmann, M.A., Sanayei, M., and Thiele, A. (2013). Attention-induced variance and noise correlation reduction in macaque V1 is mediated by NMDA receptors. *Neuron* 78, 729–739.

- Huang, T.R., and Watanabe, T. (2012). Task attention facilitates learning of task-irrelevant stimuli. *PLoS ONE* 7, e35946.
- Huttenlocher, P.R. (2002). *Neural Plasticity: The Effects of Environment on the Development of the Cerebral Cortex* (Harvard University Press).
- Ju, N.S., Guan, S.C., Tao, L., Tang, S.M., and Yu, C. (2021). Orientation tuning and end-stopping in macaque V1 studied with two-photon calcium imaging. *Cereb. Cortex* 31, 2085–2097.
- Kolb, B., Gibb, R., and Robinson, T.E. (2003). Brain plasticity and behavior. *Curr. Dir. Psychol. Sci.* 12, 1–5.
- Kolster, H., Mandeville, J.B., Arsenault, J.T., Ekstrom, L.B., Wald, L.L., and Vanduffel, W. (2009). Visual field map clusters in macaque extrastriate visual cortex. *J. Neurosci.* 29, 7031–7039.
- Law, C.T., and Gold, J.I. (2009). Reinforcement learning can account for associative and perceptual learning on a visual-decision task. *Nat. Neurosci.* 12, 655–663.
- Li, W. (2016). Perceptual learning: Use-dependent cortical plasticity. *Annu. Rev. Vis. Sci.* 2, 109–130.
- Logothetis, N.K., and Wandell, B.A. (2004). Interpreting the BOLD signal. *Annu. Rev. Physiol.* 66, 735–769.
- Maniglia, M., and Seitz, A.R. (2018). Towards a whole brain model of perceptual learning. *Curr. Opin. Behav. Sci.* 20, 47–55.
- Morales, M., and Margolis, E.B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nat. Rev. Neurosci.* 18, 73–85.
- Morris, S.R., Arsenault, J.T., Raman, R., Vogels, R., and Vanduffel, W. (2021). Electrical stimulation of the macaque ventral tegmental area drives category-selective learning without attention. *Neuron* 109, 1381–1395.e7.
- Ni, A.M., Ruff, D.A., Alberts, J.J., Symmonds, J., and Cohen, M.R. (2018). Learning and attention reveal a general relationship between population activity and behavior. *Science* 359, 463–465.
- Nishina, S., Seitz, A.R., Kawato, M., and Watanabe, T. (2007). Effect of spatial distance to the task stimulus on task-irrelevant perceptual learning of static Gabors. *J. Vis.* 7, 2.1–10.
- Raiguel, S., Vogels, R., Mysore, S.G., and Orban, G.A. (2006). Learning to see the difference specifically alters the most informative V4 neurons. *J. Neurosci.* 26, 6589–6602.
- Roelfsema, P.R., van Ooyen, A., and Watanabe, T. (2010). Perceptual learning rules based on reinforcers and attention. *Trends Cogn. Sci.* 14, 64–71.
- Sakai, K., and Miyashita, Y. (1991). Neural organization for the long-term memory of paired associates. *Nature* 354, 152–155.
- Sanayei, M., Chen, X., Chicharro, D., Distler, C., Panzeri, S., and Thiele, A. (2018). Perceptual learning of fine contrast discrimination changes neuronal tuning and population coding in macaque V4. *Nat. Commun.* 9, 4238.
- Sasaki, Y., Rajimehr, R., Kim, B.W., Ekstrom, L.B., Vanduffel, W., and Tootell, R.B. (2006). The radial bias: A different slant on visual orientation sensitivity in human and nonhuman primates. *Neuron* 51, 661–670.
- Sasaki, Y., Nanez, J.E., and Watanabe, T. (2010). Advances in visual perceptual learning and plasticity. *Nat. Rev. Neurosci.* 11, 53–60.
- Schoups, A., Vogels, R., Qian, N., and Orban, G. (2001). Practising orientation identification improves orientation coding in V1 neurons. *Nature* 412, 549–553.
- Schultz, W. (2016). Dopamine reward prediction error coding. *Dialogues Clin. Neurosci.* 18, 23–32.
- Seitz, A.R., Kim, D., and Watanabe, T. (2009). Rewards evoke learning of unconsciously processed visual stimuli in adult humans. *Neuron* 61, 700–707.
- Shen, G., Tao, X., Zhang, B., Smith, E.L., 3rd, and Chino, Y.M. (2014). Oblique effect in visual area 2 of macaque monkeys. *J. Vis.* 14, 1–16.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23 (Suppl 1), S208–S219.
- Speranza, L., di Porzio, U., Viggiano, D., de Donato, A., and Volpicelli, F. (2021). Dopamine: The neuromodulator of long-term synaptic plasticity, reward and movement control. *Cells* 10, 735.
- Szpiro, S.F.A., and Carrasco, M. (2015). Exogenous attention enables perceptual learning. *Psychol. Sci.* 26, 1854–1862.
- Tsushima, Y., Seitz, A.R., and Watanabe, T. (2008). Task-irrelevant learning occurs only when the irrelevant feature is weak. *Curr. Biol.* 18, R516–R517.
- Vanduffel, W., Fize, D., Mandeville, J.B., Nelissen, K., Van Hecke, P., Rosen, B.R., Tootell, R.B., and Orban, G.A. (2001). Visual motion processing investigated using contrast agent-enhanced fMRI in awake behaving monkeys. *Neuron* 32, 565–577.
- Vanduffel, W., Tootell, R.B., Schoups, A.A., and Orban, G.A. (2002). The organization of orientation selectivity throughout macaque visual cortex. *Cereb. Cortex* 12, 647–662.
- Vogels, R. (2010). Mechanisms of visual perceptual learning in macaque visual cortex. *Top. Cogn. Sci.* 2, 239–250.
- Watanabe, T., and Sasaki, Y. (2015). Perceptual learning: Toward a comprehensive theory. *Annu. Rev. Psychol.* 66, 197–221.
- Watanabe, T., Náñez, J.E., and Sasaki, Y. (2001). Perceptual learning without perception. *Nature* 413, 844–848.
- Wenliang, L.K., and Seitz, A.R. (2018). Deep neural networks for modeling visual perceptual learning. *J. Neurosci.* 38, 6028–6044.
- Xiao, L.Q., Zhang, J.Y., Wang, R., Klein, S.A., Levi, D.M., and Yu, C. (2008). Complete transfer of perceptual learning across retinal locations enabled by double training. *Curr. Biol.* 18, 1922–1926.
- Xu, T.X., and Yao, W.D. (2010). D1 and D2 dopamine receptors in separate circuits cooperate to drive associative long-term potentiation in the prefrontal cortex. *Proc. Natl. Acad. Sci. USA* 107, 16366–16371.
- Yan, Y., Rasch, M.J., Chen, M., Xiang, X., Huang, M., Wu, S., and Li, W. (2014). Perceptual training continuously refines neuronal population codes in primary visual cortex. *Nat. Neurosci.* 17, 1380–1387.
- Zeki, S. (1996). Are areas TEO and PIT of monkey visual cortex wholly distinct from the fourth visual complex (V4 complex)? *Proc. Biol. Sci.* 263, 1539–1544.
- Zubair, M., Morris, S.R., Isa, K., Onoe, H., Koshimizu, Y., Kobayashi, K., Vanduffel, W., and Isa, T. (2021). Divergent whole brain projections from the ventral midbrain in macaques. *Cereb. Cortex* 31, 2913–2931.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Behavioral, functional and electrophysiological data	This paper	https://data.mendeley.com/datasets/nhv5ztsznf/1
Chemicals, peptides, and recombinant proteins		
Molday ION	BioPAL Inc.; Worcester, USA	www.biopal.com/molday-ion.htm
Experimental models: Organisms/strains		
Rhesus macaque (<i>Macaca mulatta</i>)	Biomedical Primate Research Centre (BPRDC), Rijswijk, the Netherlands	https://bprc.nl/en
Software and algorithms		
MATLAB R2017B	MathWorks	RRID: SCR_001622
Python Programming Language	Python	RRID: SCR_008394
SPM 12	SPM, University College London, UK	RRID: SCR_007037
FSL	FMRIB, WIN, Oxford, UK	RRID: SCR_002823
ANTS – Advanced Normalization Tools	ANTS, University of Pennsylvania, USA	RRID: SCR_004757
AFNI – Analysis of Functional Images	AFNI, NIMH, USA	RRID: SCR_005927
Other		
A365	World Precision Instruments	RRID: SCR_008593
A362	World Precision Instruments	RRID: SCR_008593
Microwire Brush Array (MBA)		https://www.microprobes.com/products/multichannel-arrays/mba
Eye Tracking Device	ISCAN Inc	Isacaninc.com
EyeLink 1000	SR-Research	https://www.sr-research.com

RESOURCE AVAILABILITY

Lead contact

For information regarding methodology and resource sharing, questions can be directed toward the Lead Contact, Rufin Vogels (rufin.vogels@kuleuven.be).

Materials availability

No new materials were produced in this study.

Data and code availability

- The behavioral, functional, and electrophysiological datasets are available at Mendeley Data: <https://data.mendeley.com/datasets/nhv5ztsznf/1>.
- Custom scripts to generate the main figures and statistical outcomes in this study are available in the same online repository.
- Any additional information and/or raw data can be requested from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Two male rhesus macaques (*Macaca Mulatta*) participated in the current study (M1: ~7kg, 5 years old; M2: ~9kg, 6 years old). Both subjects were trained for fixation tasks for awake fMRI and electrophysiology as well as a color discrimination task for electrical micro-stimulation of the VTA (VTA-EM) and electrophysiology. An MRI-compatible headpost was fixed to their skull before starting the behavioral training, allowing stable head position during fMRI and electrophysiological studies. Animal care and experimental conditions were approved by the Animal Ethics Committee of the KU Leuven Medical School (P113-2014) and agree with national and European guidelines (Directive 2010/63/EU).

fMRI procedures

Behavioral paradigm: Grating localizer

To localize brain areas activated by the grating stimuli subsequently employed during EM and electrophysiology, we performed an fMRI mapping experiment in each animal. The monkeys were seated in an MRI-compatible chair in a sphinx position. They fixated a red square (0.14°) presented on a gray background at a distance of 57 cm for a juice reward. Five conditions were presented in equal amounts using a block design. In the fixation condition, only the fixation target was presented for 10 s. In the other four conditions, gamma-corrected circular sinusoidal gratings (2° diameter, 100% Michelson contrast, 2 cycles/degree) were presented at a 100% signal to noise ratio (SNR) in either the lower left visual field (LVF) at 225° polar angle, 5° from the center or the lower right visual field (RVF) at 315° polar angle, at 5° eccentricity for 10 s. At each location, two orientations (45° or 135°) could be shown. Thus, the four conditions were 45° _LVF, 135° _LVF, 45° _RVF, and 135° _RVF. During the 10 s block, the phase of the grating was randomly changed every 500ms. Eye position was tracked (120Hz; Iscan, Burlington, MA) during scanning. When fixating within a square window of $2^\circ \times 2^\circ$ around the fixation target, the monkeys received juice reward which increased with longer fixation times. The subjects were allowed to blink within 200ms without breaking the reward schedule. Forty-five blocks were presented in a pseudorandom order in a run with the restriction that immediate repetition of the same condition was not allowed.

MRI acquisition

Anatomical and functional images were obtained with a 3T Siemens Trio. Submillimeter resolution anatomical scans were acquired under anesthesia of ketamine and medetomidine, using a single radial transmit-receive surface coil and an MPRAGE sequence (repetition time = 2200ms, echo time = 4.05ms, flip angle = 13° , 320×260 matrix, 208 slices, 0.4 mm isotropic voxel size).

Before the functional scans, the monkeys received the intravenous contrast agent monocrystalline iron oxide nanoparticle (MION; Feraheme, AMAG pharmaceuticals, Inc., Lexington, MA, 8–11 mg/kg). Standard scanning procedures were followed (Vanduffel et al., 2001), using a custom-made eight-channel coil and a gradient-echo single-shot EPI sequence (repetition time = 2 s, echo time = 13ms, flip angle = 84° , 80×80 matrix, 40 slices, no gap, 1.25 isotropic voxel size).

VTA-EM-stimulus association procedures

Electrical micro-stimulation of the VTA (VTA-EM)

To be able to activate cells in the VTA by electrical micro-stimulation (VTA-EM), chronic electrodes were implanted in the monkeys' brains. First, MRI-compatible chambers (Crist Instruments) were implanted on the monkey's skulls. Microwire brush electrode arrays (Bondar et al., 2009) consisting of twenty 25–50-micron thick wires that fan out when inserted, were implanted into the VTA using peri-operative MRI guidance, following the procedures of (Arsenault et al., 2014). M1 received a chronic electrode in the right hemisphere (RH). M2 received an electrode in the right hemisphere and subsequently in the left hemisphere (LH). The RH electrode in M2 was misplaced (slightly too deep), leading to unreliable data. EM was performed with a constant current stimulus isolator (A365, World Precision Instruments) triggered by custom software. EM was done with the two electrodes from the electrode array with the lowest impedance and consisted of stimulation trains lasting 200ms that were composed of biphasic, square-wave pulses with a repetition rate of 200Hz for M1 and 50Hz for M2. Each pulse consisted of a 0.2ms positive and 0.2ms negative voltage with a 0.025ms interval. VTA-EM induced task-interfering pupil constriction in M2, which was reduced when stimulating with the lower frequency. Current intensity ranged from $300\mu\text{A}$ – $900\mu\text{A}$ for M1 and was kept constant at $1000\mu\text{A}$ for M2.

Behavioral paradigm: Free viewing

We tested whether the employed VTA-EM parameters affected behavior with a free-viewing task before and after pairing a visual stimulus with VTA-EM. It was shown previously that pairing a stimulus with VTA-EM can produce a preference for the VTA-EM paired image (Arsenault et al., 2014).

During the free-viewing task, two colored fractals having a size of $4^\circ \times 4^\circ$, generated with an algorithm based on (Sakai and Miya-shita, 1991), were displayed simultaneously at a position of 3° to the left and the right of the center of the screen on a gray background. The monkeys received juice rewards whenever the gaze was within the limits of the screen ($40^\circ \times 29.5^\circ$). A trial consisted of the two fractals being presented, where the fractals' positions interchanged randomly over trials. Equal numbers of trials of both position conditions were selected for analysis. Trials lasted 2 s during which the monkey had to look at the screen. Gazing outside of the screen did not count toward the 2 s. The inter-trial interval (ITI) was 1 s. Free-viewing sessions lasted for 10 minutes. Free-viewing sessions were done before and after the VTA-EM pairing blocks. The monkey's eye position was used (tracked at 1000 Hz, EyeLink, SR-Research) to measure the amount of time the monkey looked at each fractal image.

Behavioral paradigm: VTA-EM stimulus pairing

After each first free-viewing session, a 20-minute fixation task with stimulus–VTA-EM association was performed. A trial started with the presentation of a red fixation square (0.14°) on a gray background in the center of the screen. The monkeys had to fixate in a window of $2^\circ \times 2^\circ$ around the fixation point for 3500–6000ms before a fractal image was presented for 500ms in the center of the screen. ITI was 500ms. The two fractals used in the preceding free-viewing session were presented pseudo-randomly, one at a time. One of the two fractals was paired with VTA-EM (~ 60 stimulation events). When a preference for a fractal image could be detected online during the preceding free-viewing session, the non-preferred fractal was paired with VTA-EM in M1. Otherwise, one of the two fractals was chosen at random for pairing with VTA-EM. In M2, the selection of one of the two fractals for pairing with VTA-EM

was always random. The 200ms long VTA-EM started 400ms after stimulus onset. The interval between successive EM events was on average ($\pm$ SEM) 14.5 s ($\pm$ 0.27 s), avoiding habituation of the VTA-EM response. Juice reward was given every 1.5 s of fixation, independent of visual stimulus presentation times.

Main experimental procedures: PIT response properties after pairing a low-visibility grating with VTA-EM

Design

An orthogonal color discrimination task (Arsenault and Vanduffel, 2019) was used to investigate whether pairing a low visibility task-irrelevant grating with VTA-EM induced changes in the neural response properties of PIT. First, 15–20 color discrimination + VTA-EM blocks of 2 hours each were performed ($\sim$ 480 EM events), except for the STIM135 condition in M1, who performed 39 VTA-EM blocks of 2 hours and 10 blocks of 1 hour before data recording. Subsequently, daily 1-hour VTA-EM blocks ($\sim$ 240 EM events) were followed by multi-unit activity (MUA) recordings in PIT during fixation and color discrimination tasks, which were performed for 8–40 days per condition. See Table S1 for the number of VTA-EM days and events per monkey.

Initially, we alternated the MUA recordings every 4 days between hemispheres for MUA recording in both monkeys. In these VTA-EM blocks, always the same orientation was paired with VTA-EM per monkey. After those blocks, we recorded only from the hemisphere that was ipsilateral to the VTA-EM electrodes, either without preceding VTA-EM (M1 only) or after pairing of VTA-EM with an orientation orthogonal to the one paired before (both monkeys). The very first recording sessions in M2 were performed when stimulating with the RH electrode. However, as expected from the location of the wire-bundles, which were too deep, little if any effect of VTA-EM was present. Thus, we implanted VTA electrodes in its LH and started EM blocks and recordings when stimulating the LH.

The order of stimulation conditions was determined based on the following rationale: neurons might show a preference for orientations radiating from the center of the visual field relative to the perpendicular orientation, known as the radial bias in orientation preference (Sasaki et al., 2006). To control for this, the first VTA-EM paired orientations were chosen to be parallel to the radial bias orientation in M1 and perpendicular to the radial bias orientation in M2.

Noisy grating stimuli

We used circular sinusoidal gratings (2° diameter) of 100% Michelson contrast and a spatial frequency of 2 cycles/°. For each orientation, 10 equally spaced phases were randomly selected. Gratings were presented in the lower LVF at 225° polar angle, 5° from the center or in the lower RVF at 315° polar angle, 5° from the center.

The gratings were spatially masked by a dynamic noise pattern while being presented on a full-screen background of dynamic noise. The SNR of the grating stimuli was determined by the percent of the pixels of the grating that were not occluded by the noise in a given frame. The dynamic noise background consisted of a random presentation of a white noise pattern (from a pool of 1000 patterns) during each frame (60 Hz). This pool was renewed each day for the color discrimination task with VTA-EM. The dynamic background for color discrimination and fixation tasks used for recording was randomly selected from a pool of 100 noise images at 60Hz. The grating and noise stimuli were gamma-corrected.

Color discrimination task

The color discrimination task was used for VTA-EM stimulus pairing and electrophysiological recordings. The trial was initiated by fixation on a white square (0.14°) in the center of the screen. The monkeys had to respond to a colored square (0.5°) that was presented for 150ms at 7.5° above the center.

During the presentation of the color target, a grating, that was irrelevant to the monkey's task, was shown in the lower LVR or RVF at 5° eccentricity. Subsequently, the monkey had to fixate for another 200ms, a period used for either VTA-EM or neural response recording. Based on the color, the monkey had to make a saccade to one of two white target squares (0.5°), presented on the right and left of the center at 9° eccentricity for 500ms. For a trial to be accepted as aborted, the monkey needed to fixate during the 500ms pre-stimulus fixation period, the 150ms stimulus presentation period, the 200ms post-stimulus interval, and afterward make a saccade to one of the targets left or right within 500ms. Correct saccades were rewarded with a drop of juice. Training was started with the colors red and green, but the color difference was titrated daily so that the monkey's performance was $\sim$ 75%–85%, to ensure attentional effort to perform the task.

The stimulus presentation time of 150ms and SNR level of the gratings of 20% was selected based on preliminary tests with the color discrimination task using different stimulus durations (50ms–250ms in steps of 50ms) and SNR levels (0%, 5%, 10%, 15%, 20%, 50%). This was done for each monkey individually. As shown in Figure S1, the performance in the color discrimination task increased with stimulus duration, as expected. There was also an effect of SNR: a grating SNR of 50% impaired performance of the color discrimination task, which we interpret as interference by exogenous attention drawn by the grating. However, in both animals, this effect was absent at 20% SNR (compare the performance of 0% and 20% SNR in Figures S1A and S1B). Hence, no evidence for attentional capture by the grating was present for 150ms stimulus duration of a 20% SNR grating.

Color discrimination + VTA-EM: Simultaneous with the presentation of the color target (150ms), a noisy grating with an SNR of 20% was presented in the lower LVF or RVF with an orientation of 45° or 135°. After stimulus offset, the monkey had to fixate for 200ms during which the VTA-EM occurred. ITI was 2000ms. The current intensity for M1 was half the current of the EM-induced pupil dilation threshold, which was determined daily before the VTA-EM stimulus pairing block. This resulted in a range of 175–400 μ A in M1. In the left hemisphere of M2, a frequency of 50Hz with a current of 1000 μ A was used. EM was delivered in one-fourth of the trials, i.e., after the paired orientation and hemifield contralateral to the stimulation electrodes. A VTA-EM trial was always followed by a non-VTA-EM trial. The average ($\pm$ SEM) interval between successive EM events was 14.3 s ($\pm$ 0.14 s), avoiding habituation of the VTA-EM response.

Color discrimination + electrophysiology: Simultaneous with the presentation of the color target (150ms), noisy gratings with an SNR of 10%, 15%, 20% or 40% were presented in the lower visual field, contralateral to the recorded hemisphere. The orientation of the grating was 0, 45, 90 or 135°. The 16 (4 orientations x 4 SNR levels) conditions were presented randomly interleaved in blocks of 16 trials, without additional ITI between successive blocks. No VTA-EM was performed. The number of presentations per condition was on average 29.

Fixation task

High SNR: To examine changes in neural responses to different grating orientations after VTA-EM, the monkeys performed a fixation task with gratings at high visibility. During passive fixation, the monkeys had to fixate a 0.14° red square within a 2° x 2° window for a drop of juice given every 1.5 s of fixation. After fixating for 500ms, gratings in eight orientations from 0° to 157.5°, in steps of 22.5°, were presented for 200ms at 80% SNR in the lower LVF or RVF, contralateral to the recorded hemisphere at an eccentricity of 5°. ITI was 500ms. The 8 conditions were presented in random order in blocks of 8 trials without additional ITI between successive blocks. No VTA-EM was performed. For a trial to be accepted as unaborted, the monkey needed to fixate during the 500ms pre-stimulus fixation period, the 200ms stimulus presentation period, and the 300ms post-stimulus interval. The number of unaborted trials per condition was on average 23.

Low SNR: To compare MUA to low SNR gratings during the color discrimination task to responses to low SNR gratings without an orthogonal task, low SNR gratings were presented during fixation using the same trial protocol as described above for the high SNR stimuli. Noisy gratings in four orientations from 0° to 135°, in steps of 45°, were shown at 135° or 225° polar angle at 5° eccentricity with an SNR of 10%, 15%, 20% or 40% for a duration of 150ms. The 16 conditions were presented in random order in blocks of 16 trials without additional ITI between successive blocks. The number of trials per condition was on average 23. Only M2 performed the fixation task with low SNR gratings.

Receptive field mapping

Checkerboards of 3° x 3° consisting of 16 equally sized squares were presented for 200ms, with an inversion of the luminance polarity at 100ms, on a gray background. They were presented pseudo-randomly in blocks of 84 trials in 42 locations of an (invisible) grid, with locations spaced 3°. The grid consisted of 2 columns ipsilateral and 4 columns contralateral to the recorded hemisphere, 3 rows in the upper visual field, and 4 rows in the lower visual field. Checkerboards were presented with 100ms ITI. Every 1.5 s of fixation the monkeys received juice as a reward. The number of presentations per location was on average 21.

MUA recording

MRI compatible recording chambers (Crist Instruments) were implanted over the functional activations determined by the fMRI grating localizer in the posterior temporal visual cortex, ventral to the upper bank of the superior temporal sulcus (STS). Standard electrophysiological recording procedures were employed for MUA recordings using 16 channel probes (V1x16-Edge-10mm-100-177, NeuroNexus). The probe was lowered through a guide tube that was fixed into a Crist grid. Recordings started at least 60 mins after insertion of guide tube and probe into the brain to ensure stable MUA recordings. Spikes were sorted offline with Offline Sorter (Plexon), where all sorted spikes of one channel resulted in one unit. Before recording, anatomical MRI's (MPRAGE, 0.4 mm voxels) were used to project the trajectories of the grid holes in the recording chambers onto the co-registered functional activation in PIT. In the stimulated hemisphere of M1, 3 neighboring grid hole positions (spacing = 1 mm) were used. In M2, when performing VTA-EM in the RH, 3 neighboring grid holes were used in the LH. When performing VTA-EM in the LH, recording in this hemisphere was done from 1 grid hole. We recorded along different depths in each penetration, ranging from the lower bank of the STS to the PMTS (~5.5mm) in M1 and just above the PMTS (~4mm) in M2 (Figures 1E and 1F). On purpose, the penetrations were largely tangential to the cortex to avoid sampling from a single orientation column (although no evidence for such columns exists for PIT).

QUANTIFICATION AND STATISTICAL ANALYSIS

fMRI data analysis

Runs in which the animal's fixation performance was greater than 87% were selected for analysis. For M1, 36 runs were analyzed and 20 runs for M2. First, image reorientation and slice timing correction was performed (AFNI; NIH, USA) (Cox, 1996). Motion correction was performed by using non-rigid, slice-by-slice realignment within runs and an affine realignment across all runs (Kolster et al., 2009). Subsequently, a representative mean functional image was non-rigidly co-registered to the animals' anatomical image using ANTs (Advanced Normalization Tools; University of Pennsylvania, USA) (Avants et al., 2011) following a two-step procedure: 1) The mean functional image was co-registered to a GRE image acquired in the same session. 2) That GRE image was co-registered to a T1 anatomical image from the same monkey. Finally, images were smoothed in FSL (FMRIB Software Library; University of Oxford, UK) (Smith et al., 2004) employing an isotropic Gaussian kernel (FWHM: 1.5 mm). Data analysis was performed by fitting a general linear model (GLM). The grating position conditions LVF and RVF were contrasted with each other (45°_LVF+ 135°_LVF- 45°_RVF -135°_RVF).

Behavioral analysis: Free viewing

Viewing times for each fractal were compared pre-and post-VTA-EM pairing. The time that the subject's eye position was inside a 6° window centered on the fractal was measured separately for each fractal. For each free-viewing session, these viewing times per stimulus were summed and expressed by an index:

$$\text{preference index (PI)} = \frac{\text{Time}_{\text{fractal1}} - \text{Time}_{\text{fractal2}}}{\text{Time}_{\text{fractal1}} + \text{Time}_{\text{fractal2}}}$$

Fractal 1 is the fractal paired with VTA-EM. The closer the PI value is to 1, the more time the monkey looked at the paired fractal image, while 0 indicates no preference. The PI of the pre- and post-VTA-EM free-viewing sessions were compared by subtracting the PI of the pre-VTA-EM free-viewing session from the PI of the post-VTA-EM free-viewing session. This means that positive outcomes represent relatively longer viewing times post-stimulation for the VTA-EM paired fractal compared to pre-stimulation. The statistical significance of the difference between pre- and post PIs was tested with a Wilcoxon signed-rank test.

Electrophysiology data analysis: Fixation task

For unaborted trials, spikes were counted in two windows: [-200–0] for baseline and [50–250] for the gross response, 0 being stimulus onset. Responsive units were selected based on a split-plot ANOVA with as a repeated factor baseline versus gross activity and between-trial factor orientation. Units were included when either the repeated factor or the interaction between the two factors was significant ($p < 0.05$) and when the net response to at least one orientation was positive. MUA was normalized by dividing the net response (gross-baseline) of a condition by the net response in the condition with the highest net response of all conditions. The results show normalized net responses unless explicitly mentioned, but qualitatively identical results were obtained when using unnormalized net responses.

To assess the effect of VTA-EM orientation pairing we subtracted for each unit the normalized net response to 135° from that to 45° since these two orthogonal orientations were paired with VTA-EM in successive conditions (STIM45 and STIM135). The significance of the response differences between the units of the STIM45 and STIM135 conditions was tested with a Mann-Whitney U test. The 95% confidence intervals (CI) of the response differences for the STIM45 and STIM135 populations of units were computed by bootstrapping units (1000 resamplings).

To assess the contribution of the VTA-EM stimulation and radial orientation bias to the normalized net responses for the 45° and 135° orientations, we employed a linear regression model (LM) including an interaction term:

$$R = b_0 + b_1 * \text{STIM} + b_2 * \text{RB} + b_3 * (\text{STIM} * \text{RB}) + \text{error}.$$

The categorical predictor STIM and RB indicated whether the presented orientation to the unit was paired with VTA-EM (STIM) and whether it corresponded to the radial bias orientation (RB), respectively. We equated the number of units for the STIM45 and STIM135 conditions by random sampling the same number of units as that of the orientation pairing condition with the smallest number of units. Fitting the model to all data, without equation of number of units, produced highly similar results. The model was fitted to the data using the fitglm function of MATLAB (MATLAB R2017b). Since the residuals did not have a Gaussian distribution, we employed bootstrapping to estimate 95% CIs of the coefficients b_i . Bootstrapping with resampling ($n = 1000$) of unit-orientation combinations or orientations produced highly similar results. We present data obtained with the former method. The LM fitting was performed for each monkey separately. The number of data of each monkey was equated by random selection first before fitting the model.

In addition, we fitted a linear mixed-effects model, combining the data of both monkeys (after equating the number of data over monkeys and conditions with random selection), with the monkey being an additional random variable, i.e., the intercept depended on the monkey. To do this we employed the fitlme function of MATLAB with $R \sim \text{STIM} + \text{RB} + \text{STIM} * \text{RB} + (1 | M)$, M indicating the monkey. As before, bootstrapping was performed to compute 95% CIs of the coefficients.

The preferred orientation (PO) of each responsive unit was defined as the orientation out of the eight tested orientations with the largest net response. Then we computed the proportion of units for each stimulation condition (STIM45 and STIM135) that preferred 45° and 135° and subtracted the proportion of units that preferred 135° from the proportion that preferred 45°. We expected this difference in proportions to be larger in the STIM45 compared to the STIM135 orientation pairing condition. To test this, we employed a randomization test in which we pooled all units of the two orientation-EM pairing conditions and then sampled randomly, without replacement, the same number of units as in the original STIM45 and STIM135 conditions. Then, we computed the difference between the proportion of units preferring 45° and 135° for these two new samples of units and subtracted these proportion differences of the two samples. This was done for 10,000 random samples, obtaining a null distribution of the difference between conditions of the difference in the proportion of neurons preferring the two orientations. These analyses were performed for each monkey separately. 95% CIs of the difference in the proportion of units preferring the two orientations was computed by bootstrapping (resampling, with replacement, of units) for each VTA-EM pairing condition.

The Fano factor and noise correlations were computed on gross spike counts. For calculating the Fano factor (variance / mean spike count), we employed also spike rate means matching across orientations 45° and 135° by binning responses for each orientation based on spike rate (bin width = 5 spikes/s) and equating with random selection the number of observations per bin number for both orientations (see Churchland et al., 2010). This produced similar results to those obtained without equation of the mean response. Noise correlations were computed as the Pearson correlation coefficient of the single-trial responses between two simultaneously recorded units. The first trial of each session was excluded when computing noise correlations. Differences in Fano factor and noise correlations between orientations 45° and 135° were computed for each unit or pairs of units of the two orientation-VTA-EM conditions. We employed a Mann-Whitney U test to determine whether these 45-135 Fano factor and noise correlation differences depended on the VTA-EM orientation pairing condition. Statistical testing was performed on the original Fano factor data. The effect

persisted after mean matching. Noise correlations were also calculated as a function of distance between the channels per penetration.

Population peri-stimulus time histograms (PSTH) of net normalized firing rates were computed using a bin width of 20ms. The net firing rate was normalized by division of the maximum firing rate in the 20ms bin width across conditions.

To explore the possibility of spatial clustering of preferred orientation in our sample of PIT data, the difference in PO between every two channels recorded during the same day was calculated. The mean difference ($\pm$ SEM) was computed as a function of the distance between the channels of a pair. To assess the mean difference in PO that is expected without spatial clustering we permuted the POs across channels and recomputed the mean difference in PO as a function of distance. This was done 1000 times and a 95% CI was computed using the distribution of computed mean differences. This analysis was done for all RH and LH penetrations of M1 and M2, respectively.

Behavioral data analysis: Color discrimination

To investigate whether the orientation that is paired with VTA-EM affects performance by demanding attention from the monkeys, the median difference (and bootstrapped 95% CIs; n = number of sessions) in performance per session between the VTA-EM paired orientation and its orthogonal orientation was calculated for each SNR. To do this, we used the behavioral data obtained when the monkeys were performing the color discrimination task during the recordings.

Electrophysiology data analysis: Color discrimination task

For unaborted trials, spikes were counted in two windows: [-150–0] for baseline and [50–200] for gross response, 0 being stimulus onset. Responsive units were selected based on a split-plot ANOVA with as repeated-measure factor baseline versus gross activity and as between-trial factor the sixteen orientation $\times$ SNR combination conditions and a positive net response for at least one condition. MUA was normalized by dividing the net response of a condition by that of the condition with the highest response. All presented data are based on normalized net responses (gross-baseline).

As for the fixation task, we computed for each unit the difference in normalized net response between 45° and 135° and this for each of the 4 SNRs. This was performed for the STIM45 and STIM135 separately for each monkey. We ran a split-plot ANOVA on these response differences with as repeated factor SNR and between-unit factor STIM45 versus STIM135.

Population PSTHs were created using a bin width of 25ms.

Electrophysiology data analysis: Receptive field mapping

For unaborted trials, spikes are counted in two windows: [-200–0] for baseline and [50–250] for gross response, 0 being stimulus onset. Units needed to conform to two conditions to be included in the analysis. First, a split-plot ANOVA with as repeated factor baseline and gross response and between-trial factor the forty-two checkerboard positions was used to select responsive units. A unit also needed a positive net response to at least one position for inclusion. Second, the unit also needed to produce a significant response for either the fixation task or the color discrimination task. Analysis was done on normalized net responses (gross-baseline). The average receptive field maps are presented together with the grating location for the different VTA-EM conditions in [Figure S2](#), showing an overlap between the mean receptive field and the location of the presented grating stimuli.