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Quantitative analysis of functional clustering of neurons in the macaque inferior temporal cortex

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Abstract

Neurons with similar preferences for two-dimensional shapes of intermediate complexity cluster in area TE of the monkey inferior temporal cortex. To further characterize the functional structure of area TE, we quantitatively analyzed various aspects of the visual responses of closely located neurons by applying multiple single-unit recording techniques in anesthetized monkeys. Examination of the visual responses elicited with a large, predetermined set of visual stimuli confirmed previous findings that nearby neurons, on average, exhibited positively correlated preferences for a set of visual stimuli. Nearby neurons also tended to be similar in their receptive-field organization and contrast-polarity preference. In contrast, no correlation was found in the size tuning of neighboring neurons. Pooling or subtraction of activities between a pair of nearby neurons was shown to improve stimulus discriminability, if the neuron pair had positively or negatively correlated stimulus preferences, respectively. These results indicate that nearby TE neurons share some aspects of stimulus preference, but their response selectivity differ in other aspects. Both pooling and subtraction between nearby neurons can reduce across-trial response variability, if these decoding strategies are applied to appropriate neuronal pools.

Keywords: Object recognition; Functional organization; Column; Area TE; Ventral stream; Multiple single-unit recording; Monkey

1. Introduction

In the cerebral cortex, most of the neural interactions occur between neurons that are located in close proximity to one another. The probability of having synaptic contacts is much higher between nearby neurons than between distant neurons (Douglas et al., 1995; Braitenberg and Schüz, 1998). The probability and the strength of functional connectivity decrease as the distance between neurons increases (Gochin et al., 1991; Das and Gilbert, 1999; Holmgren et al., 2003). Moreover, nearby neurons often share target neurons allowing their activities to be integrated at the postsynaptic sites. These features of the anatomical and functional organization of the cerebral cortex point to an importance of the local arrangement of neurons for the neuronal representation and the decoding mechanism.

In area TE of the macaque inferior temporal cortex, two-dimensional shapes with intermediate complexity strongly activate some neurons (Schwartz et al., 1983; Richmond et al., 1987; Miyashita and Chang, 1988; Tanaka et al., 1991; Missal et al., 1997; Op de Beeck et al., 2001), whereas other neurons require more complex images, such as faces or hands, for their maximal activation (Gross et al., 1972; Desimone et al., 1984; Perrett et al., 1982; Tanaka et al., 1991; Nakamura et al., 1994; Rolls et al., 1994; Sugase et al., 1999; Vogels, 1999; Sheinberg and Logothetis, 2001; Tamura and Tanaka, 2001; Eifuku et al., 2004) or three-dimensional structure (Janssen et al., 2000; Uka et al., 2000). TE neurons with similar preferences for two-dimensional shapes of intermediate complexity are clustered (Gochin et al., 1991; Fujita et al., 1992; Fujita, 2002; but see Gawne and Richmond, 1993). However, because only two-

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dimensional shapes with intermediate complexity have been used for the evaluation of stimulus preference, neurons that preferred more complex images have been excluded from the analysis.

TE neurons vary in their sensitivity to the physical properties of stimuli, such as position, size, and contrast. Though receptive-fields (RFs) of TE neurons are typically larger than 10° in size and include the fovea; their position, size, and sensitivity profiles vary to a considerable extent between neurons (Gross et al., 1972; Desimone and Gross, 1979; Tanaka et al., 1991; Op de Beeck and Vogels, 2000; Kawasaki et al., 2000; Wang et al., 2002; DiCarlo and Maunsell, 2003). Changes in the stimulus size and contrast polarity affect the responses of a subpopulation of TE neurons, whereas the responses of other neurons are largely independent of these stimulus properties (Schwartz et al., 1983; Rolls and Baylis, 1986; Tanaka et al., 1991; Komatsu et al., 1992; Ito et al., 1994, 1995; Op de Beeck and Vogels, 2000; Baylis and Driver, 2001; Kovács et al., 2003). It remains unknown how TE neurons are spatially arranged with respect to their sensitivity to these stimulus properties.

In the present study, we simultaneously recorded extracellular responses from a group of two or more closely located neurons in area TE of anesthetized monkeys. This allowed us to quantitatively examine similarities in stimulus-image preferences, RF structures, contrast-polarity preferences, and size tuning, between pairs of nearby neurons. Stimulus-image preferences were weakly but statistically significantly correlated between nearby neuron pairs. Both RF structures and contrast-polarity preferences were also correlated between nearby neuron pairs, whereas no correlation was found for size preferences. We also examined the effects of pooling and subtraction of the activities of pairs of nearby neurons on the discriminability of the stimuli. We found that, depending on whether the stimulus preferences of nearby neurons were positively or negatively correlated, each type of integration of the activities of two nearby neurons could improve stimulus discriminability. A part of this study has been published in abstract form (Tamura et al., 2001).

2. Materials and methods

The database for most of the present analysis was shared with Tamura et al. (2004). Neuronal responses were recorded from area TE of the inferior temporal cortex in four anesthetized monkeys (*Macaca fuscata*; body weight, 5.2–7.5 kg). All experimental procedures were in accordance with the guidelines of the National Institutes of Health (1996) and the Japan Neuroscience Society. The Osaka University animal experiment committee, that included a veterinarian as a committee member, approved the procedures as appropriate and conforming to the current standard for animal experiment protocols.

2.1. Preparation and recordings

The experimental procedures were described in detail elsewhere (Tamura et al., 2004). Briefly, the monkeys were prepared for repeated recordings through initial aseptic surgery under sodium pentobarbital surgical anesthesia.

For recording experiments, monkeys were anesthetized with isoflurane in 70% N_2O–30% O_2, and were paralyzed with pancuronium bromide to prevent eye movements. Body temperature was maintained at 37–38 °C. End-tidal CO_2 was kept at 4.0–4.5%. Electrocardiograms and arterial oxygen saturation levels were continuously monitored throughout the experiment. Eyes were dilated and covered with pre-selected contact lenses.

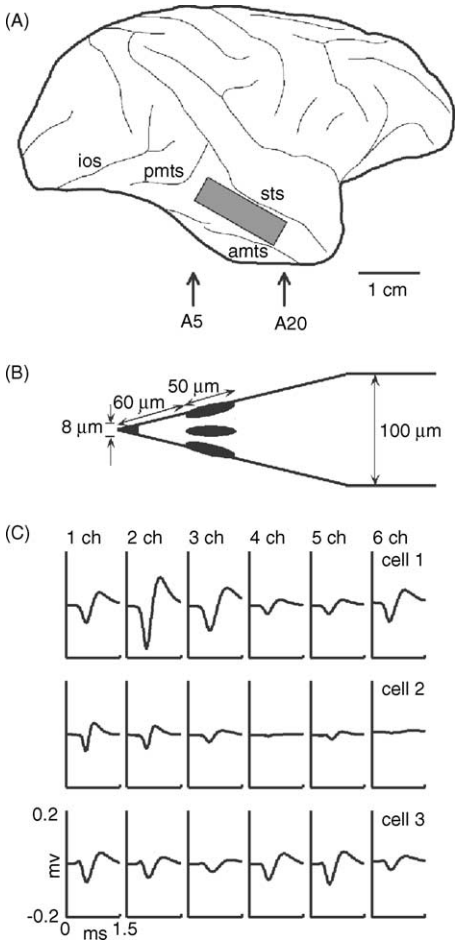

Fig. 1. Recording site, electrode, and spike shapes. (A) The shaded area on the schematic of a lateral view of the right cerebral hemisphere indicates the region in which all recordings were made. The area corresponds to the dorsal part of area TE. amts, anterior middle temporal sulcus; ios, inferior occipital sulcus; pmts, posterior middle temporal sulcus; sts, superior temporal sulcus. A5 and A20 indicate levels 5 and 20 mm anterior to the ear canal, respectively. (B) The electrode (Heptode; Thomas, Germany) has seven recording probes. One probe is located at the tip of the electrode; the other six surround the electrode at a distance of 60 µm from the tip. (C) Spike shapes of three simultaneously recorded neurons (cells 1–3). For each neuron, the average spike shapes of all the spikes recorded from each of six probes are shown. The maximal spike amplitude for cells 1–3 were recorded from Heptode channels 2, 1, and 5, respectively.

Multiple single-unit recordings were made at 103 sites in the dorsal part of area TE (Tamura and Tanaka, 2001) of the four monkeys, using a single-shaft electrode with seven recording probes (Heptode; Thomas, Germany; Fig. 1). We histologically verified that all recording penetrations were in this region. Due to the technical details of our custom-made software, we used six of the seven probes, including the tip probe, for recording. Recordings were made at intervals of 0.3 mm or greater along a penetration axis. In every penetration, sampling was made throughout the gray matter. All neurons encountered were recorded and analyzed. Recorded potentials were amplified, filtered, and digitized, before storing them on a computer. Isolation and classification of spikes from recorded signals were carried out off-line by an automated method (Kaneko et al., 1999; Tamura et al., 2004). We estimated the rate of spike isolation error from the Mahalanobis distances between clusters of spike amplitudes recorded with the six probes and analyzed in a six-dimensional space. On average, the probability of error that was defined as the proportion of overlap between two clusters was 0.038%. Out of the 1028 pairs we analyzed, 1018 pairs had the probability of error of <1%, while one pair had an exceptionally high probability of error (12.6%) and nine pairs had a probability of error of 1–5%. Although it is difficult to completely eliminate the spike isolation error, these results indicate that the contribution of isolation error to the results of the following analyses was minimal.

2.2. Visual stimuli

The stimulus set used to examine stimulus-image preferences consisted of 64 visual stimuli, 38 two-dimensional geometrical shapes (circles, squares, triangles, bars, stars), 15 textured patterns (gradation patterns and gratings), and 11 photographs of natural or man-made objects (banana, apple, monkey face, human face, cage, human hand, syringe, etc.; see Fig. 2D). Images were 4° or less of visual angle, and were encoded in 256 gray levels. The luminance of white and black were 99.2 and 0.7 cd/m^2, respectively.

Visual stimuli were individually presented for 1 s against a homogeneous gray background (15.7 cd/m^2) at the center of the receptive field hand-plotted for each recording site by referring to audio-monitored multiple unit activities recorded from the tip probe. The same homogeneous gray field was presented during the 1-s intervals between presentations of stimuli. The stimuli were presented in a pseudo-random order within a stimulus presentation block.

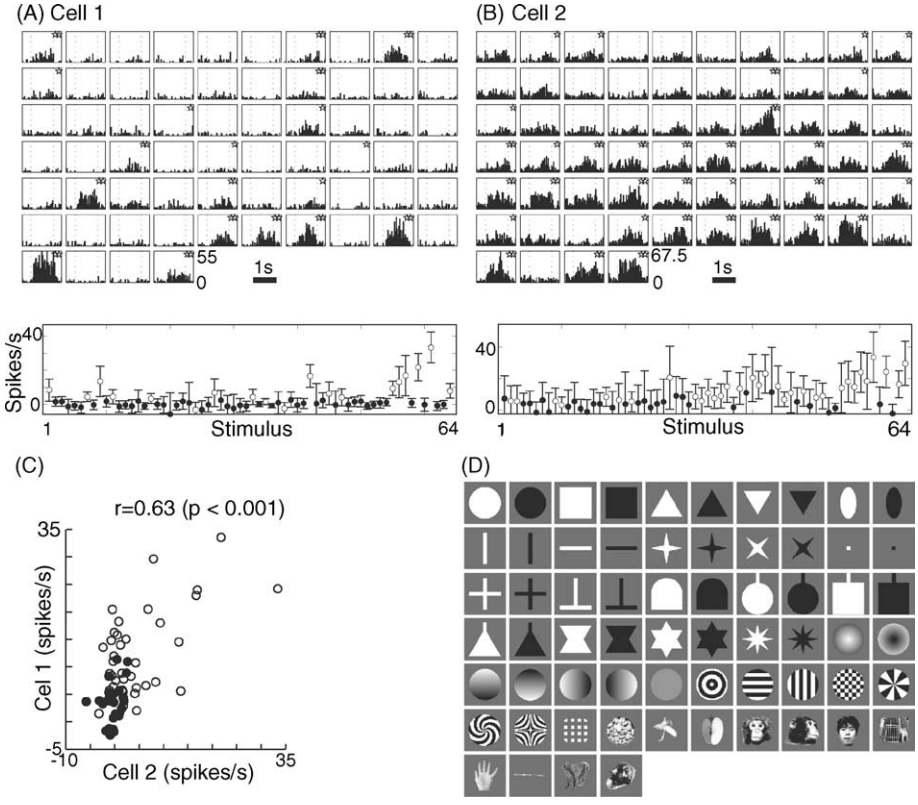

Fig. 2. A comparison of the stimulus preferences of two nearby neurons. (A and B) Peri-stimulus time histograms (PSTHs; top panels) and response profiles (bottom panels) of cell 1 (A) and cell 2 (B) to the set of 64 visual stimuli. The vertical dotted lines in the PSTHs indicate the onset and offset of each visual stimulus presentation. Stars within the PSTHs indicate significance levels (single star, $0.01 \leq p < 0.05$; double star, $p < 0.01$; Wilcoxon's signed rank test). In the response profiles, the mean number of recorded spikes ± 1S.D. during 1-s stimulus presentation periods was plotted. Open and closed circles represent significant and non-significant responses, respectively. (C) Signal correlation between the neurons. Open circles represent stimuli that evoked significant responses in at least one of the two neurons. Closed circles represent ineffective stimuli. (D) The 64 images that constituted the stimulus set.

Ten blocks were presented for each recording site. For the analysis of RF structure, three to five different stimuli were individually presented at nine positions in the visual field (fovea, 7.5° up and down, and 11° ipsilateral and contralateral). Based on multiple-unit activity, we selected the best stimulus, the stimulus that evoked 50% of the best, and the worst stimulus. In some of the cases, the second best and the stimulus that evoked 75% of the best was included. We estimated the RF as the summed RF across tested stimuli, because the extent of RF depends on stimuli in some TE neurons (Ito et al., 1995; Wang et al., 2002). RF based on a single stimulus may underestimate the region in the visual field where a neuron receives visual information. The size preference of TE neurons was investigated by presenting three different sizes of the best stimulus for each neuron (2°, 4°, and 8° of visual angle).

2.3. Analysis of visual responses

The magnitude of responses to a given stimulus was computed as the mean firing rate during visual stimulation minus the mean spontaneous firing rate over 10 trials. Statistically significant responses ($p < 0.05$, Wilcoxon's signed rank test) were determined by comparing the firing rate obtained during a 1-s period starting 80 ms after the onset of stimulus presentation with the spontaneous firing rate measured during a 0.4-s period immediately before stimulus onset. For a given pair of neurons, the overall similarity of their stimulus preferences was assessed by calculating the Pearson's correlation coefficient (r) for two sets of the response magnitudes to the same stimulus set (signal correlation). If three or more neurons were isolated at a single recording site, neurons were analyzed as multiple pairs.

3. Results

3.1. Similarity of stimulus-image preference between nearby neurons

Most (439/455) of the TE neurons responded with an increase or a decrease in their firing rate to one or more of the stimuli in the set. Simultaneously recorded, nearby neurons often shared effective stimuli. An example of the responses of two simultaneously recorded neurons is shown in Fig. 2. Both neurons showed the strongest responses to images of complex objects. Cell 1 responded to 19 stimuli among the 64 in our stimulus set ($p < 0.05$, Wilcoxon's signed rank test; Fig. 2A). The photograph of a human hand evoked the largest response (seventh row from the top, far left column), whereas the photograph of a human face (sixth row from the top, second column from the right) evoked the second-largest response. Cell 2 responded to 33 stimuli (Fig. 2B). For this cell, the photograph of a human face evoked the

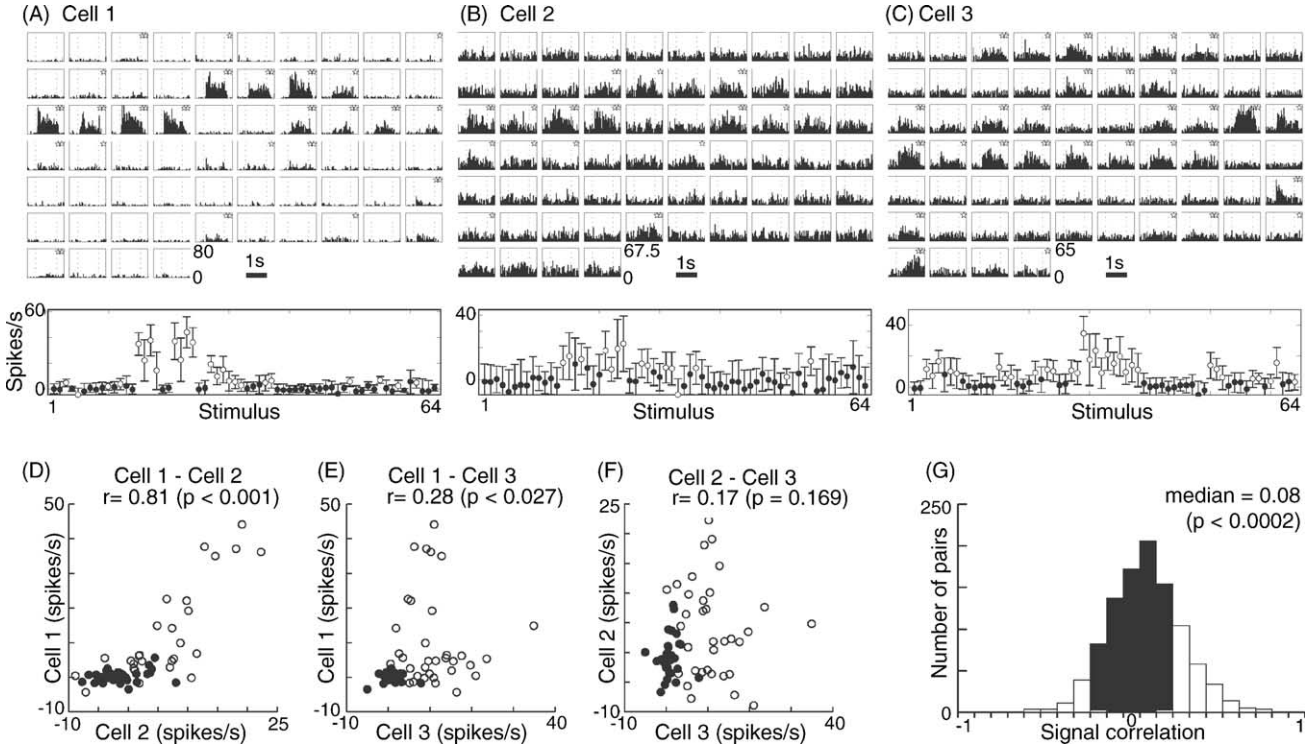

Fig. 3. Comparisons of the stimulus preferences of nearby neurons. (A–C) PSTHs (top panels) and response profiles (bottom panels) of cells 1–3 to the set of 64 visual stimuli. The spike shapes of the three neurons are shown in Fig. 1. (D–F) Signal correlation between the three neurons (D, cells 1 and 2; E, cells 1 and 3; F, cells 2 and 3). (G) Frequency distribution of signal correlation from 1028 pairs of nearby neurons. Open and closed columns indicate significant ($p < 0.05$) and non-significant correlation, respectively. Other conventions are as in Fig. 2.

largest response (sixth row from the top, second column from the right) and that of the profile of a monkey evoked the second-largest response (seventh row from the top, fourth column from the left). Fourteen images effectively stimulated both cells 1 and 2.

Another example of simultaneously recorded neurons (cells 1–3) is shown in Fig. 3. All three neurons strongly responded to images of geometrical shapes. Cell 1 responded to 24 stimulus images among the 64 in our stimulus set ($p < 0.05$; Fig. 3A). An inverted white "T"-shape evoked the largest response (third row from the top, third column from the left). Cell 2 responded to 15 stimulus images (Fig. 3B). An inverted black "T"-shape evoked the largest response (third row from the top, fourth column from the left). Ten of these 15 images were also effective for cell 1. Cell 3 responded to 31 stimulus images (Fig. 3C). A white square with a vertical protrusion on top of it evoked the largest response (third row from the top, second column from the right). Thirty-seven out of the 64 stimulus images evoked responses in at least one of the three neurons, and nine of the 37 images evoked responses in all three neurons.

We compared the stimulus-image preferences of the nearby neurons by calculating the signal correlation for each pair of neurons (r, see Section 2). Signal correlation between cells 1 and 2 shown in Fig. 2 was 0.63 ($p < 0.001$; Fig. 2C), indicating that they had positively correlated overall stimulus-image preferences. Similarly, cells 1 and 2 of Fig. 3 showed highly correlated overall stimulus-image preferences ($r = 0.81$, $p < 0.001$; Fig. 3D). Cells 1 and 3 of Fig. 3 also showed a positive, albeit a weak, signal correlation ($r = 0.28$, $p = 0.027$; Fig. 3E). Finally, although some stimuli effectively activated both cells 2 and 3 of Fig. 3, the overall stimulus-image preferences of the cells were independent of each other ($r = 0.17$, $p = 0.169$; Fig. 3F).

We evaluated the similarity of stimulus-image preferences for 1028 pairs of nearby neurons. The signal correlation ranged from −0.61 to 0.81, and the median was 0.08. Among the 1028 pairs, 222 pairs (21.6%) had a significant positive correlation ($p < 0.05$; white columns on the right part of the histogram in Fig. 3G). A significant negative correlation (white columns on the left part of Fig. 3G) was observed in 61 pairs (5.9%), a proportion marginally larger than chance level ($p = 0.05$). An example of a neuron pair with significant negative correlation ($r = -0.37$, $p = 0.003$) is shown in Fig. 4. The remaining 745 pairs showed no significant correlation between their stimulus-image preferences.

To test the null hypothesis that the observed median is similar to the median obtained with randomly sampled neuron pairs, we performed a permutation test in the following way. We generated 1028 "artificial" pairs by randomly sampling the 455 neurons. This process was repeated 5000 times. None of the calculated medians from the 5000 random sampling cases exceeded the median of actual nearby neuron pairs (the maximum of the artificially generated medians was 0.02). Thus, although the median

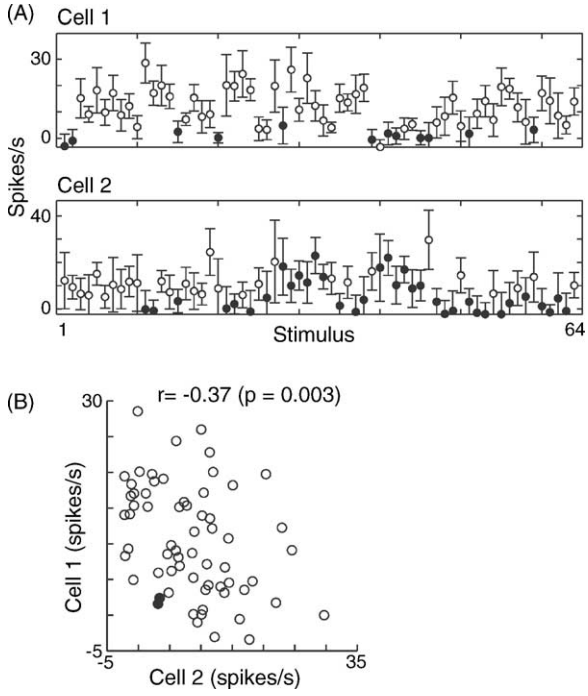

Fig. 4. Signal correlation between nearby neurons with negatively correlated stimulus preferences. (A) Response profiles of cell 1 (top) and cell 2 (bottom) to the set of 64 visual stimuli. A white vertical bar evoked the largest response in cell 1 (11th stimulus), whereas it was not effective for cell 2. Concentric circles evoked the largest response in cell 2 (46th stimulus), whereas they were not effective for cell 1. (B) Signal correlation between the neurons. Other conventions are as in Fig. 2.

signal correlation of nearby pairs was small, it was statistically significantly larger than that of random pairs ($p < 0.0002$, permutation test). The results indicate that, on average, nearby neurons show more similar stimulus-image preferences than distant neurons.

The degree of correlation differed among electrode tracks ($p = 0.022$, Kruskall–Wallis test), and was ranged from 0.19 to −0.06 (median). The difference in the signal correlation was not correlated with the anterior-posterior axis of area TE ($p \geq 0.05$, Mann–Whitney's U-test).

We also calculated signal correlation based on the 53 shape stimulus (*r-shape*) and that based on the 11 object stimulus (*r-object*). The median *r-shape* and *r-object* was 0.08 and 0.09, respectively, and they were not different from each other ($p = 0.64$, Wilcoxon's signed rank test). The *r-shape* was correlated with *r-object* ($r = 0.51$, $p < 0.001$, $n = 1028$). The analysis indicates that if a neuron had a correlated stimulus preference for shape stimuli, the neuron tend to have a similarly correlated stimulus preference for object stimuli.

3.2. Similarity of receptive-field structure, contrast preference, and size preference

Next we compared RF structures, contrast-polarity preferences, and size preferences of nearby neurons. Fig. 5 shows an example of the RF structures of a pair of simultaneously

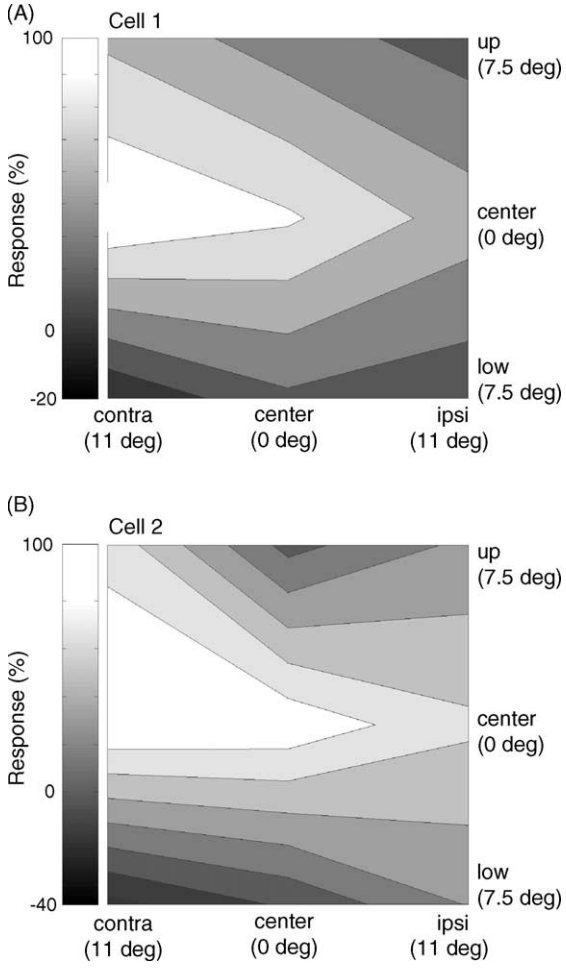

Fig. 5. A comparison of the receptive-field (RF) structures of nearby neurons. (A) RF structure of a neuron (cell 1). (B) RF structure of a simultaneously recorded neuron (cell 2). RF structure was examined with nine locations (fovea, 7.5° up and down, and 11° contralateral and ipsilateral). Averaged responses across four stimuli were plotted with interpolation. Responses were normalized with the maximum averaged response.

recorded neurons. Cell 1 responded with a maximum firing rate to stimuli presented at 11° contralateral to the fovea (Fig. 5A). Cell 2, a simultaneously recorded neuron at the same site, also responded with a maximum firing rate to stimuli at the same position (Fig. 5B). Both neurons also responded well to stimuli presented at the fovea. The two neurons showed a highly similar RF structure (two-dimensional correlation $r = 0.92$, $p < 0.001$). The median correlation of RF structures for 1817 neuron pairs analyzed in the same way was 0.11 (Fig. 8A). This positive correlation may have been derived from an overall bias of TE neurons towards the contralateral visual field. To test this possibility, we performed a permutation test in the same way as described above for stimulus-image preferences. The median correlation was larger between nearby neurons than between randomly selected neurons ($p < 0.0002$), indicating that nearby neurons tend to have similar RF structures.

Next, responses to the 38 black and white geometrical figures were analyzed for contrast-polarity preference. Among the 455 neurons used for contrast-polarity analysis, 248 neurons displayed a consistent preference for black or white stimuli ($p < 0.05$, ANOVA). These 248 neurons formed 381 nearby neuron pairs. Fig. 6 shows four examples of contrast-polarity preference from simultaneously recorded neurons. Both neurons of the first pair (cells 1 and 2 of Fig. 6A) preferred white stimuli. Their shape preferences were also similar to each other, and signal correlation evaluated with responses to 19 white stimuli (*r-ww*) was 0.61 ($p = 0.005$). A second neuron pair (cells 3 and 4 of Fig. 6B) had independent stimulus-image preferences (*r-ww* = 0.084, $p = 0.732$) but both preferred white stimuli. Sixty-two percent (238/381) of the pairs had similar contrast preferences (102 pairs for white and 136 pairs for black; Fig. 8B). Preference for stimulus contrast was not identical between neurons in the remaining 143 pairs (38%). For example, a neuron (cell 5) in the third pair of Fig. 6C preferred white shapes, whereas the other neuron in the pair (cell 6) preferred black shapes. Interestingly, their shape preferences were similar to each other and signal correlation between responses to 19 white stimuli of cell 5 and those to 19 black stimuli of cell 6 (*r-wb*) was 0.72 ($p = 0.001$). The contrast-polarity preference of a neuron (cell 7) in the fourth pair of Fig. 6D also differed from that of the other neuron in the pair (cell 8 of Fig. 6D). Shape preference of cell 7 was different from that of cell 8 (*r-wb* = 0.216, $p = 0.375$). At the population level, nearby TE neurons tended to prefer the same contrast polarity (contingency coefficient = 0.24, $p < 0.001$, χ^2-test). Some of the pairs with similar contrast preference had positively correlated shape preferences, i.e., significant *r-ww* or *r-bb*. The incidence of the correlated shape preference of pairs with similar contrast preference was 11% and that of pairs with different contrast preference was 10%. The incidence was not different between them ($p = 0.867$, χ^2-test).

Tuning for the stimulus size was not correlated among nearby neurons. An example of size-tuning curves of three simultaneously recorded neurons is shown in Fig. 7A. Among the three sizes tested (2°, 4°, and 8°), cells 1–3 had the largest responses to the largest, to the medium, and to the smallest size, respectively. Their size preferences were not correlated ($r = -0.88$, $p = 0.320$ for cells 1 and 2; $r = -0.66$, $p = 0.544$ for cells 1 and 3; $r = 0.21$, $p = 0.864$ for cells 2 and 3). Another example of size tuning curves of simultaneously recorded neurons is shown in Fig. 7B. The two neurons (cells 4 and 5) preferred the largest stimuli, and had a correlated size tuning ($r = 0.99$, $p = 0.003$). The median correlation of size preference (0.62) for the 109 pairs of nearby neurons tested was not significantly different from that of random pairs ($p = 0.389$, permutation test; Fig. 8C).

3.3. Effects of gathering activities of neurons on discriminability of stimuli

Responses of a single neuron to a given stimulus vary across repeated presentations. Variation in neuronal

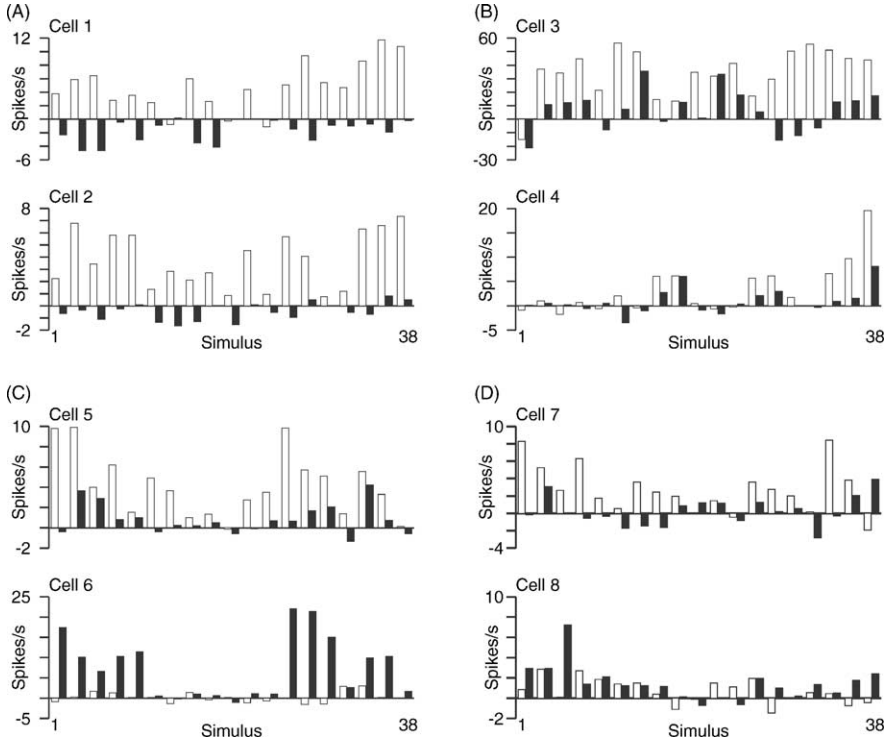

Fig. 6. A comparison of contrast polarity preferences of four pairs (A–D) of nearby neurons using the first 38 visual stimuli from Fig. 2D. Open and closed columns indicate responses to stimuli in white and black, respectively.

responses to the same stimulus limits the ability of the decoding mechanism to discriminate different visual images. A possible solution for the decoding system to reduce response variability and thereby improve discriminability is to gather activities from multiple neurons. We examined whether gathering of activities of two nearby TE neurons could improve the ability of the neurons to discriminate between different visual images. Pooling, calculating the mean firing rate of two neurons, and subtraction, calculating the difference between the firing rates of two neurons, have been proposed as possible mechanisms to integrate information from neuron pairs (Werner and Mountcastle, 1963; Tolhurst et al., 1983; Paradiso, 1988; Britten et al., 1992; Shadlen et al., 1996; Reich et al., 2001; Panzeri et al., 2003; Romo et al., 2003).

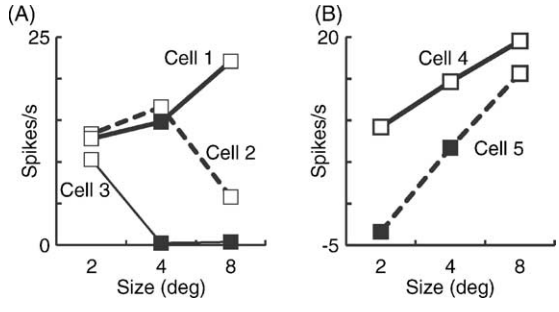

Fig. 7. (A) Size-tuning curves of three simultaneously recorded neurons (cell 1, thick line; cell 2, dotted line; cell 3, thin line). (B) Size-tuning curves of two simultaneously recorded neurons (cell 4, thick line; cell 5, dotted line). A stimulus was presented in three different sizes (2°, 4°, and 8° of visual angle). Open and closed squares represent significant and non-significant responses, respectively.

Stimulus discriminability of a single neuron or of neuron pairs was quantified using the Mahalanobis distance (MD) between spike-count distributions obtained during repeated presentations of a stimulus. If two stimuli evoke different spike counts, an observer can guess the identity of each stimulus by counting the number of spikes. If two stimuli evoke similar spike counts, it becomes difficult to discriminate between them. Thus the distance between spike-count distributions can be used as a measure of discriminability. MD was calculated with the following equation:

$$\mathrm{MD} = \frac{|m_1 - m_2|}{\sqrt{(\mathrm{var}_1 + \mathrm{var}_2)/2}}$$

m_i and var_i are the mean and the variance, respectively, of the spike-count distribution obtained through 10 presentations of stimulus i. MD was calculated for each stimulus pair. The mean Mahalanobis distance (MMD) across all the distribution pairs (pair-wise combinations of 64 stimuli yield 2016 stimulus pairs) represents the discriminability of a neuron. We compared the MMD from single-neuron activities and gathered (pooled or subtracted) activities to evaluate the effectiveness of gathering.

Pooling increased the MMD in 146 of the 1028 neuron pairs (14%) ($p < 0.05$, one-tailed Wilcoxon's rank sum test; open column in Fig. 9A). The neuron pairs with an increased

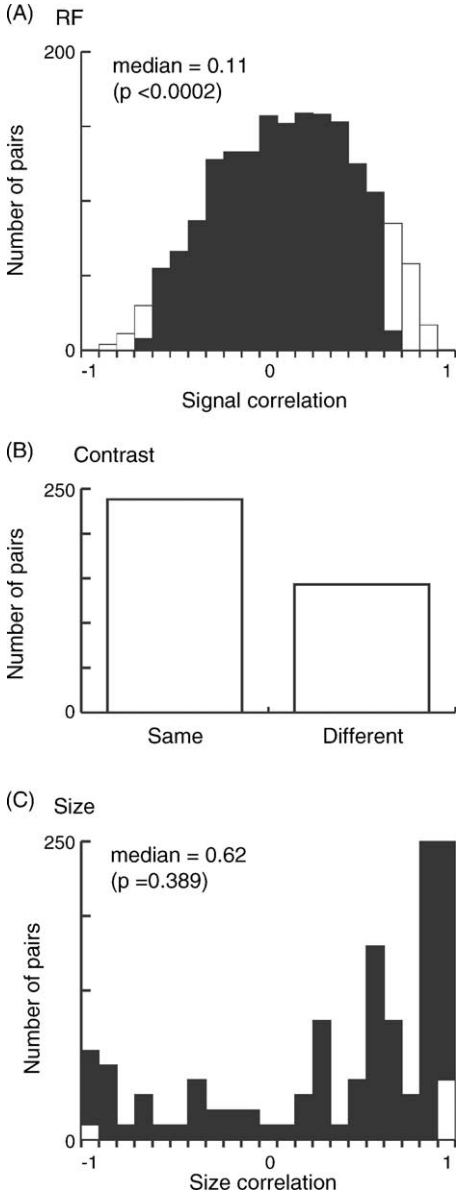

Fig. 8. (A) Frequency distribution of RF signal correlation from 1817 pairs of nearby neurons. (B) Incidence of neuron pairs with same contrast preference and that with different contrast preference. Three hundred and eighty one neuron pairs were analyzed. (C) Frequency distribution of size signal correlation from 109 pairs of nearby neurons. Other conventions are as in Fig. 3.

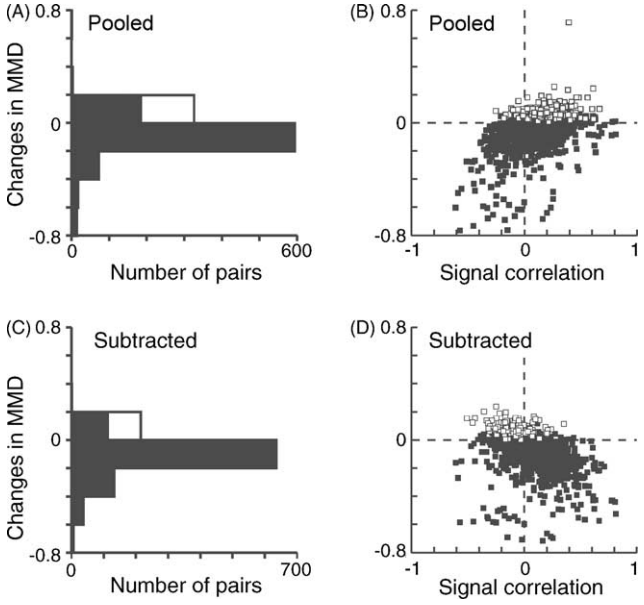

Fig. 9. Effects of pooling and subtraction on the mean Mahalanobis distance (MMD). (A) Frequency distribution of changes in the MMD [(pooled − single)/single] induced by pooling ($n = 1028$). If pooling increased the MMD, the index was larger than 0, otherwise it was equal to or less than 0. Open and closed columns indicate significant ($p < 0.05$, one-tailed Wilcoxon's rank sum test) and non-significant increases in the MMD, respectively. (B) The relationship between pooling induced changes in the MMD and signal correlation. Changes in the MMD were plotted against signal correlation. Open and closed squares indicate significant ($p < 0.05$) and non-significant increases in the MMD, respectively. (C) Frequency distribution of changes in the MMD induced by subtraction ($n = 1028$). (D) The relationship between changes in the MMD induced by subtraction and signal correlation.

MMD tended to have positive signal correlation (open squares in Fig. 9B). Signal correlation and changes in the MMD [(pooled MMD − single MMD)/single MMD] were positively correlated ($r = 0.45$, $p < 0.001$). Signal correlation between neurons in pairs with an increase in the MMD [$r = 0.22 \pm 0.18$ (S.D.)] differed from that between neurons in pairs without an increase in the MMD ($r = 0.06 \pm 0.22$; $p < 0.001$, U-test: Fig. 9B). The results indicate that pooling of activities from two neurons with positively correlated stimulus preferences could improve the stimulus discriminability of the neurons.

Subtraction of activities between two neurons increased the MMD in 105 of the 1028 pairs (10%; $p < 0.05$; open column in Fig. 9C). These neuron pairs tended to have a negative signal correlation (open squares in Fig. 9D). Signal correlation and changes in the MMD were negatively correlated ($r = -0.37$, $p < 0.001$). Signal correlation between neurons in pairs with an increase in the MMD ($r = -0.10 \pm 0.15$) differed from that between neurons in pairs without an increase in the MMD ($r = 0.11 \pm 0.21$; $p < 0.001$; Fig. 9D). The results indicate that subtraction of activities between two neurons with negatively correlated stimulus preferences could improve the stimulus discriminability of the neurons.

Analysis of the maximum MD among the 2016 MDs, instead of the MMD, yielded similar results (data not shown).

4. Discussion

The stimulus-image preferences of nearby TE neuron pairs were, on average, more similar than those of random neuron pairs. The presence of neurons with positively correlated stimulus preferences as well as those with

negatively correlated stimulus preferences at a local cortical site could be beneficial for stimulus discrimination, when their activities are appropriately combined and decoded by downstream neurons.

4.1. Clustering of neurons with similar stimulus preferences in area TE

Clustering of neurons with similar two-dimensional shape preferences in area TE has been reported (Gochin et al., 1991; Fujita et al., 1992; Fujita, 2002). In the previous studies, stimulus preferences were examined with two-dimensional shapes of intermediate complexity. In the present study, which was based on more than 1000 neuron pairs, we confirmed clustering of neurons with similar stimulus preferences by quantitatively investigating stimulus-image preferences with a large stimulus set including photographs of complex images. We used the same stimulus set for all neurons tested, thus allowing us to extract population measures (e.g., median signal correlation) and assess the similarity with no experimenters' bias.

Previous studies also showed that TE neurons along penetrations perpendicular to the cortical surface tend to have similar stimulus preferences, suggesting that area TE consists of functional columns (Fujita et al., 1992; Wang et al., 1998; Tsunoda et al., 2001; Fujita, 2002). The overall similarity of closely located neurons demonstrated in the present study may reflect this columnar organization. The present results may also reflect the laminar organization of neuronal response properties. In the primary visual cortex, for example, direction selective neurons are predominantly found in layers 4B and 6 (Hawken et al., 1988). The possibility that TE neurons in the same layer share some aspects of stimulus selectivity should be explored in future studies.

In the present experiments, activities from nearby neurons were simultaneously recorded, whereas those from distant neurons constituting most of the random neuron pairs were recorded in different sessions. If the animals were not maintained at stable conditions, changes in their conditions may modulate the activities of simultaneously recorded neurons in a similar way, resulting in a high correlation of their responses. We cannot rule out a possible bias towards overestimating the response correlation of simultaneously recorded neurons. We, however, confirmed in some of our neurons that the firing rate, stimulus preference, and cross-correlation remained unchanged over 1280 s between the initial five trial blocks and the latter five trial blocks (Kaneko et al., 2003). If the changes in conditions of the animals induced the correlated stimulus preferences, the effect should have been found in the other response characteristics tested. It should also be noted that anesthetized animals have some advantages for correlation analysis compared to awake, behaving animals. For example, other possible causes of overestimation of correlation measures such as fixational eye movements and changes in pupil size, accommodation, or attention level (Gur et al., 1997) are small in anesthetized animals.

4.2. Existence of neurons with different stimulus preferences in local regions of area TE

We showed that neurons with dissimilar preferences were also present in local regions of area TE. There were neuron pairs with a wide variety of degrees in response similarity. Some neuron pairs had positive signal correlation; others had independent signal correlation or negative signal correlation. Thus, the similarity of stimulus-image preferences between nearby neurons is weak on average. This weak correlation compared to the high similarity of stimulus selectivity reported earlier (Fujita et al., 1992; Wang et al., 2000) can be explained by several factors. First, we did not optimize shape, color, size, and orientation of stimulus for each neuron. Thus the estimate of stimulus preference may not be perfect and the correlation coefficient might be underestimated from the true value. Second, the weak similarity might result from the usage of photographs of complex natural images as visual stimuli. Because an photograph of complex natural image contains a variety of visual features and an interaction between features may affect the response of neurons, stimulus preference evaluated with the present stimulus set may well be different from that evaluated with two-dimensional shapes, despite of the positive correlation between *r-shape* and *r-object*. Finally, the weak correlation may result from the usage of correlation coefficient as an estimate for similarity of stimulus selectivity between neurons. For example, although all three cells in Fig. 3 responded best to shapes with an upward protrusion, they showed only a weak low correlation coefficient, because cell 1 preferred a white inverse T, cell 2 preferred a black one, and cell 3 preferred a square with an upward protrusion. A simple calculation of signal correlation will miss the similarity in such cases.

Weak similarity of stimulus preferences between nearby neurons has also been shown for other cortical regions. Nearby neuron pairs in the perirhinal cortex have a range of similarity of stimulus preferences, and the average similarity is low (Erickson et al., 2000). Even in the primary visual cortex, when neurons are examined with a variety of stimulus dimensions, the stimulus preferences of a neuron are not identical to all the other nearby neurons (Gawne et al., 1996; Maldonado and Gray, 1996; DeAngelis et al., 1999; Freeman, 2003). These results suggest that clustering of neurons according to their stimulus selectivity is not perfect in the cortex; there are a significant number of neurons with independent or negatively correlated stimulus preferences within local regions of the cortex.

Nearby TE neurons show similarity in their sensitivity to contrast and the RF organization (present study) and selectivity for binocular disparity (Yoshiyama et al., 2004). In contrast, the preference for stimulus size was not more positively correlated among nearby neurons than

random pairs. It is not clear why some stimulus properties are clustered and others are not. Differences in the need for response pooling as suggested by DeAngelis et al. (1999), and/or differences in the mechanisms that generate stimulus selectivity may be reflected in the different degrees of clustering. The result of size preference analysis, however, must be interpreted with caution. In the present study, we tested neurons only with three different sizes. The small number of stimuli may result in an inaccurate estimate of correlation coefficient.

Nearby neurons often project to the same target neurons where their activities can be integrated. One possible benefit of clustering of neurons with similar stimulus preferences is to decrease response variability and improve stimulus discriminability by response pooling at target neurons (Werner and Mountcastle, 1963; Tolhurst et al., 1983; Paradiso, 1988; Britten et al., 1992; Shadlen et al., 1996; Reich et al., 2001; Panzeri et al., 2003). We found that pooling improved stimulus discriminability in 14% of TE neuron pairs. Although the proportion of pairs with increased discriminability was low, the result was obtained by pooling all the possible combinations of nearby neuron pairs. Selective neural connections may more effectively improve stimulus discriminability by pooling.

If local intracortical interactions reduce response variability, clustering of neurons with the same stimulus preferences would be preferable. The role of local computation, however, is not restricted to the reduction of response variability; it can also contribute to the generation or elaboration of stimulus selectivity. From this point of view, the presence of neurons with different stimulus preferences in proximity is desirable (Troyer et al., 1998; Tamura et al., 2003, 2004).

We found that subtraction increased the stimulus discriminability in 10% of TE neuron pairs. Subtraction was effective, when it was performed selectively among neurons with negatively correlated stimulus preferences. Romo et al. (2003) have shown that subtraction between neurons with different stimulus-tuning properties increases stimulus-coding accuracy in the secondary somatosensory cortex. They recorded neural activity while monkeys performed a tactile discrimination task and estimated psychophysical and neuronal performance based on the signal detection theory. They showed that subtraction of activity between neurons with negatively correlated frequency tuning was beneficial. With subtraction, neurons with different stimulus preference can engage in the reduction of response variability.

In the present study, we explored the possibility of pooling and subtraction of activity of nearby neurons as a decoding method used by their target neuron. Another possible decoding scheme is the multi-dimensional decoding in which inputs from each neuron are decoded without loosing neuron's identity (Rolls et al., 1997; Reich et al., 2001; Schneidman et al., 2003). In this decoding strategy, the presence of neurons with different stimulus preference is advantageous, because it allows the dimensions of the discrimination space more orthogonal. Exact estimation of ability of population coding is, however, difficult, because of the limited sampling problem (Panzeri and Treves, 1996).

In conclusion, nearby neuron pairs in area TE had a variety of degrees in correlation of their stimulus-image preferences. On average, they exhibited a weak, positive correlation in stimulus preference. Neurons with similar as well as dissimilar stimulus preferences can contribute to the improvement of stimulus discriminability, if these decoding strategies are applied to appropriate neuronal pools.

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