

# Current Biology

## Differential Impact of Behavioral Relevance on Quantity Coding in Primate Frontal and Parietal Neurons

### Highlights

- Neurons in monkey PFC and VIP were recorded before and after numerosity training
- PFC showed improved responses to numerosity during active discrimination
- VIP neurons continued to respond to numerosity just as before training
- Flexible numerical PFC representations contrast with stable VIP coding

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### In Brief

Viswanathan and Nieder describe how the monkey prefrontal cortex shows elevated responses to magnitude categories during active discrimination, whereas the parietal cortex encodes quantity categorically, regardless of behavioral relevance. This indicates that quantity is perceived as a special “natural” category.



# Differential Impact of Behavioral Relevance on Quantity Coding in Primate Frontal and Parietal Neurons

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## SUMMARY

Prefrontal cortex (PFC) and posterior parietal cortex are key brain areas for magnitude representations. Whether active discrimination of numerosity changes neuronal representations is still not known. We simultaneously recorded from the same recording sites in the PFC and ventral intraparietal area (VIP) before and after monkeys learned to actively discriminate the number of items in a set. Only PFC neurons, and not VIP neurons, exhibited heightened representation of number after numerosity training. Increased responsiveness of PFC was evidenced by enhanced differentiation of numerosity by the population of neurons, as well as increased numerosity encoding by individual selective neurons. None of these effects were observed in the VIP, in which neurons responded invariably to numerosity irrespective of behavioral relevance. This suggests elevated PFC participation during numerical task demands and executive control, whereas VIP encodes quantity as a perceptual category regardless of behavioral relevance.

## INTRODUCTION

Assessing the number of elements in a set, its numerosity, requires a high level of sensory abstraction. Studies in behaviorally trained nonhuman primates identified a cortical network in the prefrontal (PFC) and posterior parietal cortex (PPC) with individual neurons selectively responding to the number of items [1, 2]. Such numerosity-selective neurons have also been traced indirectly in the human brain using fMRI [3, 4]. Whereas it was tacitly assumed that neuronal responses to numerosities were shaped by or even caused by extensive behavioral training, neurons in PFC and the intraparietal sulcus have recently been reported to encode numerosity even in monkeys that were never trained to discriminate numerosities [5]. Furthermore, the tuned coding of preferred numerosities in numerically naive monkeys was strikingly similar to that found in experienced animals. Together with psychophysical findings that numerosity representations resemble perceptual categories like color and shape and are susceptible to adaptation [6, 7], the spontaneous presence of numerosity-selective neurons in untrained animals argues

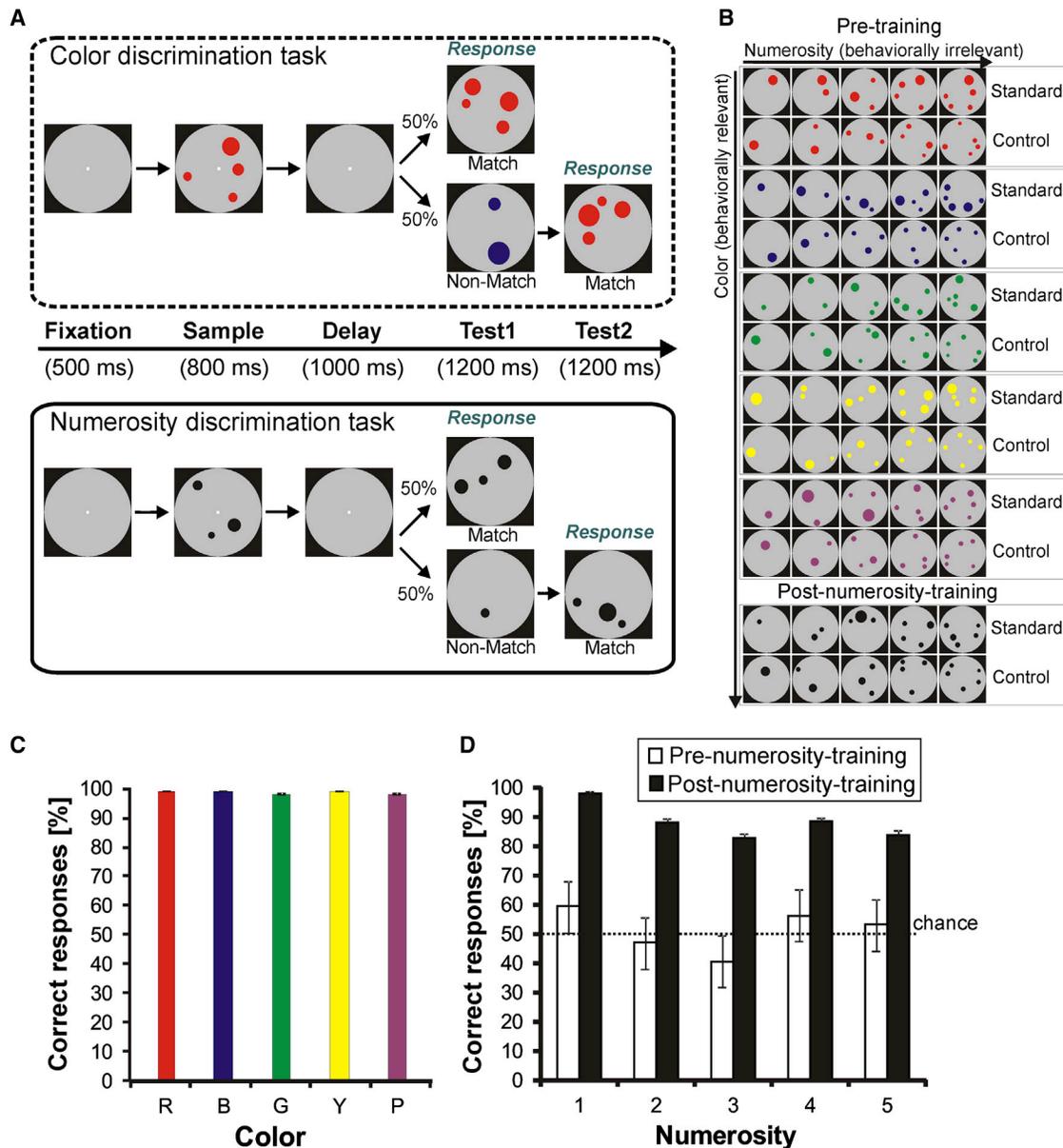
for a “sense of number,” the faculty to perceive numerosity intuitively [8, 9].

Whereas much has been learned about numerosity coding by neurons in the fronto-parietal cortex, the role of behavioral relevance and learning in the modulation of neuronal selectivity remains unexplored. Both parietal and PFC neurons show increased responses to behaviorally relevant as opposed to irrelevant stimuli [10, 11]. Experience-dependent plasticity is further suggested by observations that visual neurons in prefrontal [12] and parietal [13] visual areas can respond highly selectively to familiar and well-trained visual stimuli. Not only do response properties change, but also the proportion and location of selective neurons change with learning [14]. Moreover, prefrontal and posterior parietal neurons robustly reflect the learned category membership of visual stimuli, and visual selectivity shifts after monkeys were retrained to group the same stimuli into two new categories [13, 15, 16]. Whether abstract representations of quantity experience modifications with behavioral relevance, learning, and familiarity, however, remains elusive.

To address this, we simultaneously recorded from the same recording sites in the PFC and ventral intraparietal area (VIP) while numerically naive monkeys discriminated the color of a set of dots and, after numerosity training, responded to the number of items of equivalent dot collections. We found contrasting neuronal effects for PFC and VIP neurons as a result of learning to discriminate numerosity explicitly. In addition, the observed findings were not predicted by experiments using arbitrary perceptual categories as discriminative stimuli.

## RESULTS

We analyzed single-neuron activity from the parietal and prefrontal cortices of two monkeys before and after training on a numerosity-delayed match-to-sample task. Before numerosity training, monkeys matched the color of sequentially presented multi-dot displays (color task; Figure 1A, top). After numerosity training, they matched the number of all black dots in the sequentially presented multi-dot displays (numerosity task; Figure 1A, bottom). The task structure stayed the same for both discrimination protocols: monkeys watched a sample display after a fixed period of visual fixation. The sample was followed by a 1-s memory delay, after which the test display appeared. In the color task, the test display matched the sample in color in 50% of the trials (match trials) and did not match in the other 50% of the trials (non-match trials). Importantly, the number of dots also varied systematically in the dot displays but was



**Figure 1. Behavioral Task Design, Example Stimuli, and Behavioral Performance**

(A) Task: the delayed match to sample task involved an initial fixation period of 500 ms followed by a sample period where the visual dot arrays were presented. The monkeys were required to remember the sample through the subsequent delay period and respond only to matching test stimuli. If a non-match stimulus followed, they were required to withhold response until the match appeared. The color discrimination task (top panel) was used for all the pre-training data and the numerosity discrimination task (bottom panel) after the monkeys were trained to discriminate numerosity, for the post-training data.

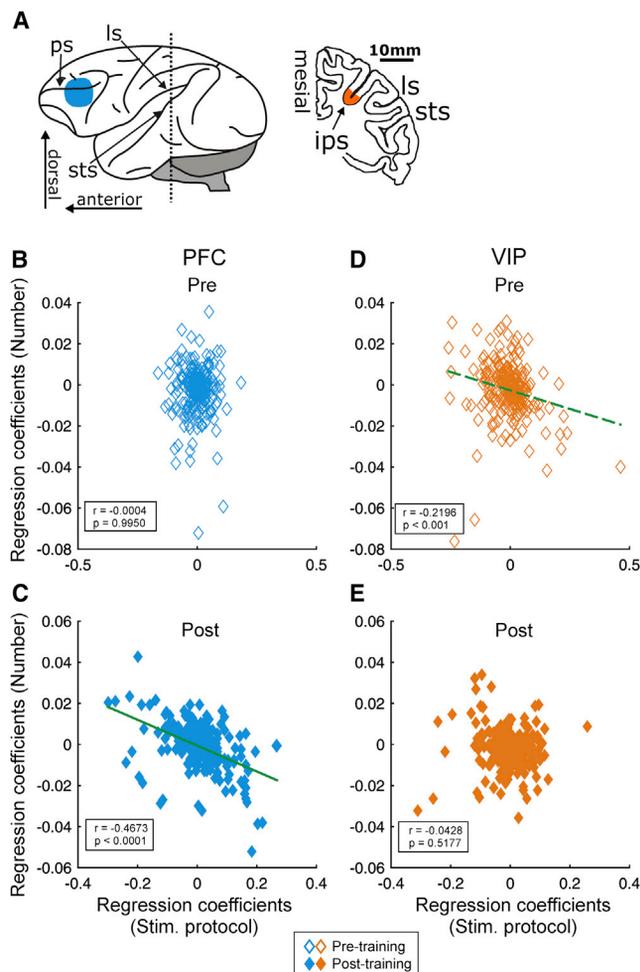
(B) Examples of the dot array stimuli used. For the color-discrimination task, all five colors were tested in all five numerosities and across two stimulus protocols. The standard stimuli (odd rows) consist of randomly sized and spaced dots. The control stimuli (even rows) are such that the colored area and the dot density are equalized across numerosities.

(C) Behavioral performance on color discrimination task with the various colors as sample, averaged across monkeys, as a percentage of total trials. Error bars denote SEM.

(D) Behavioral performance on the numerosity-discrimination task as tested before and after numerosity training (empty bars denote chance level performance before training; filled bars denote performance after training; dashed horizontal line denotes 50% chance level) for each number as sample numerosity. Error bars denote SEM.

behaviorally irrelevant and was not used by the monkeys (that were not trained to respond to numerosity at that stage) to solve the task. In the numerosity task, the test displays matched the sample with respect to the number of items (50% match trials),

whereas the numerosity did not match in the remaining trials (50% non-match trials). In the non-match trials, the non-match test1 item was always followed by a match test2 item. The monkeys had to respond to the matching item (matching



**Figure 2. Recording Areas and Neuronal Populations**

(A) Schematic diagram of the macaque brain, illustrating the locations where recordings were performed. Abbreviations: AS, arcuate sulcus; IPS, intraparietal sulcus; PS, principal sulcus; STS, superior temporal sulcus.

(B) De-noised regression coefficients for the factor numerosity plotted against those for the factor stimulus protocol for each recorded neuron. The coefficients describe how much of the trial-by-trial firing rate of the neuron is affected by the plotted factors “protocol” and “number”. Each dot on the plot denotes a PFC neuron. Correlations between coefficients are shown ( $p < 0.05$ ; Pearson’s correlation coefficient,  $r$ ) and significant correlations are indicated by the regression lines in green.

(C) The same layout as in (B) for the PFC neurons recorded post-training.

(D and E) The same as (B) and (C) for area VIP.

See also [Figures S1](#) and [S2](#).

color—before training; matching number—after training) by releasing a bar that they held throughout the trial. To exclude that the monkeys were responding to low-level visual features that co-varied with numerosity, control stimuli were applied (in 50% of the trials) in addition to the pseudo-randomized standard stimuli. Both in the color task and the numerosity task, the total dot area and density were equated across numerosities in the control stimuli ([Figure 1B](#)). All task parameters (match versus non-match, color versus numerosity, and standard versus control stimuli) were balanced and presented pseudo-randomly to the monkeys.

### Behavioral Performance before and after Numerosity Training

In the color task, before numerosity training, color discrimination performance ([Figure 1C](#)) was well above chance for both monkeys (monkey L:  $99.19\% \pm 0.24\%$ ; monkey S:  $97.93\% \pm 0.34\%$ ; binomial test;  $p < 0.001$ ) for all color combinations (as reported previously in [5]). We confirmed that none of the monkeys learned to judge numerosity in the color task by confronting the monkeys with colorless black dots. During the color task, numerosity performance tested on two sessions was at chance level for both monkeys (monkey L:  $43.8\% \pm 12.7\%$ ; monkey S:  $58.8\% \pm 12.4\%$ ; two-tailed binomial test;  $p > 0.05$ ). This suggests that the monkeys were unable to use numerosity as discriminating stimulus feature during the color task.

After single-cell recordings during the color task were completed, the same monkeys were retrained to discriminate numerosity. Color information was eliminated to avoid Stroop-like effects. After approximately 2 months of training (monkey L: 41 sessions; monkey S: 30 sessions), both monkeys reached a high level of numerosity discrimination performance (monkey L:  $91.4\% \pm 0.78\%$ ; monkey S:  $84.5\% \pm 0.99\%$ ; two-tailed binomial test;  $p < 0.001$ ; same sample numerosities as in the color task; numerical distance between sample and non-match of two or more; [Figure 1D](#)). Performance also showed the classical effects reported in earlier studies, such as the numerical distance and size effects [17]. These results collectively show that the monkeys were numerically naive during the color task but numerically competent and able to discriminate the number of items after numerosity training.

### Representation of Task Variables in the Neuronal Populations

We recorded single-cell activity from the lateral PFC and the VIP before and after numerosity training, i.e., during the color and the numerosity task, from the same two monkeys ([Figure 2A](#)). We targeted the same electrode penetration coordinates and depths in the color and the numerosity task in both individual monkeys. This allowed for recordings from the same recording sites post-numerosity training from where the majority of neurons were sampled before numerosity training.

We applied multi-variable linear regression analysis to the trial-by-trial firing rates of all single neurons [18] to first explore the contributions of the recorded neuronal populations in encoding the behaviorally irrelevant features of number and stimulus protocol during the color task. We then applied the same analysis for the same features, which became behaviorally relevant during the number task. We calculated the weights with which the various stimulus features affected the neuronal activity and used principal-component analysis (PCA) to estimate the most informative (first 12 PCAs) of these weights at each time point within the analysis period. We call these estimated weights “de-noised regression coefficients” of number and stimulus protocol. In particular, we examined the correlations between these de-noised weights of number and stimulus protocol. We compared these correlations in the pre-training and the post-training periods as they reflect how well the neuronal population was able to extract the numerosity of the stimuli from the co-varying lower level visual features to solve the number task.

We compared sample activity of a total of 268 PFC cells recorded during pre-training and 245 cells recorded post-training with the multi-variable linear regression analysis without pre-selecting neurons for any response properties. The weights of the “number” or “protocol” predictors did not show a significant difference between the pre-training and post-training population (Mann-Whitney U test;  $p = 0.08$  and  $p = 0.20$ , respectively). The regression coefficients (beta values) for the factors “stimulus protocol” and “numerosity” were not correlated pre-training (Pearson’s correlation coefficient;  $r < 0.001$ ;  $p = 1.00$ ; [Figure 2B](#)). However, the coefficients were significantly and negatively correlated post-training ( $r = -0.4673$ ;  $p < 0.0001$ ; [Figure 2C](#)). The correlations for the population indicate that the neuronal units that are strongly regressed by one of the factors are less or sometimes conversely affected by the other factor. Excluding color as a predictor in the pre-training linear model did not change the main findings. The improvement in the PFC population as evident in the weak negative correlation between the predictors for number and stimulus protocol remained ( $r = -0.1355$ ;  $p = 0.03$ ; [Figures S1A and S1B](#)).

In contrast, the comparison of the population of 238 VIP cells pre-training and 231 cells post-training showed the opposite effect when performing the same multi-variable linear regression analysis. The regression coefficients were significantly and negatively correlated pre-training ( $r = -0.2196$ ;  $p < 0.001$ ; [Figure 2D](#)) but were no longer correlated post-training ( $r = -0.0428$ ;  $p = 0.52$ ; [Figure 2E](#)). The weights of the number or protocol predictors do not show a significant difference between the pre-training and post-training population (Mann-Whitney U test;  $p = 0.44$  and  $p = 0.26$ , respectively). Excluding color as a predictor in the pre-training period only enhanced the negative correlation observed in the VIP population pre-training ( $r = -0.6954$ ;  $p < 0.0001$ ; [Figures S1C and S1D](#)).

We used an ANCOVA (at  $\alpha = 0.05$ ) to test the regression lines pre- and post-training for the two areas (green lines, [Figure 2](#)). We found that, for both areas, PFC and VIP, the slopes of the regression line were significantly different with active numerical discrimination. For PFC, the slope post-training was significantly higher ( $p = 0.0001$ ), and for VIP, the slope post-training was significantly lower than pre-training ( $p = 0.0394$ ). Additionally, the slope of PFC population post-training was also significantly higher than that of VIP pre-training ( $p = 0.0359$ ).

### Proportions of Numerosity-Selective Cells Increased Only in PFC with Behavioral Relevance

To identify individual neurons that were selective to numerosity and presumably maximally contributed to the observed effects found in the population analysis, we performed an ANOVA based on the trial-by-trial firing rates for each neuron separately. For the pre-training data (color task), a three-factor ANOVA with main factors “sample color” (five colors), “sample numerosity” (numerosity 1–5), and “stimulus protocol” (standard versus control stimuli) was calculated (at  $\alpha = 0.01$ ). For the post-training condition (numerosity task), a two-factor ANOVA with main factors “sample numerosity” and “stimulus protocol” was applied. Numerosity-selective cells were determined to be those cells that displayed a main effect for the factor sample numerosity. In [Figure 3](#), example numerosity-selective neurons and their

respective tuning curves from the PFC ([Figure 3A](#)) and the VIP ([Figure 3B](#)) can be seen.

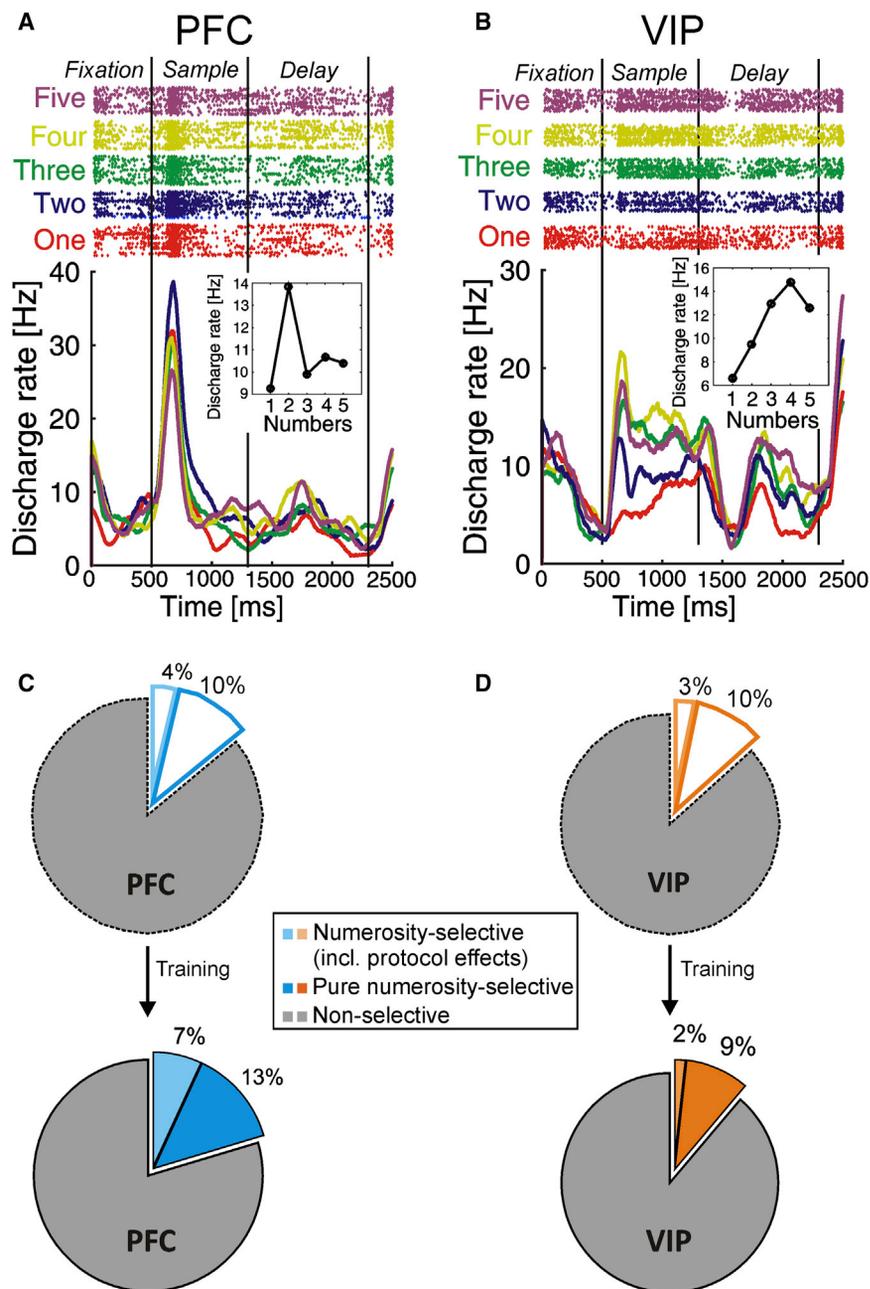
To confirm that the results from the multi-variable linear regression analysis of the population mainly relied on the contributions of numerosity-selective neurons, we calculated the correlations based solely on the numerosity-selective neurons identified by the ANOVA. In PFC, the coefficients were significantly negatively correlated post-training, but not pre-training ([Figures S2A and S2B](#)). In VIP, we found significantly negatively correlated coefficients pre-training, but not post-training ([Figures S2C and S2D](#)). For both post-training PFC and pre-training VIP, the magnitude of Pearson’s correlation coefficients were higher for the population of numerosity-selective neurons than the entire population of all recorded neurons. This suggests that numerosity-selective neurons contributed significantly to the observed population effects and thus were probably most important to convey numerosity information.

We found evidence that the proportion of numerosity-selective cells in the PFC increased from 14% (38/268) pre-training to 20% (50/245) post-training (chi-square test;  $p = 0.06$ ; [Figure 3C](#); [Table S1](#)). The majority of these cells were unaffected by the co-varying lower visual features of the stimuli and thus showed no effect of the stimulus protocol or an interaction of numerosity with stimulus protocol. In the PFC, the proportion of such “pure” numerosity-selective cells was 10% pre-training and 13% post-training.

We did not observe a change in the proportion of numerosity-selective neurons in the parietal cortex. Numerosity-selective cells in the VIP were 14% (32/238) pre-training and 11% (26/231) post-training (chi-square test;  $p = 0.47$ ). Pure numerosity proportions, i.e., without effects or interactions of stimulus protocol, were 10% pre-training and 9% post-training ([Figure 3D](#)). We report the results of the ANOVAs in detail in [Table S1](#).

### Sharpness of Numerosity Tuning Was Unchanged by Relevance

Active discrimination has been shown to change tuning properties of sensory neurons. We therefore investigated whether active numerosity discrimination resulted in an increase in the strength of tuning to numerosity in our selective population. Numerosity-selective cells have displayed tuned responses to the number of items in dot displays [19], in item sequences [20], and across modalities [21]. Such tuning is characterized by a maximal response toward a preferred numerosity with a gradual decrease of activity for numerosities with increasing numerical distance to the preferred numerosity. We also found tuned responses to numerosity in our selective population before and after numerosity training ([Figures 4A–4D](#)). The frequencies of preferred numerosities were also similar in both areas pre- and post-training. We compared the tuning sharpness pre-training and post-training in PFC and VIP from population-tuning curves created by normalizing and averaging all individual tuning curves around the preferred numerosity and the graded responses expressed as a factor of numerical distance. The pre-training and post-training population tuning functions for PFC ([Figure 4E](#)) and VIP neurons ([Figure 4F](#)) were indistinguishable (except for few arbitrary numerical distances; Mann-Whitney U test;  $p < 0.05$ ).



**Figure 3. Numerosity-Selective Neurons**

(A) An example numerosity-selective cell in PFC. Trials are sorted by sample numerosity (top panel) in the raster plot, and each dot denotes an action potential. Vertical lines mark the various task phases. The discharge is thus averaged across trials to create a peri-stimulus time histogram (bottom panel) for each sample numerosity. The inset shows the numerical tuning function for that neuron by averaging the activity across time and trials.

(B) The same as (A) for an example neuron recorded in VIP.

(C) Pie charts showing the proportions of numerosity-selective neurons among those recorded in the PFC. The dashed contours enclose the proportions found in the PFC pre-training (top), and the solid contours enclose the proportions found post-training (bottom). The colored areas depict the numerosity-selective proportions found with ANOVA. The darker shaded areas depict the “purely” numerosity-selective proportions, and the lighter shaded areas depict the proportions sensitive to stimulus protocol effects, i.e., co-varying low-level visual features of the stimulus.

(D) Same layout as (C) for area VIP.

See also [Figure S2](#) and [Table S1](#).

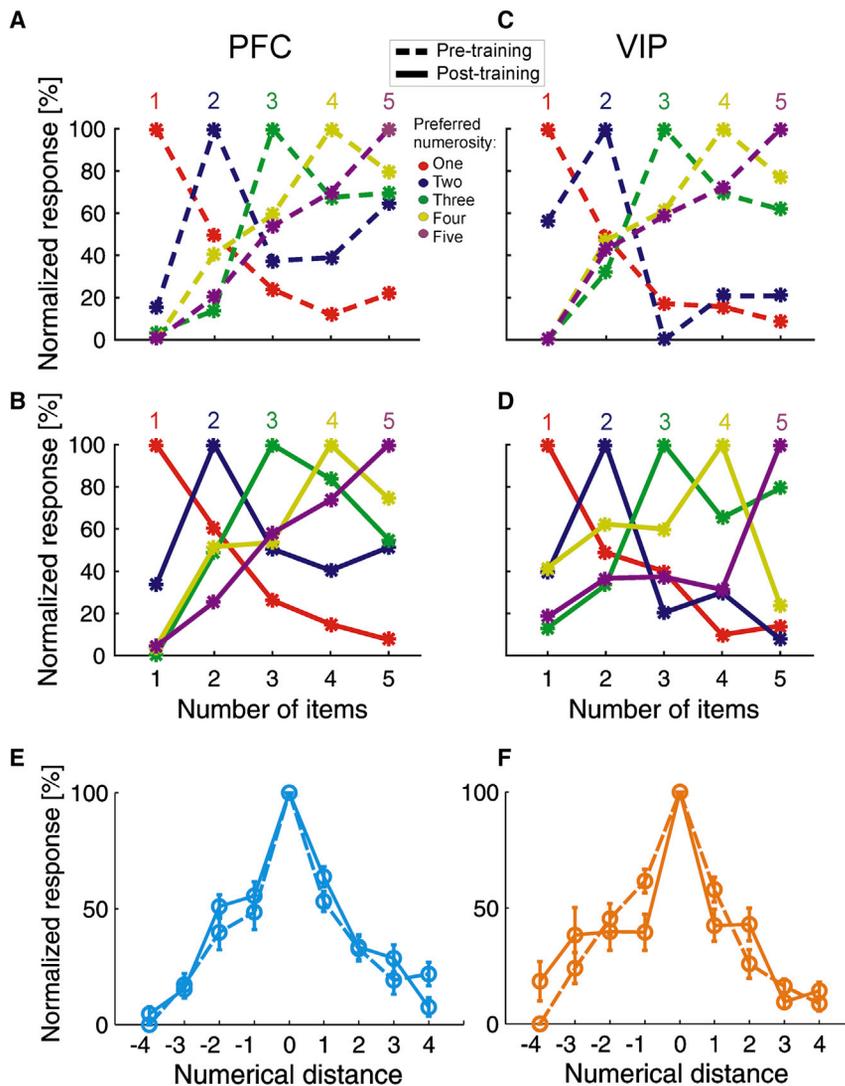
### Explained Variance Measures

Because raw tuning curve measures do not necessarily take the strength of response modulation into account, we also calculated the proportion of explained variance ( $\omega^2$  PEV) [22]. It quantifies how much information about the sample numerosity was carried by the discharge rates of the population of numerosity-selective neurons. We used a two-way ANOVA with the factors sample numerosity and stimulus protocol to additionally explore the interaction term between stimulus protocol and numerosity.

The sliding-window analysis in [Figure 5A](#) shows that the  $\omega^2$  values for PFC neurons increased during the sample period, as expected for selective neurons. Interestingly, however, the  $\omega^2$

PEV values were higher during post-training compared to pre-training. This difference was significant when compared in an 800-ms interval covering the entire sample period (median 0.0591 pre-training,  $n = 38$ ; median 0.0640 post-training,  $n = 50$ ; Mann-Whitney U test;  $p = 0.025$ ; [Figure 5A](#), inset). This difference was still present when only the purely numerosity-selective neurons were analyzed (two-tailed Mann-Whitney U test;  $p = 0.024$ ;  $n = 28$  pre-training and  $n = 33$  post-training). The explained variance for the stimulus protocol and interaction did not show any significant changes ([Figure 5A](#), purple and black functions). The explained variance for the whole population of PFC cells did not change post-training (all cells, median 0.0025 pre-training; median 0.0039 post-training;  $p = 0.24$ ).

In the VIP, however, the result was different ([Figure 5B](#)). The  $\omega^2$  PEV for the factor numerosity did not change for pre-compared to post-training (median 0.0577 pre-training,  $n = 32$ ; median 0.0605 post-training,  $n = 26$ ; two-tailed Mann-Whitney U test;  $p = 0.52$ ; [Figure 5B](#), inset). For purely numerosity-selective neurons, there was no difference in  $\omega^2$  PEV between pre- and post-training (two-tailed Mann-Whitney U test;  $p = 0.96$ ;  $n = 24$  pre-training and  $n = 22$  post-training). The explained variance for the stimulus protocol and interaction did not show any significant changes. The explained variance for the whole population of VIP cells did not change



**Figure 4. Tuning Curves of Selective Neurons**

(A–D) The numerosity-selective neurons are grouped according to the preferred number eliciting the maximal response, indicated here by the different colors. Their responses to the various numerosities are then normalized and plotted here as tuning curves. (A) PFC neurons recorded pre-training ( $n = 38$ ). (B) PFC neurons recorded post-training ( $n = 50$ ). (C) VIP neurons pre-training ( $n = 32$ ). (D) VIP neurons post-training ( $n = 26$ ). (E) The neuronal responses to various sample numerosities are normalized (preferred numerosity = 100% and least preferred numerosity = 0%) and centered to the preferred numerosity such that the other sample numerosities are expressed as numerical distance from the preferred numerosity. Dashed lines depict selective cells pre-training and solid lines post-training in the PFC. Error bars denote SEM. (F) The same as (E) for VIP neurons.

post-training (all cells, median 0.0039 pre-training; median 0.0028 post-training;  $p = 0.24$ ).

#### Numerosity Discriminability Changes in PFC and VIP

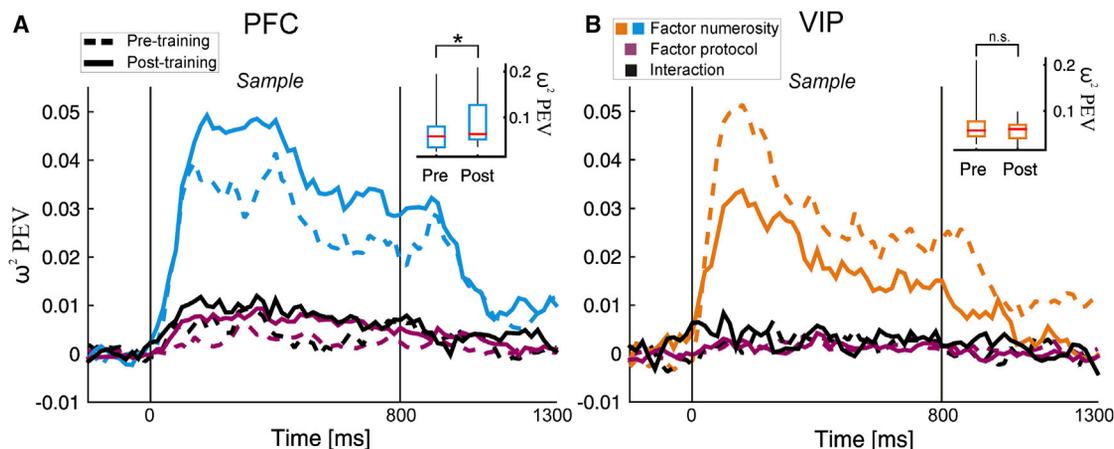
We applied an ROC analysis derived from signal detection theory to quantify the neuronal discriminability for numerosity in the same sample time windows as used for the other analyses. The values of the area under the ROC curve (AUC) could range from 0.5 (no discriminability between most- and least-preferred magnitude value) to 1.0 (perfect discriminability).

In the PFC, the AUC values were significantly higher post-training compared to pre-training (median pre-training = 0.693 to post-training = 0.724; two-tailed Mann-Whitney U test;  $p = 0.016$ ; Figure 6A). This significant improvement in discriminability was also seen for purely numerosity-selective neurons alone (two-tailed Mann-Whitney U test;  $p = 0.024$ ). The AUC value also increased significantly across the whole population of PFC neurons, irrespective of numerosity selectivity (for all recorded PFC cells, median pre-training = 0.586 to median post-training = 0.595;  $p < 0.05$ ). This indicates that numerosity

discriminability robustly increased post-training in the PFC for neuronal populations containing numerosity-selective neurons. This improvement did not arise from differences between firing-rate distributions of the two recording periods. We tested the means of distributions (t test;  $p > 0.05$ ) and the shape of the distributions (Kolmogorov-Smirnov test;  $p > 0.05$ ) and found no significant differences between the pre-training and post-training samples. Additionally, this change in the AUC values was stable during the entire recording period (Figure 6C) and did not change over time (regression analysis;  $p > 0.1$ ). For number-selective PFC cells, the AUC calculated for error trials post-training had a

median of 0.708 and was not significantly different from those calculated for correct trials ( $p = 0.11$ ). For the whole population of PFC cells, median AUC for error trials was 0.517 and significantly different from those calculated for correct trials ( $p < 0.0001$ ).

The neuronal discriminability in VIP, on the other hand, did not change with training (Figure 6B). The AUC values pre- and post-training were comparable for numerosity-selective neurons (median pre-training = 0.715; post-training = 0.702; two-tailed Mann-Whitney U test;  $p = 0.120$ ) and also for the population of purely numerosity-selective neurons (two-tailed Mann-Whitney U test;  $p = 0.286$ ). Similarly, no difference was detectable for the entire population of all recorded VIP neurons (for all recorded VIP cells, median pre-training = 0.597 to median post-training = 0.598;  $p < 0.05$ ). For number-selective VIP cells, the AUC calculated for error trials post-training had a median of 0.618 and was not significantly different from those calculated for correct trials ( $p = 0.06$ ). For the whole population of VIP cells, median AUC for error trials was 0.515 and significantly different from those calculated for correct trials ( $p < 0.001$ ).



**Figure 5. Numerosity Information in Selective Neurons**

(A) Proportion explained variance ( $\omega^2$  PEV) calculated with a sliding window of 100 ms slid by 20 ms steps for the numerosity-selective cells in PFC. Dashed lines in the plot depict pre-training data, and solid lines depict post-training data. Cyan lines show the  $\omega^2$  PEV values for the factor numerosity, purple lines the factor stimulus protocol, and black lines the interaction (numerosity  $\times$  stimulus protocol). The inset boxplot describes the numerosity  $\omega^2$  PEV calculated during the sample period for all the selective neurons. The horizontal red lines indicate the medians within the boxes spanning the 25<sup>th</sup>–75<sup>th</sup> percentiles of the data. The whiskers span the 5<sup>th</sup>–95<sup>th</sup> percentiles.

(B) The same as (A) for area VIP with orange lines depicting the  $\omega^2$  PEV values for the factor numerosity.

### Broad Spiking Cells Show Improvement by Numerosity Training in PFC

Finally, we investigated the training effects for the two major cortical cell classes [23–25]. We grouped the recorded neurons based on their extracellularly recorded waveforms into narrow spiking (NS) (23% of all neurons pre-training and 23% post-training), i.e., putative interneurons, and broad spiking (BS) (74% of all neurons pre-training and 73% post-training), i.e., putative pyramidal cells (Figure 7). We calculated an averaged and normalized waveform for each recorded neuron and used a linear classifier to classify the neurons into the two different classes. This method of classification has been used in recent studies to investigate the involvement of different neuronal classes in different aspects of a task [26].

In the PFC (Figure 7A), BS cells showed a slight increase in AUC values (0.693 to 0.718;  $p = 0.0505$ ) post-training. NS cells, however, did not show changes (0.694 to 0.735;  $p > 0.1$ ) with behavioral relevance. In the VIP (Figure 7B), neither cell class showed a corresponding effect (BS cells 0.722 to 0.694;  $p = 0.0985$ ; NS cells 0.705 to 0.708;  $p > 0.1$ ).

## DISCUSSION

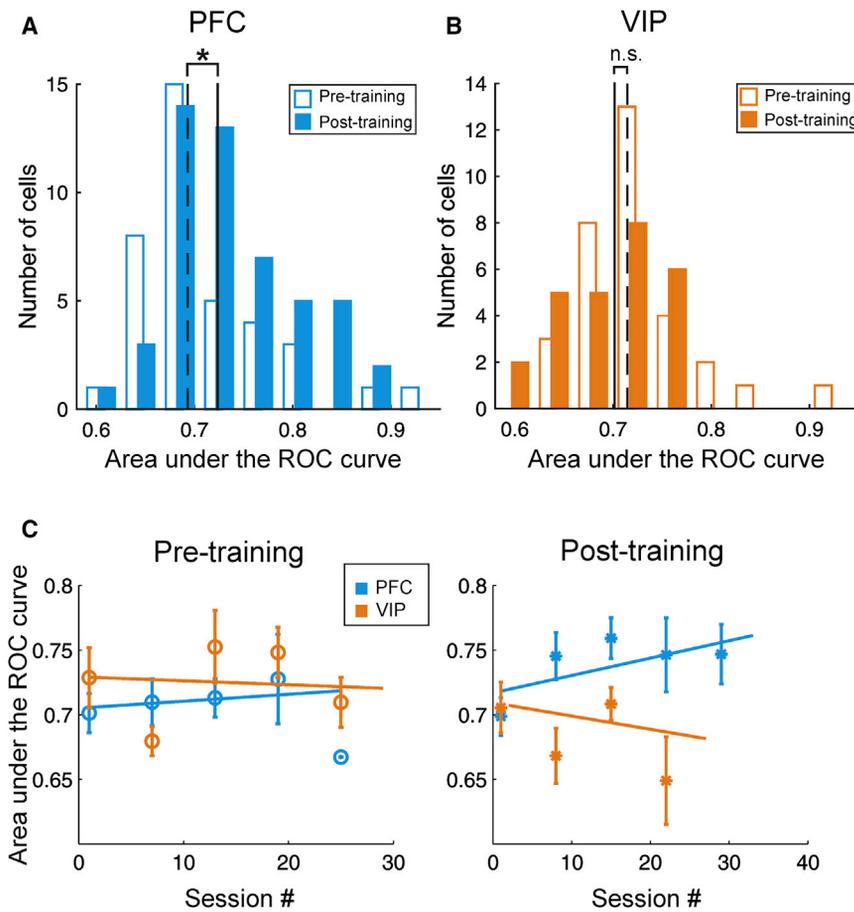
We hypothesized that active discrimination of numerosity would change response properties of neurons in the PFC and/or VIP, two areas known to be engaged in processing numerical information. We report that only the PFC became more responsive to numerosity during active numerosity discrimination. The regression analysis performed for the entire neuronal population showed that the PFC improved in its ability to differentiate between numerosity and co-varying lower visual parameters. Closely following this finding, numerosity-selective neurons in PFC also became more frequent and more informative about numerosity. This improve-

ment was due mostly to broad-spiking putative pyramidal neurons.

In contrast to the PFC, none of these effects were observed for VIP neurons, even though VIP neurons were also responsive to numerosity. As a population, VIP neurons were not effective in discriminating between numerosity and co-varying lower visual parameters after numerosity training whereas individual numerosity-selective cells maintained their selectivity. Neither the proportion of numerosity-selective cells, nor numerosity discriminability of VIP neurons changed with active discrimination of numerosity. This lack of modulation of quantity categories in the parietal cortex through behavioral relevance stands in contrast to previous findings obtained with arbitrary perceptual categories.

### PFC Encodes Behaviorally Relevant Numerical Information

Our population analysis of the task variables and their effect on trial-by-trial firing rates yielded diametrically opposite results in prefrontal and posterior parietal lobe. The post-training emergence of a neuronal PFC population that was differentially influenced by the factors number and stimulus protocol contrasted with the lack of such correlated activity post-training in VIP. As the de-noised regression coefficients describe how much of the trial-by-trial firing rate of the unit depends on the task variables at hand [18], the correlations between the regression coefficients to the different factors are telling of the mixed selectivity experienced by the units [27]. The emergence of this property in PFC during active numerosity discrimination indicates that prefrontal neurons distinguished between the numerosity of the stimuli and the co-varying visual features much more strongly post-training. Thus, our results are indicative of the PFC playing a role in actively discriminating behaviorally relevant numerical categories from the co-varying visual features with decreased behavioral relevance.



**Figure 6. Coding Quality Assessed by Area under the ROC Curve**

(A) Histograms showing AUC values in PFC colored by recording periods; empty bars, pre-training; filled bars, post-training. Black vertical lines indicate the median values; dashed lines, pre-training; solid lines, post-training.

(B) The same as (A) for area VIP.

(C) AUC values plotted as a function of days (session numbers) pre-training (left panel) and post-training (right panel). Each data point represents an average across all neurons recorded in a bin of 6 days (pre-training) or 7 days (post-training); cyan data points indicate PFC cells, and orange data points indicate VIP cells. Error bars show SEM. Solid lines represent the linear regression; none of the slopes was significantly different than zero.

This is consistent with the PFC conveying top-down signals to parietal neurons to exert cognitive control during rule-based tasks [16].

### Selective Neurons in PFC, but Not VIP, Improve during Active Numerosity Discrimination

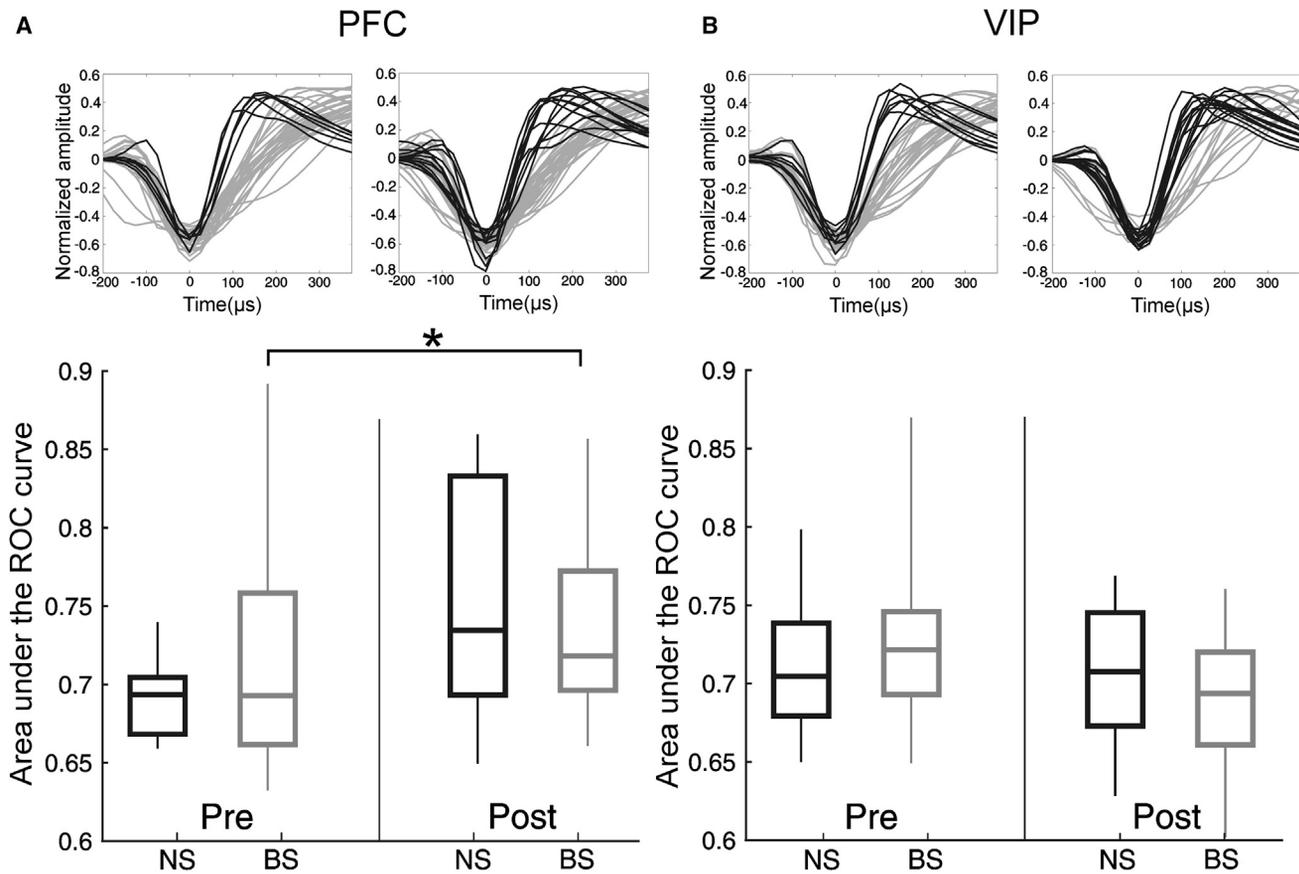
After the color-discrimination task, we retrained the monkeys to discriminate numerosity. This introduced numerosity as a behaviorally relevant stimulus feature and increased the monkeys' experience with numerosity. One might expect that these changes also had an impact on the response properties of neurons in such classical association areas like the PFC and the VIP. Experience-dependent sharpening of neuronal selectivity has been described in early (V1) [28] and intermediate (V4) [29, 30] visual cortex. Also in the inferior temporal cortex (area IT), the termination zone of the ventral visual pathway, learning to discriminate among complex objects was found to enhance object selectivity of neurons [31, 32]. Similarly, neurons in the PPC of the dorsal visual pathway have been shown to reflect behavioral relevance [10, 33] and learned arbitrary category membership of visual motion stimuli [13, 34]. In the PFC, behavioral relevance sometimes has dramatic effects on neuronal responses and can even re-tune cells according to changed boundaries of arbitrary perceptual categories [35]. An increase in proportions of responsive neurons when switching from a passive fixation

task to an active working memory task has also been found in PFC [14].

Our data show that learning- and relevance-dependent neuronal plasticity does not hold true for all possible visual stimulus features, particularly in the PPC. After analysis of several neuronal parameters, we could not detect enhancement for numerical categories in VIP. VIP neurons steadily encoded numerosity during both the color- and the numerosity-discrimination tasks but independent of whether numerosity was behaviorally relevant or not. This also

suggests that numerosity selectivity in VIP evolves along the visual pathway through a bottom-up process not requiring top-down modulation by the PFC [36]. This is in agreement with the observation that, sometimes even in trained animals, parietal signals of visual categories do not arise as a result of feedback from PFC [34]. Response latency data support this hypothesis because neurons in the intraparietal cortex represent their preferred numerosity on average about 50 ms earlier than PFC neurons, both in numerically naive [5] and numerically trained monkeys [37, 38]. Collectively, this suggests that sensory representations of numerosity are rapidly and automatically encoded in VIP, irrespective of task demands. Of course, this is not to say that VIP neurons cannot be modulated whenever numerical information needs to be processed according to the rules of other cognitive control functions.

In contrast to VIP, active discrimination of set size significantly enhanced the representation of numerosity in PFC. Surprisingly, this enhancement was only modestly based on an increase in the frequency of selective neurons but rather caused by a higher quality of numerosity encoding by a relatively stable set of numerosity-selective neurons. This relevance-induced improvement in numerosity discriminability of PFC neurons was primarily found in BS (putative pyramidal) neurons. This suggests a preferential modulation of BS neurons with active numerosity processing and corresponds with our previous finding that putative pyramidal cells showed a higher degree of numerosity selectivity



**Figure 7. Change in AUC Mediated by Different Neuronal Classes**

(A) PFC neurons classified into narrow spiking (NS) (black) and broad spiking (BS) (gray) by their normalized waveforms (top panel) and boxplots depicting the AUC values (bottom panel) for the two cell classes pre-training (left) and post-training (right). The horizontal lines indicate the medians within the boxes spanning the 25<sup>th</sup>–75<sup>th</sup> percentiles of the data. The whiskers span the 5<sup>th</sup>–95<sup>th</sup> percentiles.

(B) The same as (A) for VIP neurons.

[23]. BS neurons in PFC also seem to contribute to other PFC functions, such as learning to memorize stimuli [14], motion discrimination [26], and decision making [39]. Sensory prefrontal neurons are also differentially affected by dopaminergic modulation [24, 40].

Our results contrast activity changes found in ventral PFC of monkeys before (i.e., during passive fixation) and after training on a spatial working memory task. Qi et al. [14] observed a doubling of the proportion of activated neurons (from 10% to 20%) but also a degradation of the neurons' stimulus selectivity after training. In our study, however, we witnessed only a very moderate increase of the proportion of numerosity-selective neurons but a clear enhancement of the coding quality of such neurons after numerosity training. A possible explanation for this discrepancy may include differences in the discriminative stimulus (numerical versus spatial stimulus feature) but perhaps more importantly differences in the cognitive states the monkeys needed to adopt, because passive fixation (as applied by Qi et al.) demands only little attention and/or arousal compared to an active discrimination task. We, therefore, had the monkeys engaged in equally demanding delayed discrimination tasks pre-training (color discrimination) and post-training (numerosity discrimination) to exclude general internal state differences.

Our data also diverge from results obtained with perceptual category training. Strong categorical representations of stimuli in PFC have been described in monkeys trained to recognize binary category membership of sensory stimuli, such as “up versus down” motion directions [34, 41] or “cats versus dogs” classes [15]. Both in IPS and PFC, such categorical discharges are not present in naive animals but emerge with training to encode behaviorally relevant stimulus groups. Changing category boundaries also causes adaptive changes in PFC neurons [15]. The encoding of numerical categories differed from these findings because numerosity-selective neurons in the IPS and PFC are already present in numerically naive monkeys [5] and they exhibit a stable labeled-line code irrespective of stimulus context (PFC) [42] or training status (current study). We suspect that this coding stability is related to numerosities being “natural” categories, which—unlike arbitrary perceptual categories that necessarily need to be conditioned—possess an inherent meaning with permanent category boundaries. In addition (and unlike VIP), PFC numerosity-selective neurons did experience enhancement of coding quality. We interpret this improved neuronal selectivity as a reflection of increased relevance of numerical categories post-training. This improved selectivity might help the PFC exert top-down influence on downstream

cortical stages and guide executive functions via numerical information.

### Quantities as Stable Natural Categories

The current data suggest that numerosity representations in the PFC and VIP rely on a sparse code [43] with dedicated and relatively stable “labeled lines” [44]. Sensory numerosity representations in the parietal lobe seem to be largely independent from task relevance, thus supporting the idea of a visual “number sense,” the faculty to perceive visual collections intuitively [8, 9]. Visual numerosity-selective neurons may develop spontaneously and naturally within visual neural structures of the primate brain, prior to learning how to use this information. In agreement with this idea and based on psychophysical findings, Burr and Ross [6] suggested visual numerosity as a sensory attribute that is susceptible to adaptation just like color, contrast, or speed. Perhaps numerosity, like faces [45], constitutes an exceptionally relevant type of information with adaptive value. The numerical category “set size” could therefore emerge as a natural category represented spontaneously in a dedicated parieto-frontal network. Just as face selectivity, numerosity selectivity could potentially be present at birth [46]. In the PFC, however, numerosity selectivity is enhanced during explicit processing of sensory numerical information. This plasticity potentially enables PFC networks to emphasize behavioral relevance of numerosity during executive functions.

### EXPERIMENTAL PROCEDURES

All procedures complied with the European Communities Council Directive 2010/63/EC and the German Law for Protection of Animals and were approved by the national authorities, following appropriate ethics review. For detailed methods, please see the [Supplemental Experimental Procedures](#).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and one table and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.03.025>.

### AUTHOR CONTRIBUTIONS

A.N. and P.V. designed the experiments. P.V. performed research and analyzed data. P.V. and A.N. wrote the manuscript.

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### REFERENCES

- Nieder, A. (2013). Coding of abstract quantity by ‘number neurons’ of the primate brain. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 199, 1–16.
- Ansari, D. (2008). Effects of development and enculturation on number representation in the brain. *Nat. Rev. Neurosci.* 9, 278–291.
- Piazza, M., Izard, V., Pinel, P., Le Bihan, D., and Dehaene, S. (2004). Tuning curves for approximate numerosity in the human intraparietal sulcus. *Neuron* 44, 547–555.
- Eger, E., Michel, V., Thirion, B., Amadon, A., Dehaene, S., and Kleinschmidt, A. (2009). Deciphering cortical number coding from human brain activity patterns. *Curr. Biol.* 19, 1608–1615.
- Viswanathan, P., and Nieder, A. (2013). Neuronal correlates of a visual “sense of number” in primate parietal and prefrontal cortices. *Proc. Natl. Acad. Sci. USA* 110, 11187–11192.
- Burr, D., and Ross, J. (2008). A visual sense of number. *Curr. Biol.* 18, 425–428.
- Arrighi, R., Togoli, I., and Burr, D.C. (2014). A generalized sense of number. *Proc. Biol. Sci.* 281, 20141791.
- Dantzig, T. (1930). *Number: The Language of Science*. (New York: Free Press).
- Dehaene, S. (1997). *The Number Sense*. (Oxford: Oxford University Press).
- Mountcastle, V.B., Andersen, R.A., and Motter, B.C. (1981). The influence of attentive fixation upon the excitability of the light-sensitive neurons of the posterior parietal cortex. *J. Neurosci.* 1, 1218–1225.
- Rainer, G., Asaad, W.F., and Miller, E.K. (1998). Memory fields of neurons in the primate prefrontal cortex. *Proc. Natl. Acad. Sci. USA* 95, 15008–15013.
- Rainer, G., and Miller, E.K. (2000). Effects of visual experience on the representation of objects in the prefrontal cortex. *Neuron* 27, 179–189.
- Freedman, D.J., and Assad, J.A. (2006). Experience-dependent representation of visual categories in parietal cortex. *Nature* 443, 85–88.
- Qi, X.L., Meyer, T., Stanford, T.R., and Constantinidis, C. (2011). Changes in prefrontal neuronal activity after learning to perform a spatial working memory task. *Cereb. Cortex* 21, 2722–2732.
- Freedman, D.J., Riesenhuber, M., Poggio, T., and Miller, E.K. (2001). Categorical representation of visual stimuli in the primate prefrontal cortex. *Science* 291, 312–316.
- Crowe, D.A., Goodwin, S.J., Blackman, R.K., Sakellaridi, S., Sponheim, S.R., MacDonald, A.W., 3rd, and Chafee, M.V. (2013). Prefrontal neurons transmit signals to parietal neurons that reflect executive control of cognition. *Nat. Neurosci.* 16, 1484–1491.
- Nieder, A., and Miller, E.K. (2003). Coding of cognitive magnitude: compressed scaling of numerical information in the primate prefrontal cortex. *Neuron* 37, 149–157.
- Mante, V., Sussillo, D., Shenoy, K.V., and Newsome, W.T. (2013). Context-dependent computation by recurrent dynamics in prefrontal cortex. *Nature* 503, 78–84.
- Nieder, A., Freedman, D.J., and Miller, E.K. (2002). Representation of the quantity of visual items in the primate prefrontal cortex. *Science* 297, 1708–1711.
- Nieder, A., Diester, I., and Tudusciuc, O. (2006). Temporal and spatial enumeration processes in the primate parietal cortex. *Science* 313, 1431–1435.
- Nieder, A. (2012). Supramodal numerosity selectivity of neurons in primate prefrontal and posterior parietal cortices. *Proc. Natl. Acad. Sci. USA* 109, 11860–11865.
- Siegel, M., Warden, M.R., and Miller, E.K. (2009). Phase-dependent neuronal coding of objects in short-term memory. *Proc. Natl. Acad. Sci. USA* 106, 21341–21346.
- Diester, I., and Nieder, A. (2008). Complementary contributions of prefrontal neuron classes in abstract numerical categorization. *J. Neurosci.* 28, 7737–7747.
- Jacob, S.N., Ott, T., and Nieder, A. (2013). Dopamine regulates two classes of primate prefrontal neurons that represent sensory signals. *J. Neurosci.* 33, 13724–13734.
- Womelsdorf, T., Ardid, S., Everling, S., and Valiante, T.A. (2014). Burst firing synchronizes prefrontal and anterior cingulate cortex during attentional control. *Curr. Biol.* 24, 2613–2621.

26. Hussar, C.R., and Pasternak, T. (2009). Flexibility of sensory representations in prefrontal cortex depends on cell type. *Neuron* 64, 730–743.
27. Rigotti, M., Barak, O., Warden, M.R., Wang, X.-J., Daw, N.D., Miller, E.K., and Fusi, S. (2013). The importance of mixed selectivity in complex cognitive tasks. *Nature* 497, 585–590.
28. Gilbert, C.D., Li, W., and Piech, V. (2009). Perceptual learning and adult cortical plasticity. *J. Physiol.* 587, 2743–2751.
29. 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.
30. Yang, T., and Maunsell, J.H.R. (2004). The effect of perceptual learning on neuronal responses in monkey visual area V4. *J. Neurosci.* 24, 1617–1626.
31. Freedman, D.J., Riesenhuber, M., Poggio, T., and Miller, E.K. (2006). Experience-dependent sharpening of visual shape selectivity in inferior temporal cortex. *Cereb. Cortex* 16, 1631–1644.
32. Kobatake, E., Wang, G., and Tanaka, K. (1998). Effects of shape-discrimination training on the selectivity of inferotemporal cells in adult monkeys. *J. Neurophysiol.* 80, 324–330.
33. Toth, L.J., and Assad, J.A. (2002). Dynamic coding of behaviourally relevant stimuli in parietal cortex. *Nature* 415, 165–168.
34. Swaminathan, S.K., and Freedman, D.J. (2012). Preferential encoding of visual categories in parietal cortex compared with prefrontal cortex. *Nat. Neurosci.* 15, 315–320.
35. Freedman, D.J., Riesenhuber, M., Poggio, T., and Miller, E.K. (2002). Visual categorization and the primate prefrontal cortex: neurophysiology and behavior. *J. Neurophysiol.* 88, 929–941.
36. Roitman, J.D., Brannon, E.M., and Platt, M.L. (2007). Monotonic coding of numerosity in macaque lateral intraparietal area. *PLoS Biol.* 5, e208.
37. Nieder, A., and Miller, E.K. (2004). A parieto-frontal network for visual numerical information in the monkey. *Proc. Natl. Acad. Sci. USA* 101, 7457–7462.
38. Tudusciuc, O., and Nieder, A. (2009). Contributions of primate prefrontal and posterior parietal cortices to length and numerosity representation. *J. Neurophysiol.* 101, 2984–2994.
39. Ding, L., and Gold, J.I. (2012). Neural correlates of perceptual decision making before, during, and after decision commitment in monkey frontal eye field. *Cereb. Cortex* 22, 1052–1067.
40. Ott, T., Jacob, S.N., and Nieder, A. (2014). Dopamine receptors differentially enhance rule coding in primate prefrontal cortex neurons. *Neuron* 84, 1317–1328.
41. Mendoza-Halliday, D., Torres, S., and Martinez-Trujillo, J.C. (2014). Sharp emergence of feature-selective sustained activity along the dorsal visual pathway. *Nat. Neurosci.* 17, 1255–1262.
42. Moskaleva, M., and Nieder, A. (2014). Stable numerosity representations irrespective of magnitude context in macaque prefrontal cortex. *Eur. J. Neurosci.* 39, 866–874.
43. Olshausen, B.A., and Field, D.J. (2004). Sparse coding of sensory inputs. *Curr. Opin. Neurobiol.* 14, 481–487.
44. Nieder, A., and Merten, K. (2007). A labeled-line code for small and large numerosities in the monkey prefrontal cortex. *J. Neurosci.* 27, 5986–5993.
45. Moeller, S., Freiwald, W.A., and Tsao, D.Y. (2008). Patches with links: a unified system for processing faces in the macaque temporal lobe. *Science* 320, 1355–1359.
46. Rodman, H.R., Skelly, J.P., and Gross, C.G. (1991). Stimulus selectivity and state dependence of activity in inferior temporal cortex of infant monkeys. *Proc. Natl. Acad. Sci. USA* 88, 7572–7575.