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Correspondence and requests for materials should be addressed to J.N.P. (joe.perry@bbsrc.ac.uk).

Cortical activity reductions during repetition priming can result from rapid response learning

Ian G. Dobbins[1], David M. Schnyer[2,3], Mieke Verfaellie[2] & Daniel L. Schacter[4]

[1]*Psychological and Brain Sciences, Duke University, Durham, North Carolina 27708, USA*
[2]*Memory Disorders Research Center, Boston VA Healthcare System and Boston University School of Medicine, Boston, Massachusetts 02118, USA*
[3]*Athinoula A. Martinos Center for Biomedical Imaging, MGH/MIT/HMS, Charlestown, Massachusetts 02129, USA*
[4]*Department of Psychology, Harvard University, Cambridge, Massachusetts 02138, USA*

Recent observation of objects speeds up their subsequent identification and classification[1,2]. This common form of learning, known as repetition priming, can operate in the absence of explicit memory for earlier experiences[3,4], and functional neuroimaging has shown that object classification improved in this way is accompanied by 'neural priming' (reduced neural activity) in prefrontal, fusiform and other cortical regions[5–10]. These observations have led to suggestions that cortical representations of items undergo 'tuning', whereby neurons encoding irrelevant information respond less as a given object is observed repeatedly[10], thereby facilitating future availability of pertinent object knowledge. Here we provide experimental support for an alternative hypothesis, in which reduced cortical activity occurs because subjects rapidly learn their previous responses[11]. After a primed object classification (such as 'bigger than a shoebox'), cue reversal ('smaller than a shoebox') greatly slowed performance and completely eliminated neural priming in fusiform cortex, which suggests that these cortical item representations were no more available for primed objects than they were for new objects. In contrast, prefrontal cortex activity tracked behavioural priming and predicted the degree to which cue reversal would slow down object classification—highlighting the role of the prefrontal cortex in executive control.

We contrasted tuning and response-learning hypotheses by examining the response sensitivity to a cue change following primed object-size judgements, under the assumption that a response-learning strategy would be abandoned when prior responses became inappropriate, even if retrieval remained directed towards the same object properties[12] (Fig. 1). Importantly, the cue change did not alter the target knowledge (object size) or the comparison item (the shoebox); it merely rendered the previous responses inappropriate. If information regarding target object properties becomes increasingly available with priming (that is, tuning occurs), then this cue change should have little effect on performance (speed of object size classification) or on neural priming, because the same object knowledge is targeted by either cue. In contrast, if subjects rapidly come to rely upon learned response associations, then the switch should generate considerably slowed performance in response time and re-engagement of the same cortical networks employed during initial processing should occur.

Behaviourally, reaction times (RT) were examined for cue type (start, switch, return) and priming type (novel, low, high) within

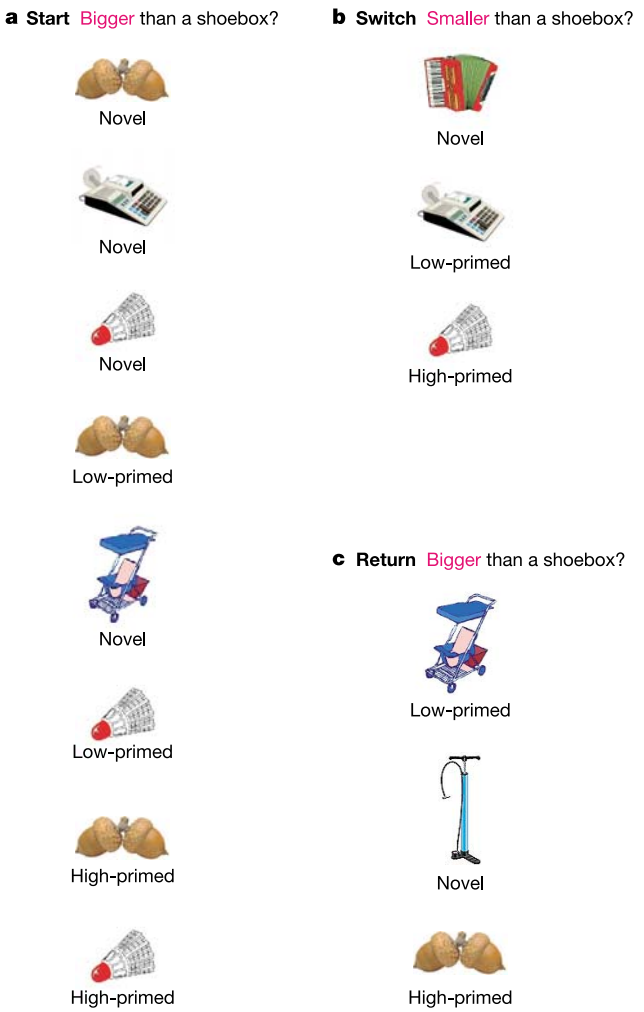

Figure 1 Experiment design. Subjects encountered three sequential phases during scanning ('Start', 'Switch' and 'Return'), during which the retrieval cue direction ('bigger than' or 'smaller than' judgement, needing a yes/no response) was reversed or consistent with respect to the initial start phase cue. **a**, During the start phase, objects were seen either once ('Novel'), or three times ('High-primed'). **b**, During the subsequent switch phase, half of the items from the previous start phase that had initially been classified as novel or high-primed were re-presented along with a new set of novel items. Thus the switch phase contained items that were viewed for the first time (novel), items viewed for the second time ('Low-primed') and items making their fourth appearance (high-primed). **c**, Similarly, the final return phase used the remaining novel and high-primed items from the initial start phase along with a completely new set of novel items.

each cue type ($F_{2.4, 35.9} = 5.75$, $P < 0.005$) (Fig. 2a). Although RT to novel stimuli did not vary with cue type ($F_{1.4, 21.2} = 2.7$, $P > 0.10$), low- and high-primed RT values slowed during the switch, and partially recovered when the cue was returned to the original form for the remaining items (return phase), as confirmed by quadratic trends for high-primed ($F_{1, 15} = 31.50$, $P < 0.0001$) and low-primed ($F_{1, 15} = 16.52$, $P < 0.01$) items across cue type. Although noticeably slowed by the cue switch, priming was not completely eliminated (high- and low-primed RTs versus novel, P values <0.001).

Event-related functional magnetic resonance imaging (fMRI) demonstrated reduced cortical activity in prefrontal, parietal, occipito-temporal and fusiform regions for (both low- and high-) primed relative to novel items during the start phase, and an interaction demonstrating significant disruption of this neural priming for the high-primed items in prefrontal cortex (PFC), fusiform and other regions as a result of the cue reversal. Priming disruptions were more evident in the left hemisphere, and potentially correspond to the behavioural RT changes (Fig. 3).

To determine whether regional activity paralleled response behaviour, regions of interest (ROIs) were extracted from regions near those implicated in previous priming studies[7,13] that demonstrated a start–switch phase interaction, namely, left posterior PFC (BA 9/44) and left fusiform (BA 37). For these regions, the mean priming signals (novel minus high- or low-primed) were subjected to quadratic trend analysis (Fig. 3c). For posterior PFC, the trend was marginally significant for the high-primed condition ($F_{1, 15} = 3.98$, $P = 0.065$), suggesting a significant decline in the priming signal during the cue switch that only partially recovered during the return phase. The priming signal for the low-primed condition in PFC, although demonstrating a significant decline during the switch phase, showed no sign of recovery during the return phase (quadratic trend $P > 0.29$). The trends for the fusiform region priming responses were more robust (high-primed $F_{1, 15} = 17.74$, $P < 0.001$; low-primed $F_{1, 16} = 4.61$, $P < 0.05$), suggesting elimination of neural priming followed by recovery of the neural difference between novel and primed items when the cue was restored to the original form for both high- and low-primed items respectively. This pattern rules out simple causes such as forgetting and shows that priming-like reductions in fusiform activity were heavily dependent on the stability of the associated response, and not on the requirement to access the same item knowledge across exposures[14].

Although PFC and fusiform reductions are common in priming studies, they have not been reliably linked to individual performance. To investigate this issue, the mean priming signals (novel minus high-primed) during the start phase were entered into a multiple regression to examine their relation to each subject's behavioural priming scores for the same items (Table 1). Both regions uniquely predicted priming scores, accounting for 63% of the variance. Additionally, the same signal differences were also used in an attempt to predict the subsequent behavioural cost of switching the cue. Only the posterior PFC region predicted switching costs, with regression accounting for 59% of the variance (Table 1).

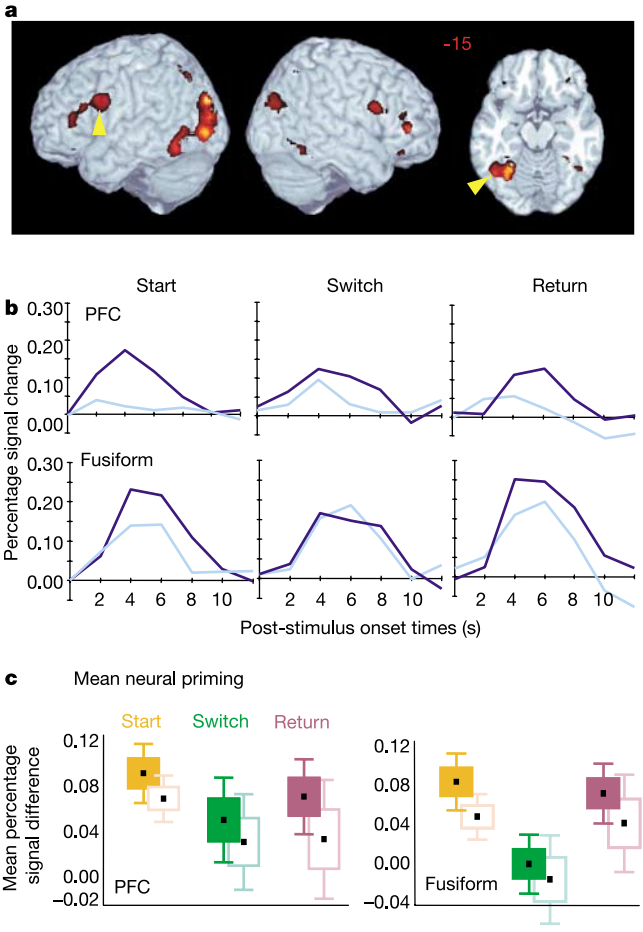

Figure 3 SPM results for novel versus high-primed responses across start, switch and return phases. During the start phase, neural priming was evident in large regions of the PFC, parietal, inferotemporal and fusiform areas. A minor change in cue direction ('bigger than' to 'smaller than') during the switch phase disrupted neural priming, particularly in posterior regions. Returning the cue to its original form (return phase) resulted in partial recovery of neural priming, especially noticeable in the fusiform and other posterior regions. **a**, SPM interaction map showing regions that demonstrated a significant disruption of neural priming signal in the switch relative to start phase overlaid on a high-resolution three-dimensional canonical brain in Montreal Neurological Institute (MNI)152 space and displayed on an axial slice (−15), both thresholded at 0.001. Yellow arrows denote approximate location of ROIs used for the time courses and box plots. **b**, Haemodynamic time courses. Dark blue lines indicate response to novel items; pale blue lines indicate response to high-primed items. The left PFC region consisted of 80 voxels (MNI −45, 6, 27; BA 9/44). The left fusiform region consisted of 62 voxels (MNI −24, −57, −15; BA 19/37). **c**, Box plots indicate mean percentage signal difference between novel and high-primed (filled boxes) and novel and low-primed (open boxes) items for each phase, averaged from 4 to 8 s post-stimulus onset for start, switch and return phases. Box indicates one standard error of the between-subjects mean; box plus error bars indicates two standard errors.

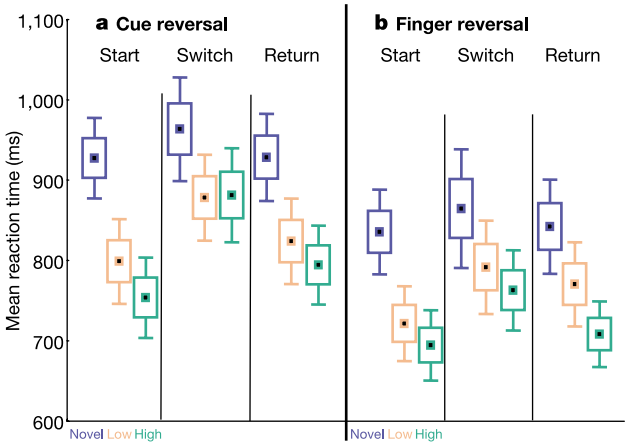

Figure 2 Behavioural reaction time data for scanned and finger-reversal experiments as a function of cue phase. Box indicates one standard error of the between-subjects mean; box plus error bars indicates two standard errors.

Thus in posterior PFC, a greater neural response reduction indicates larger behavioural facilitation, and anticipates a larger relative impairment when subjects encounter a cue that still targets the same semantic content, but is modified such that the previous response has become inappropriate. This observation supports the contention that reductions in this region do not indicate increasingly available size information, since this would not anticipate larger costs when switching the cue. Instead, the PFC data indicate a shift from controlled working memory/executive operations thought to be mediated by this area[15], to the reliance on more automatic learned response associations.

In contrast to PFC, the activity difference in left fusiform yielded a negative relation with priming scores in the start phase, and was unrelated to the later cue-reversal performance cost. This inverse relation to priming scores probably resulted from the negative relation of fusiform activity to RTs for novel items across subjects (fusiform $r = -0.64$, $P < 0.01$) with increased fusiform activity associated with quicker, not slower, responding. This negative relation was not due to general cortical arousal differences across subjects because it remained even when the left PFC region was also added to the regression, suggesting that increased fusiform activity predicted rapid novel-item RTs (Table 1). By the third item repetition however, fusiform activity no longer inversely predicted RT. This suggests that the negative regression weight of the fusiform when predicting priming scores occurred primarily because of the initial association between greater fusiform activity and quicker responding to novel items, a pattern no longer present by the third trial, by which time responses were presumably well learned (Table 1).

This negative relation between fusiform response and subject responses to novel stimuli has not previously been reported and its meaning with respect to the role of fusiform in long-term object-knowledge is unclear. An informal item analysis suggested that, for each subject, RTs to novel stimuli were largely governed by the size difference between the referent (the shoebox) and probe, with distant probes (such as a pencil or strawberry) responded to more quickly than close ones (such as Kleenex box or dustpan). However, fusiform activity did not parametrically change with RTs to individual novel stimuli, even at liberal thresholds, suggesting that it was insensitive to the trial-by-trial difficulty of the judgement.

Another possibility is that the mean fusiform response for each subject was governed by the overall availability of pre-existing object representations[16]. If so, then subjects with more experience of many of the items would be expected to show more activity and respond more quickly. Although speculative, this explanation fits with evidence that similar fusiform regions increase response during the gradual acquisition of expertise with objects[17]. In contrast to the null parametric fusiform response to trial difficulty, posterior PFC demonstrated a robust positive parametric relation with individual novel-item RTs—this again suggests a trial-by-trial role for PFC in executive mechanisms that are increasingly critical as referent and probe sizes approach one another.

Overall, the data strongly suggest that subjects rapidly learn their responses. To examine the specificity of this learning, we examined whether similar slowing would occur when just the finger assignment for 'yes' and 'no' responses was reversed, but cue direction remained constant ('bigger than a shoebox?') using a non-scanned group of participants ($n = 16$). A similar interaction between cue type and priming type was observed ($F_{2.7, 40.9} = 3.91$, Mse = 991.35, $P < 0.05$). The finger-reversal manipulation did not affect novel-item RTs ($F_{2.0, 29.8} = 1.97$, $P > 0.15$), but did yield a quadratic trend for high- and low-primed item RTs across cue type ($F_{1, 15} = 53.18$, $P < 0.0001$; $F_{1, 15} = 12.68$, $P < 0.01$) (Fig. 2b). On average, subjects were quicker in the finger-reversal task than in the scanner ($F_{1, 30} = 5.72$, $P < 0.05$); however, there were no interactions across the groups. These findings suggest that response learning extends to the level of finger assignment and are consistent with previous behavioural observations of extreme specificity ('hyperspecificity') in the expression of some repetition priming effects[18,19].

Our findings demonstrate that a considerable proportion of the behavioural and cortical consequences of object-knowledge priming paradigms may not result from increasing availability of object-related knowledge, as suggested by tuning explanations of neural priming[10,20]. Instead we suggest a different conceptualization for repetition-related neural-activity reductions[11]. Using response learning to explain neural priming, we suggest that subjects bypass recovery and analysis of detailed object knowledge whenever the task can be more easily accomplished via a learned association between object identity and prior response. This association was well established after three exposures (high-primed), but the response of the fusiform region, and the behavioural data, suggest that some learning may have occurred even after only a single exposure (low-primed), although the effect was clearly less robust.

The response-learning explanation we offer is probably not directly applicable to previous designs that have not required classification[16], or that have had orthogonal response requirements across encounters[7,21–23]. However, more generally, signal reductions across a range of priming tasks may be due to a shift in strategy that results in rapidly bypassing local neuronal systems rather than tuning them on the basis of short-term environmental contingencies.

The response-learning mechanism outlined here would conserve capacity-limited executive functions by eliminating the need to recover and evaluate detailed representations of object-related knowledge, and would make the practised version of the task fundamentally different from the initial task[5]. The ability to rapidly learn one's prior responses to recurrent stimuli has strong adaptive value and may be a largely unavoidable consequence of deliberative processing in the service of response goals, particularly when the response set is limited and therefore easily learned, and the items are relatively distinctive. Given this perspective, evidence for rapid changes in the representation of object knowledge as a function of task repetition will require the study of subjects who are unable to learn responses effectively, or experimental designs in which such a mechanism is implausible. Only under such conditions can we be confident that activity reductions in fusiform or related posterior structures are not the indirect result of the response-learning mechanism demonstrated here. □

Table 1 **Regression results for predicting behavioural scores with cortical responses**

Dependent variable	Region	Beta	Error	*t*-test value for beta weight	*P* value	R^2
Priming score*	**Left PFC**	**0.75**	**0.174**	**4.33**	**0.0008**	
	Left fusiform	**−0.52**	**0.174**	**−3.01**	**0.0099**	0.63
Switch cost†	**Left PFC**	**0.79**	**0.184**	**4.27**	**0.0009**	
	Left fusiform	−0.29	0.184	−1.57	0.1412	0.59
Novel RT‡	Left PFC	−0.14	0.219	−0.41	0.6822	
	Left fusiform	**−0.61**	**0.219**	**−2.80**	**0.0153**	0.41
High-prime RT§	Left PFC	−0.21	0.25	−0.83	0.4227	
	Left fusiform	−0.37	0.25	−1.46	0.1681	0.21

For each subject, independent variables in the regressions were constructed using the response of left PFC and fusiform regions from 4–8 s post-stimulus onset. These were then used to predict different behavioural scores in four separate regression analyses as outlined below. Boldface text denotes variables that were significant in each of the four regressions.

*Independent variables were the mean response difference for novel and high-primed items during the start phase. The dependent variable was the corresponding behavioural priming score: novel RT minus high-primed RT during the start phase.

†Independent variables were the same as above. The dependent variable was the switch cost, defined as the difference in behavioural priming scores between switch and start phases: start (novel RT minus high-primed RT) minus switch (novel RT minus high-primed RT).

‡Independent variables were the mean response for novel stimuli relative to baseline during the start phase. The dependent variable was RT to novel stimuli during the start phase.

§Independent variables were the mean response to high-primed stimuli relative to baseline during the start phase. The dependent variable was RT to high-primed stimuli during the start phase.

Methods

Subjects

Sixteen right-handed, 18- to 35-year-old, native-English-speaking volunteers were paid participants ($50). Informed consent was obtained, as required by the Human Subjects Research Committee at Massachusetts General Hospital.

Materials

Materials consisted of 408 colour drawings of common objects used in previous studies of object-size priming[24].

Study procedure

The overall design employed four scans with three experimental phases within each scan. The initial start phase was designed to closely match a standard object-priming paradigm and yield the expected neural and behavioural priming effects.

During the start phase subjects indicated via key-press whether each displayed picture was bigger than an average-sized shoebox ('bigger than a shoebox?') using the left key for a yes response and the right key for a no response. Items presented during this phase were seen once (novel) or three times (high-prime).

Subsequently, for the switch phase, and during the same scanning run, this cue was replaced with a 'smaller than a shoebox?' cue requiring subjects to respond to novel items, and half of the novel and high-primed items from the previous phase. This critical phase was predicted either to incur a cost if subjects were using response learning, or to have very little effect if priming was the result of increasingly efficient size knowledge retrieval (that is, tuning). Behaviourally, subjects reversed 92 and 95% of their responses in the switch phase for low- and high-primed items respectively.

Finally, for the return phase, during the same scanning run, the cue was returned to the original ('bigger than a shoebox?'), and subjects again responded to novel items, and the remaining novel and high-primed items from the initial phase. The behavioural data were Greenhouse–Geisser-corrected for sphericity violations. During each trial, the item remained on the screen for 2,200 ms. The experimental trials were interspersed with blank trials, using an optimal sequencing program[25]. Each scan lasted 10.5 min.

Data acquisition

Scanning was performed on a 3T Siemens Trio system using a standard head coil. Functional data were collected using a gradient-echo echo-planar pulse sequence (TR = 2,200, TE = 30 ms, 31 axial slices parallel to the AC–PC plane, 3.125 × 3.125 mm, 0.3 mm interslice gap). Before functional data collection, four dummy volumes were discarded to allow for T1 equilibration. Head motion was restricted using a pillow and foam inserts.

fMRI data analysis

Data were preprocessed using statistical parametric mapping—SPM99 (Wellcome Department of Cognitive Neurology, London). Slice-acquisition timing was corrected by resampling slices in time relative to the middle slice collected, followed by a rigid-body motion correction across all runs. Functional data were spatially normalized to a canonical EPI template using a 12-parameter affine and nonlinear cosine basis function transform. Volumes were then spatially smoothed with an 8-mm full-width at half-maximum (FWHM) gaussian kernel. Each session was rescaled such that the mean signal was 100.

The data were analysed by treating subjects as a random effect. Volumes were treated as a temporally correlated time series and modelled using a canonical haemodynamic response function and its first-order time derivative for each event onset. These functions were used along with a basis set of cosine functions that were used to high-pass filter the data, and a covariate representing session effects. The least squares parameter estimates of the response for each condition were then used to construct contrast images based on the canonical HRF for each subject. These images were tested against a null of no difference between contrast conditions. Regions were considered significant, and potentially submitted to a later ROI analysis, if they exceed five or more contiguous voxels at an alpha threshold of 0.001.

ROIs were extracted using peristimulus averaging for voxels significant in the canonical model within an 8-mm-radius sphere of SPM-identified maxima selected based on prior literature.

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Correspondence and requests for materials should be addressed to I.G.D. (ian.dobbins@duke.edu).

Protein-only transmission of three yeast prion strains

Chih-Yen King & Ruben Diaz-Avalos

Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida 32306, USA

Key questions regarding the molecular nature of prions are how different prion strains can be propagated by the same protein and whether they are only protein[1–3]. Here we demonstrate the protein-only nature of prion strains in a yeast model, the [*PSI*] genetic element that enhances the read-through of nonsense mutations in the yeast *Saccharomyces cerevisiae*[4,5]. Infectious fibrous aggregates containing a Sup35 prion-determining amino-terminal fragment labelled with green fluorescent protein were purified from yeast harbouring distinctive prion strains. Using the infectious aggregates as 'seeds', elongated fibres were generated *in vitro* from the bacterially expressed labelled prion protein. *De novo* generation of strain-specific [*PSI*] infectivity was demonstrated by introducing sheared fibres into uninfected yeast hosts. The cross-sectional morphology of the elongated fibres generated *in vitro* was indistinguishable from that of the short yeast seeds, as visualized by electron microscopy. Electron diffraction of the long fibres showed the 4.7 Å spacing characteristic of the cross-beta structure of amyloids. The fact that the amyloid fibres nucleated *in vitro* propagate the strain-specific infectivity of the yeast seeds implies that the heritable infor-