

Using perfusion fMRI to measure continuous changes in neural activity with learning

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Abstract

In this study, we examine the suitability of a relatively new imaging technique, *arterial spin labeled perfusion imaging*, for the study of continuous, gradual changes in neural activity. Unlike BOLD imaging, the perfusion signal is stable over long time-scales, allowing for accurate assessment of continuous performance. In addition, perfusion fMRI provides an absolute measure of blood flow so signal changes can be interpreted without reference to a baseline. The task we used was the serial response time task, a sequence learning task. Our results show reliable correlations between performance improvements and decreases in blood flow in premotor cortex and the inferior parietal lobe, supporting the model that learning procedures that increase efficiency of processing will be reflected in lower metabolic needs in tissues that support such processes. More generally, our results show that perfusion fMRI may be applied to the study of mental operations that produce gradual changes in neural activity.

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1. Introduction

Some mental operations of interest to the cognitive neuroscientist evolve over relatively long time-scales. Examples of these include changes in emotional state, adoption of a particular cognitive “set” during the performance of a task, or the effects of sleep and alertness. A particularly salient case is the cognitive process of learning, examples of which produce enduring changes in performance that accumulate slowly over minutes to hours. Continuous motor sequence learning, in which a subject becomes skilled at the execution of an ordered set of motor movements, is of this kind (Nissen & Bullemer, 1987).

Attempts to study learning, or other slow changes in neural activity, with BOLD fMRI face the obstacle that BOLD

fMRI data are rather unstable at long time scales, as the signal tends to drift up and down over time (Zarahn, Aguirre, & D’Esposito, 1997). This presents an obvious limitation for studies that attempt to discern the slow neural changes that are associated with continuous learning: it is very difficult to discriminate the changes in imaging signal that are due to learning from those that are present as drift noise.

In this study, we examine the suitability of a relatively new imaging technique for the study of continuous, gradual changes in neural activity. Arterial spin labeled (ASL) perfusion imaging permits the noninvasive quantification of regional brain tissue perfusion using labeled inflowing arterial protons as an endogenous tracer (Alsop & Detre, 1998). “Label” images include a radiofrequency irradiation,¹ aimed at the carotid and vertebral arteries, that precedes the image

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¹ It should be noted that radioactive isotopes are not used in perfusion imaging.

acquisition. Label images are alternated with “control” images, and the difference in signal between adjacent image pairs yields the signal due to perfusion. The perfusion effects of ASL are independent of the pulse sequence used to obtain the image after the label. For example, if echoplanar images are used, then the raw image data also contain BOLD contrast, which is attenuated during subtraction of control and labeled pairs. This allows BOLD and perfusion effects to be compared within the same data set (Wong, Buxton, & Frank, 1997). We have previously shown (Aguirre, Detre, Zarahn, & Alsop, 2002) that the perfusion fMRI signal is stable at long time scales, indicating that it may be useful for the study of slow changes in neural activity.

We obtained neuroimaging data from subjects while they performed a motor serial response time (SRT) task. During scanning, subjects performed a series of finger movements in response to visual cues over a 20 min period. Unbeknownst to the subject, there was a repeated pattern to the movements. Under these circumstances, subjects generally demonstrate a gradual and continuous decline in response time (e.g., Nissen & Bullemer, 1987). Prior imaging studies of subjects performing SRT tasks have demonstrated a reduction of neural activity in response to motor execution after training as compared to the start of training. Because the change in performance is slow and continuous, we assumed for this study that the neural correlate of performance improvement during SRT training is a gradual reduction in regional activity. Our goal in the current study was to use perfusion fMRI in an attempt to detect these neural changes. A positive result would demonstrate the ability of perfusion fMRI to measure dynamic changes in CBF over long time scales. Any effects found in the perfusion data can be contrasted with effects present in the simultaneously acquired BOLD data in each subject. Finally, because BOLD imaging is better suited to the detection of transient neural activity, we examined if the BOLD data could be used to detect trial-wise differences between correct and incorrect responses.

To foreshadow our results, insufficient power was present to detect learning effects at a map-wise level in the per-

fusion data. However, within regions of interest defined with a lowered map-wise threshold, separate statistical tests revealed significant correlations of CBF changes with reaction time measures of learning. These effects could not be detected within the BOLD data. However, there were measurable, event-related differences in the BOLD signal between correct and incorrect responses. Thus, this study serves as a demonstration of the potential of perfusion fMRI data to simultaneously examine rapid changes in neural activity with BOLD contrast and slow changes in neural activity with perfusion contrast.

2. Methods

2.1. Participants

Ten participants (ages 19–40, average age = 25, 5 males) were recruited from the University of Pennsylvania and received payment for participation. All subjects had normal or corrected-to-normal visual acuity, were right handed, and were free of any history of neurological or psychiatric disease. Written consent was given according to an Institutional Review Board approval from University of Pennsylvania.

2.2. Experimental procedure: SRT task

The classic serial-response (SRT) task was adopted as the learning task during perfusion fMRI scans. Subjects were required to use four fingers (left middle, left index, right middle, right index) to press keys as quickly and as accurately as possible in response to the presentation of visual cues that consisted of four white outline squares ($2.65^\circ \times 2.65^\circ$, separated by 0.05°) that were arrayed horizontally on a medium gray (RGB 127) background (see Fig. 1). Each square would illuminate with a color that corresponded to those present on the response box: red, green, blue, or yellow. The spatial organization of the colors remained consistent throughout the experiment. The color target appeared for 500 ms and was followed by a 300 ms

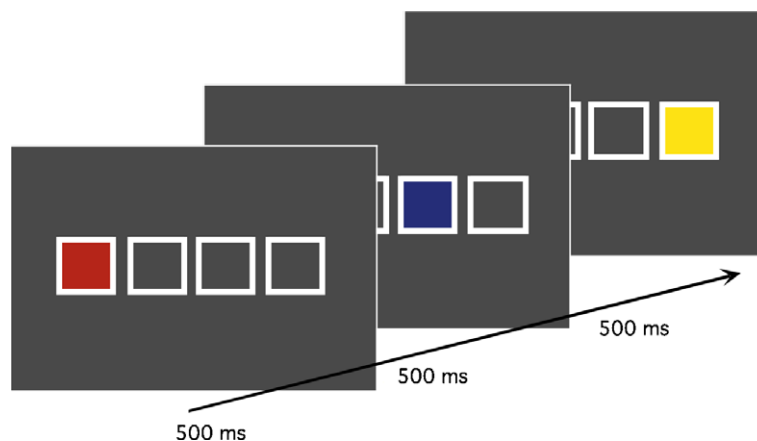


Fig. 1. Schematic drawing of the task (stimuli are not to scale). Four horizontally arrayed boxes lit up sequentially to indicate the required keypress.

blank response window. This was followed by a 200 ms ITI after which the next color target was shown.

Prior to scanning, each subject participated in 11 practice trials and task-related questions were answered. The practice trials used a different sequence than that presented during scanning. The scanning protocol consisted of three, 25.4 min long, functional scans and one, 6 min anatomical scan at the end of the scan session. Each functional scan had three ordered parts: (1) a 2.5 min long baseline fixation condition; (2) a 20.9 min long SRT task condition; and (3) a final, 2 min long fixation condition.

The two baseline fixation conditions consisted of a gray screen with a white, central fixation cross. There was no task requirement. The SRT task condition consisted of two parts. The first part, the *sequence learning session*, consisted of an 11-item fixed-order sequence that was repeated 86 times for a total time of 15.8 min. Subjects were not told that the sequence would repeat. The repeated sequence was computer-generated and designed to meet these criteria: a color target could not repeat itself (e.g., green, red, red, blue) and each color was used at least one time per 11-item sequence. This sequence was repeated without break until 86 repetitions had occurred. The second part of the SRT task was the *transfer session*, consisting of 28 randomly generated, 11-item sequences (5.1 min). All surface features of the transfer session were identical to that used in the sequence learning (except the stimulus order) and the subject was given no indication that the task had changed. These sequences were subject to the same criteria as the repeated sequences. The purpose of the transfer phase was to ensure that any gains in RT were sequence-specific, and not due to general task, or procedural learning. The three functional scans were identical except for the particular order of color cues in the SRT task.

2.3. Behavioral data analysis

All analyses were performed on data that were collapsed across the three runs. For data modeling and display, the sequence learning session was binned in 12 epochs and transfer session was binned into 4 epochs (each epoch included seven 11-item sequences). Incorrect trials were discarded in the RT analysis and the mean RT for each epoch during the SRT task was calculated. Overall trends were analyzed in a repeated measures ANOVA using SPSS 12.0 software (SPSS, Chicago, IL).

2.4. Experimental procedure: Recognition task

To assess explicit knowledge of repeated sequences, all subjects participated in a recognition test immediately after the completion of scanning. Subjects were asked to respond by keypress to six 11-item sequences, just as they had in the scanner. Three sequences were from the repeated portion of the scan session, and three were newly generated. Immediately after completing keypresses to each sequence, subjects were required to indicate by a yes/no response whether or not they recognized the sequence from the scan session.

2.5. Imaging parameters and analysis

2.5.1. Image acquisition

The functional imaging was conducted on a Siemens 3.0T Trio whole-body scanner (Siemens AG, Erlangen, Germany), using a standard Transmit/Receive head coil. A continuous arterial spin labeling (CASL) technique was used for perfusion fMRI scans (Alsop & Detre, 1998). Interleaved images with and without labeling were acquired using a gradient echo echo-planar imaging sequence with acquisition parameters: FOV = 22 cm, matrix = 64×64 , TR = 3 s, TE = 17 ms, flip angle = 90° . Fourteen 8 mm thick slices with a 2 mm gap were acquired from inferior to superior in a sequential order to cover the whole brain. Each subject performed three CASL scans each with 508 acquisitions that took 25.4 min. After the functional scans, high-resolution T1-weighted anatomic images were obtained using 3D MPRAGE: TR = 1620 ms, TI = 950 ms, TE = 3 ms, flip angle = 15° , 160 contiguous slices of 1.0 mm thickness, FOV = $192 \times 256 \text{ mm}^2$, matrix = 192×256 , 1 NEX with a total scan time of 6 min.

2.5.2. Imaging data pre-processing

Functional image processing and analysis were carried out primarily with the Statistical Parametric Mapping software package (SPM99, Wellcome Department of Cognitive Neurology, UK implemented in Matlab 5, Math Works, Natick, MA), with some modifications to provide for perfusion analysis (<http://cfn.upenn.edu/perfusion/software.htm>) Functional images were realigned to correct head motion, co-registered with the anatomical image, and smoothed in space with a three-dimensional, 8 mm full width at half maximum (FWHM) Gaussian kernel. The images at this stage of processing are dominated by BOLD signal changes and served as the dependent data in the BOLD-based analysis of learning effects and error responses. These time-series data have a TR of 3 s. The perfusion weighted image series was then generated by pair-wise subtraction of the label and control images, followed by conversion to absolute CBF image series based on a single compartment CASL perfusion model (Wang et al., 2005-in press). Pair-wise subtraction was employed to minimize auto-correlation and because BOLD contamination of the perfusion signal was not expected to be a factor (as the neural signal change was continuous low-frequency, as opposed to blocked with higher-frequency elements (Liu & Wong, 2005). Thus, the resulting CBF data sets contained 254 images for each scan with an effective TR of 6 s. Both the original BOLD and CBF images were normalized to a $2 \times 2 \times 2 \text{ mm}$ Montreal Neurological Institute (MNI) template in the standard Talairach space using bilinear interpolation.

2.5.3. Perfusion data analysis

Map-wise analysis of the perfusion imaging data was conducted by first dividing the learning condition coarsely into it early, middle, and late learning periods. Thus, each 25.4 min scan had six conditions: *first fixation*, *early*

learning, middle learning, late learning, transfer session, and final fixation. A voxel-wise general linear model (GLM) was conducted on the CBF data of each individual subject. Temporal filtering and smoothing were not employed. Two contrasts were defined for each subject. The first contrast was designed to identify brain areas that had a *general* response to the task, and was constructed as the early learning and transfer condition versus the fixation periods. Middle and late learning conditions were excluded due to potential effects of motor habituation on the neural signal. The second contrast was designed to identify modulation of CBF signal during the learning period. It was constructed as an *F* test that evaluated the early and late learning periods conjointly. The middle period was excluded to improve the statistical power of the contrast, as the greater variance provided by the extremes of the response range are more detectable. The individual contrast images (e.g., β maps for each contrast) were entered into a group analysis of random effects using a one-sample *t* test or two-sample *F* test. For the former contrast between SRT task and baseline, the SPM(*t*) map was calculated to obtain the brain activation pattern (CBF signal increase) induced by the SRT task. For the latter contrast between early learning and later learning, the SPM(*F*) map was calculated to obtain the brain activation changes across learning (either CBF signal increase or decrease). Areas of signal change were identified at the cluster level for the *p* value smaller than .001, uncorrected, and the cluster extent size larger than 15 voxels. These uncorrected threshold levels were selected after map-wise analyses at a corrected level of significance failed to identify areas of signal change. The activation clusters revealed by both contrasts were defined as the regions of interest (ROIs) for subsequent ROI analysis. For each ROI, the CBF signal intensities of the 12 learning epochs and 4 transfer epochs were extracted by the SPM Marsbar toolbox (Brett, Anton, Valabregue, & Poline, 2002). The average CBF time course in each ROI during the learning period was then correlated with the RT function described in the behavioral data to examine the relationship between brain activation and performance.

2.5.4. BOLD data analysis

Two analyses were conducted with the “unsubtracted” time-series data in which BOLD effects predominate. The first analysis was performed as described above for the perfusion data, and aimed to identify both general responses to task as well as signal changes related to the early and late learning periods. Just as for the perfusion data, the average BOLD signal present during long experimental epochs (e.g., fixation, early learning, transfer) was obtained from each subject then analyzed across subjects in a random effects analysis.

A second, “event-related” analysis was performed to identify neural responses associated with error trials in which the incorrect button was pressed in response to the SRT stimulus. Only those trials for which a true error occurred, e.g., an incorrect button press, were included

(2.6% of all trials). The rate of error responses was fairly constant over the learning and transfer periods. Because subjects made responses continuously during the experiment, this analysis will identify differences in neural activity between correct and error responses. For each subject, these error trials for each run were modeled as delta functions at the onset time of the events, convolved with the standard SPM hemodynamic response function. Both the label and control images were included in the analysis to provide 3 s temporal resolution. The signal changes induced by the ASL label were removed by adding a covariate that modeled a constant difference between label and control images. Group analysis of random effects was then conducted using a one-sample *t* test based on the individual contrast images (β maps for the event of error trials) to allow population inferences. Areas of significant activation associated with error trials were identified at the cluster corrected, map-wise $p < .05$.

3. Results

3.1. Behavioral data

Overall accuracy was 73.6%. Participants self-reported that their low accuracy was attributed to two factors: (1) anticipatory responses that were counted as errors; and (2) fatigue due to the lengthy run time. Anticipatory and fatigue-related errors would be shown as the absence of a response, whereas “true errors” would be shown as an incorrect response. We examined the error data over the 12 epochs of the learning phase and found that there were more anticipatory/fatigue related errors (23.8%) than true errors (2.6%), $F(1, 9) = 80.59$, $p < .0001$. The former type of error decreased over time, whereas true errors changed very little, as illustrated by the interaction of error type and epoch, $F(11, 99) = 4.19$, $p < .0001$.

The mean reaction times of the SRT task (12 learning epochs and 4 transfer epochs, see Fig. 2) showed the expected decrease in RT from the early learning to late

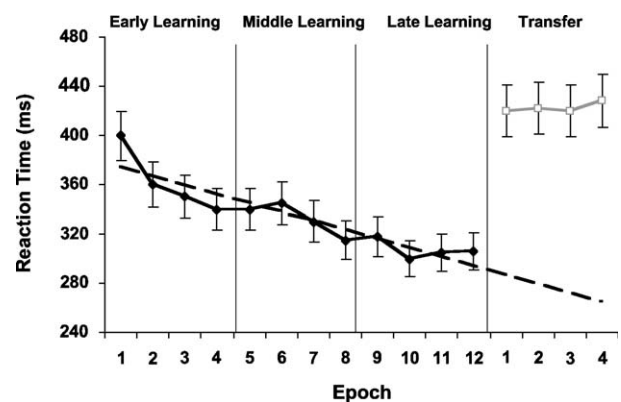


Fig. 2. Reaction time during the 12 sequence learning epochs and 4 transfer session epochs. Each “epoch” corresponds to a 75 s period during which the 11 item sequence was repeated seven times. Error bars represent standard error of the mean.

learning epochs and a robust RT increase from the later learning epochs to the transfer epochs. Learning was assessed by a repeated-measures 1-factor (epoch, 1–12) ANOVA. There was a significant main effect of epoch ($F(11,99)=7.52$, $p<.001$) due to faster RTs over time. Response times were speeded by an average of 87ms over the course of learning (see Fig. 2). This gain was lost when the random sequences were introduced; response times slowed by 110ms between the last epoch of learning and the first epoch of transfer. A paired t test between the mean RTs of late learning (the last four learning epochs) and the mean RT of the 4 random epochs revealed a significant difference ($t(9)=5.071$, $p<.001$).

Was this learning explicit or implicit? Hits (e.g., the number of yes responses to repeated sequences) and false positives (e.g., the number of yes responses to random sequences) were calculated for the recognition data collected outside of the scanner. The average number of hits (53%) exceeded the average number of false positives (27%), $p<.037$, suggesting that there was some, albeit limited, explicit recognition of the repeating sequences.

3.2. Perfusion fMRI results

3.2.1. Map-wise analyses

We first determined whether the perfusion CBF measure could show changes that differentiated sensory-motor processing. This comparison grouped the early learning and transfer session and compared them to the fixation conditions using a t test. Middle and late learning were excluded due to potential effects of motor habituation on the neural

signal. There were no areas of signal change that exceeded a map-wise level of significance. At a lowered level ($t>4.3$, uncorrected $p<.001$, cluster size >15 voxels) signal changes were found in bilateral supplementary motor area (SMA), L. precentral cortex, and L. parietal cortex. These activation clusters were defined as three motor processing related ROIs for the subsequent ROI analysis. When the threshold was decreased to (uncorrected) $p<.005$, additional activations were found in bilateral visual cortex, R. cerebellum, L. insular/putamen, R. postcentral cortex, and R. ventral premotor/inferior frontal cortex. The activation results from the group analysis are illustrated in Fig. 3A and listed in Table 1.

A map-wise analysis was also conducted to identify areas where signal changes occurred during the learning task. An F test was used to evaluate the effect of the early and late learning conditions. Again, no significant results were obtained using a map-wise threshold of significance in the random-effects analysis. With a lowered threshold (uncorrected $p<.001$; cluster size >15 voxels), signal changes were found in the R. inferior parietal lobe, R. ventral premotor (inferior frontal), and R. superior temporal gyrus (Fig. 3B and Table 2). These three activation clusters were defined as the sequence learning ROIs for the subsequent ROI analysis.

3.2.2. ROI analysis: Relationship to performance

Within the regions defined by our map-wise analysis, we examined the relationship between changes in CBF that occurred during the learning and transfer sessions to changes in reaction time performance. The sequence

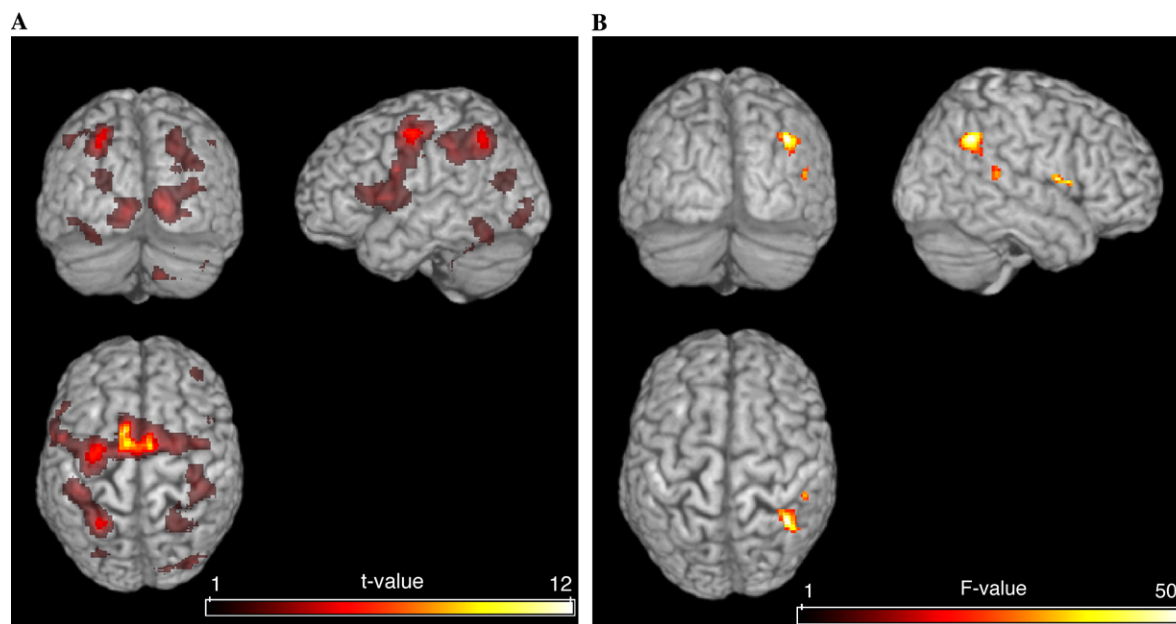


Fig. 3. (A) Brain areas activated by visuomotor processing compared to the fixation baseline (e.g., motor ROIs). The areas in bright red and yellow exceeded our uncorrected threshold of $t>4.3$ (uncorrected $p<.001$, cluster size >15 voxels). Areas with signal changes that were below this threshold (but exceeded .005) are indicated with a darker red. (B) Brain areas showing modulation (uncorrected $p<.001$, cluster size >15 voxels) of CBF by the early and late sequence learning periods (e.g., learning ROIs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Table 1
Brain areas showed significant activations associated with visuomotor processing in the SRT task

Region	Brodmann's area	Cluster size	MNI coordinates			T value (Z-score)
			X	Y	Z	
Bilateral SMA/cingulate	BA6/24	576	-10	6	46	12.02 (4.95)
			-10	-2	52	9.45 (4.54)
			8	-4	54	9.16 (4.48)
L. precentral	BA6	299	-30	-10	52	5.80 (3.65)
			-25	-10	42	5.29 (3.48)
L. parietal	BA40	179	-26	-60	48	4.90 (3.34)
L. insula/putamen	BA13	44	-30	6	6	5.24 (3.46)
L. occipital	BA17/18	39	-8	-82	-2	4.09 (3.00)
L. parietal	BA40	29	-42	-36	44	3.74 (2.83)
R. occipital	BA17/18	101	18	-84	0	4.52 (3.18)
R. cerebellum		82	26	-52	-32	4.04 (2.98)
			24	-48	-46	3.83 (2.88)
R. postcentral	BA3	67	34	-22	36	5.19 (3.44)
R. premotor/inferior frontal	BA44/13	34	42	10	14	3.77 (2.85)

L, left; R, right; uncorrected $p < .005$; extent threshold $k > 15$ voxels.

Table 2
Brain areas showed significant changes in CBF associated with the F contrast evaluating early and late learning

Region	Brodmann's area	Cluster size	MNI coordinates			F value
			X	Y	Z	
R. inferior parietal	BA40	148	40	-54	36	52.04
R. ventral premotor/insula	BA44/13	47	36	2	10	44.79
R. superior temporal gyrus	BA13	15	52	-38	14	32.47

L, left; R, right; uncorrected $p < .001$; extent threshold $k = 15$ voxels.

learning session was divided into 12 small epochs, as in the behavioral analysis. The CBF time course in each ROI over the learning session was averaged across subjects and then correlated with the RT time course to assess whether regional CBF changes in these regions tracked the gradual behavioral changes. Mean CBF signal intensities were calculated and compared.

First, there were no significant correlations in any of the general sensory/motor ROIs: bilateral SMA ($r = -0.399$, $p = .20$); L. precentral cortex ($r = 0.343$, $p = .28$), and L. parietal cortex ($r = 0.155$, $p = .63$) suggesting that these regions do not significantly contribute to behavioral changes associated with sequence learning. An example of one of these regions (the right SMA) is shown in Fig. 4.

Second, all three learning defined ROIs showed significant correlations between CBF and response times over the sequence learning session: R. IPL ($r = 0.757$, $p = .004$), R. premotor/inferior frontal cortex ($r = 0.74$, $p = .006$), and R. superior temporal gyrus ($r = 0.655$, $p = .021$); see Fig. 5. Interestingly, the final minutes of sequence learning were associated with blood flow levels that were equal to, or less than, that measured during simple fixation.

Were the CBF changes due to sequence learning or general task learning? Sequence specific learning should show a

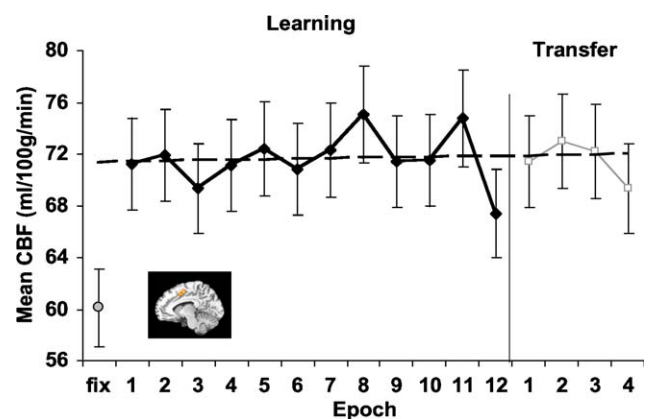


Fig. 4. The CBF time courses in the SMA, one of the motor ROIs, during a 2 min, baseline fixation period, followed by learning and transfer periods. Error bars represent standard error of the mean. Each "epoch" corresponds to a 75 s period during which the 11 item sequence repeated seven times.

decrease between activations in later learning and activations during the transfer phases, whereas general task learning should show no difference between late learning and transfer. A significant CBF increase from late learning to the transfer session ($p < .05$) was found in the R. ventral premotor/inferior frontal cortex. The onset of the transfer

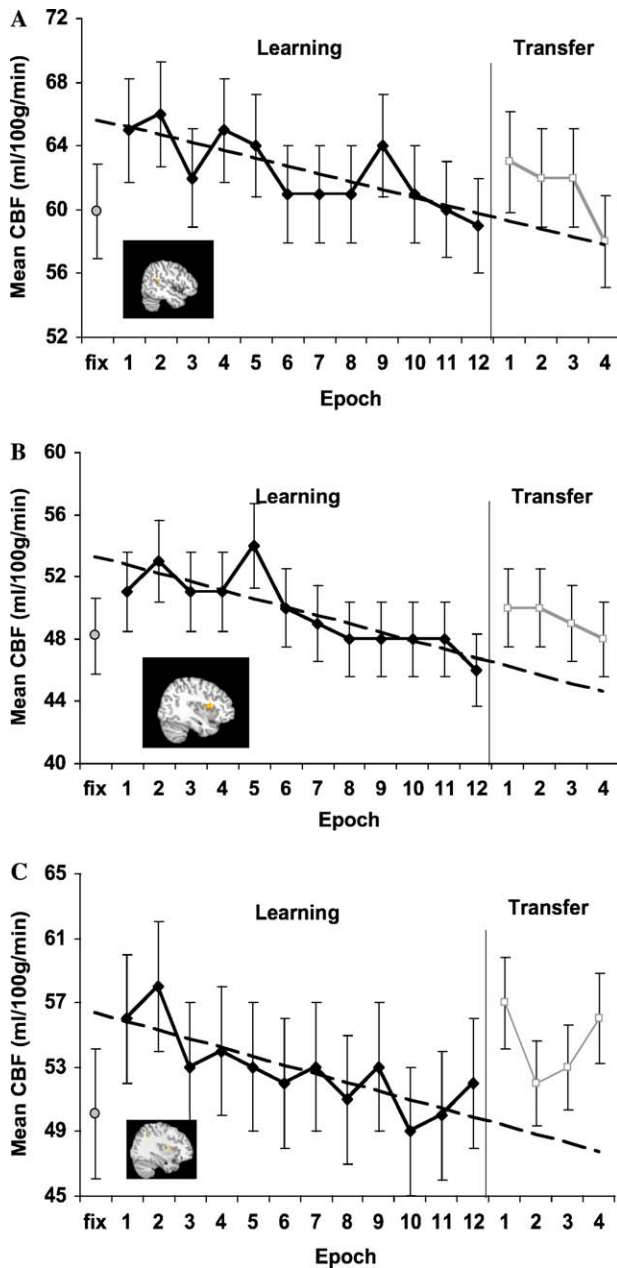


Fig. 5. The CBF time courses in the three learning related ROIs showed gradual CBF decrease during learning: (A) Right superior temporal gyrus; (B) right ventral premotor cortex (extending to insula); (C) right inferior parietal cortex. Error bars represent standard error of the mean.

period did not, however, significantly alter the signal within the R IPL ($p = .12$) or R STG ($p = .25$).

In sum, time-dependent monotonic decreases in CBF were observed in association with the learning period, and in one instance this could be shown to increase significantly with the onset of the transfer phase. These effects were subtle, however, and could only be detected using a ROI-based analysis.

3.3. BOLD fMRI results

While the study had borderline power to detect slow changes in neural activity with perfusion, we might ask if

this nonetheless represents an improvement over previously available MRI methods. Because echoplanar imaging was used to measure the effects of arterial spin labeling, BOLD fMRI time-series was acquired simultaneously with the perfusion data. We analyzed the BOLD fMRI data in a manner exactly analogous to that employed for the perfusion data to determine if learning effects might be observed using this more traditional MR imaging method. No observable effects of learning were present in the BOLD data either at a map-wise level of significance or the lowered criterion employed in the perfusion data. Further reductions of the threshold failed to reveal activation that corresponded to that seen in the perfusion data.

This result is not surprising given the known instability of the BOLD signal at long time scales. However, the ability to acquire perfusion and BOLD contrast simultaneously raises the possibility that each contrast might be assayed for effects at different time scales. To illustrate this approach, we analyzed the BOLD data to determine if event-related differences between correct and error responses could be observed. During the learning period subjects occasionally made errors in which they pressed the wrong button. These errors were infrequent (2.6% of trials), and occurred at an essentially constant rate during the learning session. Differences in BOLD signal between these error responses and correct responses were sought in the analysis.

Fig. 6 shows the one area of significant (map-wise 0.05, cluster filter $k=15$) signal change located within the L. putamen/insula. BOLD responses to error trials were observed in other regions when a more lenient threshold (uncorrected $p < .001$, cluster size >40 voxels) was used. These activations were in the bilateral thalamus, bilateral inferior temporal lobe, and R. precuneus.

4. General discussion

The primary purpose of this study was to assess the utility of perfusion fMRI for studies of cognition. There are several features of perfusion fMRI that make it suitable for the study of particular mental operations. First, the perfusion fMRI signal is stable over long time scales. As such, perfusion fMRI has the same power to detect a change in neural activity whether 30 s has elapsed or 30 min (Aguirre et al., 2002). Second, perfusion fMRI provides an absolute measure of neural change, so variations in CBF over time can be directly compared across subjects, without reference to a baseline control condition (Aguirre et al., 2002). These features suggest that perfusion fMRI may be best suited for between-subject designs, studies that utilize an intervention or longitudinal design, or studies that require the comparison of observations at widely different time points such as studies of sleep or learning. In this study, we assessed the utility of perfusion fMRI for one particular type of learning: motor learning.

The serial response time task (SRT) is a model task for studying motor sequence learning. This task focuses on

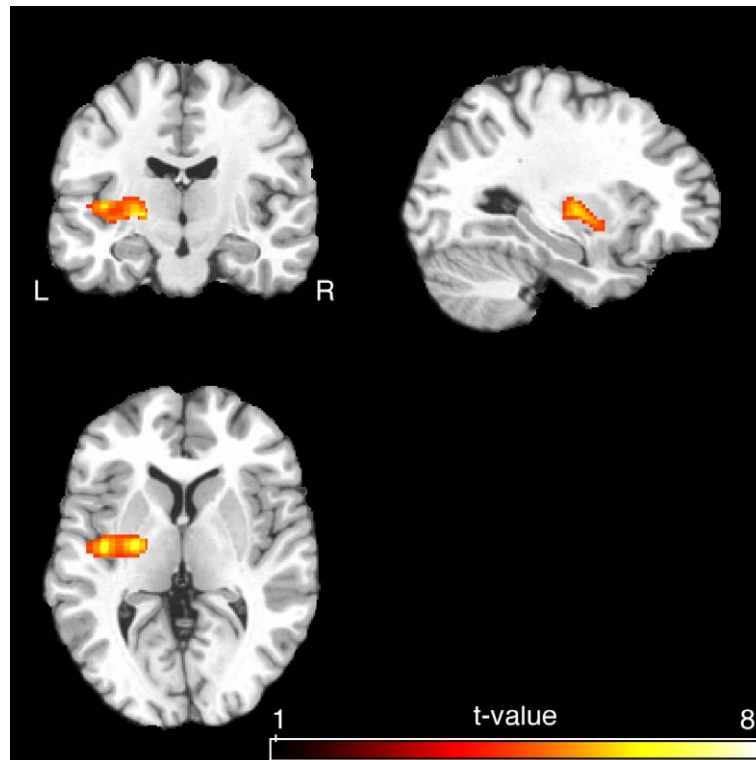


Fig. 6. The BOLD response to errors in the left insula/putamen ($p < .05$, corrected).

incremental changes in the execution of a learned motor sequence without evoking additional processes such as chunking or working memory (Lee & Quessy, 2003). In this study, we used the SRT task as a tool to induce slow changes in neural activity. Our goal was to test the ability of perfusion imaging to detect slow changes in mental states. As such, we were agnostic as to whether the observed changes in CBF reflected true learning of motor sequences or simply reductions in RT associated with learning motor sequences.

4.1. Learning-related changes in neural activity

The strongest evidence for the involvement of any particular region in learning comes from assessing the temporal profile of signal change in a particular neural region: if it replicates the temporal profile of behavioral change then this provides good evidence that the region is involved in the plastic change. To assess learning-related changes in neural activity, we correlated the CBF time course in each ROI with the RT time course. This allowed us to examine both specific and general learning changes. R. ventral premotor cortex, and R. inferior parietal lobe showed monotonic decreases in CBF that recovered with the onset of the transfer phase. When CBF was correlated with RT, there were significant positive correlations between decreases in RT over time and decreases in CBF over time suggesting that specific learning in these regions guides response speeding to learned sequences. In the R STG, there was a significant decline in signal during learning, although the

switch to the transfer session was not associated with a significant rise in signal. This might suggest that signal changes in the R STG reflect more general learning processes (faster reaction times in general). However, focused, inter-region statistical comparisons would be needed to support this assertion, and insufficient power was present in this study to do so.

The ventral premotor cortex/inferior is richly interconnected with the parietal cortex (Cavada & Goldman-Rakic, 1989). Single units studies in monkeys have showed that cells in this region fire prior to movement initiation, anticipating the eventual motor response (Godschalk, Lemon, Kuypers, & van der Steen, 1985). Ventral premotor cortex also plays an important role in movement execution (Kurata, 1994), and in translating visual coordinates to motor coordinates for action (Kurata & Hoshi, 2002). Neuroimaging studies have frequently linked this region to sequence learning (Eliassen, Souza, & Sanes, 2001; Iacoboni, Woods, & Mazziotta, 1998; Muller, Kleinhans, Pierce, Kemmotsu, & Courchesne, 2002; Thomas et al., 2004). Toni, Krams, Turner, and Passingham (1998), as well as Honda, Deiber, Ibanez, Pascual-Leone, and Zhuang (1998) found that activity in premotor cortex decreased with learning, with a response function that looked similar to that reported here (Fig. 5B). It is possible that the sequence-specific learning observed in ventral premotor cortex reflects changes in the ability of “preparatory neurons” to anticipate a target.

The activation found in the inferior parietal lobe is interesting because one of the most consistent findings in the

sequence learning literature is that activity in the inferior parietal lobe is modulated by sequence learning (Thomas et al., 2004). There are various hypotheses about the meaning of learning related changes in the inferior parietal lobe. It is well known that portions of the parietal lobe are involved in shifting attention (Nobre, 2001; Rushworth, Paus, & Sipila, 2001). Procedural learning such as tested here leads to a decreased need for attention as the task becomes automatized (Logan, 1995). Thus decreases seen in the inferior parietal lobe may reflect decreases in attentional requirements as the task progresses. A second hypothesis is that learning related decreases are related to an increased ability to prepare for the upcoming stimulus. Portions of the parietal cortex, especially the region near the intraparietal sulcus, are involved in preparing for action (Andersen, 1997; Snyder, Batista, & Andersen, 1997; Wolpert, Goodbody, & Husain, 1998). Our data tend to favor the attentional hypothesis because the activations reported in this study were more inferior to that reported in studies of preparatory processing.

Only a few other BOLD fMRI or PET studies have correlated the time course of learning and behavior in the SRT task. Muller and colleagues (Muller et al., 2002) compared early learning to late learning, finding that late learning was associated with decreased activations in superior parietal cortex, inferior parietal lobe, precentral gyrus, and middle and superior frontal gyri. Increases were less notable (Sakai et al., 1998). Toni et al. (1998) examined more gradual changes that accompany the time-course of learning in the SRT task and found learning related changes in prefrontal, premotor and parietal cortex and cerebellar areas. Shortcomings of that study include the small number of participants ($N=3$) and the lack of statistical analyses (see also Honda et al., 1998).

4.2. Comparison with findings from BOLD imaging

We did not find robust activations in some regions, such as portions of the basal ganglia, motor cortex, and thalamus, that have been reported in prior BOLD fMRI studies of the SRT task (Doyon & Ungerleider, 2002). Sequence learning tasks have historically provided disparate neural results (Doyon & Ungerleider, 2002) and ascertaining the source of this variability is difficult. However we offer a few speculations that may account for differences between our study and other published sequence-learning studies. First, our study differs from prior studies of sequence learning in that we assessed learning over a continuous 21-min run rather than over 5–6 min runs. It is possible that the type of learning studied here is associated with a slow decline in hemodynamic response that peaks each time there is a need to pay attention to new stimuli. When there are shorter blocked on–off learning sessions, the hemodynamic response function might be characterized as slowly declining with intermittent spikes correlated with the onset of each new block. Thus, some of the regions activated during blocked on–off

learning sessions drop out when learning is continuous due to neural adaptation. Second, it is possible that the lower signal to noise ratio of perfusion fMRI did not allow for detection of neural activity in some regions identified by BOLD imaging.

Regardless of the fact that some areas activated in prior studies were not found here, the areas that were activated showed good correspondence to findings reported in prior studies, as reviewed above, suggesting that perfusion fMRI is a good method for assessing learning-related changes. In the next section, we discuss experimental designs and questions for which perfusion imaging is a good choice.

4.3. Limitations of ASL perfusion fMRI for studies of cognition

Several limitations of perfusion fMRI are highlighted by the design and findings reported here. One limitation is the lower signal to noise ratio which was notable in this study. Despite the presence of convincing regional changes in CBF that were well correlated with task performance, these correlations could not be detected without the assistance of a region-of-interest based analysis. Several technical advances promise improvements in the SNR of ASL perfusion (reviewed in Aguirre, Detre, & Wang, 2005).

Modification and improvement of the task paradigm can benefit future studies. Improvements in power might be obtained by increasing the frequency of stimuli and subject responses to induce more “time on task”. Subject fatigue might be reduced by providing occasional rest periods during scanning. Although this study was meant to contrast with experimental designs in which the subject alternates between task performance and a control condition, these “chopped” designs might be studied with perfusion fMRI as well. Instead of a high frequency of alternation between task and control conditions (e.g., 30 s of task, 30 s of control), the design might use 5-min blocks of learning. Although this would entail interrupting performance, perfusion fMRI could still be used to measure slow changes in CBF during each learning block, and the absolute CBF during control and task periods could be evaluated, allowing the study to test for the possibility that there are changes in the response to the control condition over time.

Perfusion fMRI is also marked by relatively poor temporal resolution. This is a consequence of the time required for labeling and blood transit time, as well as the need to take the difference of label and control images to reveal the perfusion effect. Because perfusion fMRI is best suited to the study of slow changes in neural activity, this limitation does not in practice severely limit the method. Further, and as was demonstrated here, it is possible to test for more rapid changes in neural activity through evaluation of the BOLD signal change present in the raw perfusion data. The perfusion signal is present in the difference between sequential label and control images, but these images themselves can be acquired using a gradient echo sequence that is sensitive to BOLD contrast.

Thus, the original, unsubtracted time-series contains signal changes sampled at a higher temporal resolution (here, at a TR = 3 s) with superior SNR. Of course, the unsubtracted BOLD signal is dominated by low temporal frequency noise, making it unsuitable to detect slow changes in neural activity. It can be used, however, to test for higher frequency, “event-related” signal changes, as was done here to evaluate signal changes associated with error responses. This analysis showed that neural activity associated with errors on a trial-by-trial basis was reflected in the left putamen. Interestingly, activation in the left putamen has been a consistent finding in studies that have manipulated the temporal predictability of rewarding stimuli (McClure, Berns, & Montague, 2003).

There have been several prior demonstrations of ASL perfusion used to detect functional changes in neural activity, although only more recently has the technique allowed acquisition of multi-slice data sets (Garraux, Hallett, & Talagala, 2005). Although we have shown previously that the perfusion fMRI signal is stable over long time periods (Wang et al., 2003), the important finding in this study is that slow, continuous changes in signal might be detected. With further technical improvements, perfusion based fMRI should prove a useful tool for cognitive neuroscience applications.

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