Top-Down Regulation of Laminar Circuit via Inter-Area Signal for Successful Object Memory Recall in Monkey Temporal Cortex

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SUMMARY

Memory retrieval in primates is orchestrated by a brain-wide neuronal circuit. To elucidate the operation of this circuit, it is imperative to comprehend neuronal mechanisms of coordination between area-to-area interaction and information processing within individual areas. By simultaneous recording from area 36 (A36) and area TE (TE) of the temporal cortex while monkeys performed a pair-association memory task, we found two distinct inter-area signal flows during memory retrieval: A36 spiking activity exhibited coherence with low-frequency field activity in either the supragranular or infragranular layer of TE. Of these two flows, only signal flow targeting the infragranular layer of TE was further transmurally coupled with gamma activity in the supragranular layer of TE. Moreover, this coupling was observed when monkeys succeeded in the retrieval of the sought object but not when they failed. The results suggest that local translaminar processing can be recruited via a layer-specific inter-area network for memory retrieval.

INTRODUCTION

Memory retrieval in primates is orchestrated by a brain-wide neuronal network (Squire et al., 2007; Maunsell and Newsome, 1987; Logothetis and Sheinberg, 1996; Moscovitch et al., 2006; Burke et al., 2015; Miyashita, 2004). To elucidate the operation of this network, it is imperative to understand not only which brain areas are interacting (Kornblith et al., 2013; Igarashi et al., 2014; Salazar et al., 2012) but also how inter-area signals affect local information processing within the target area. Converging evidence from neuropsychological, anatomical, and lesion studies has suggested that memory processes including consolidation and retrieval are implemented by the interaction between the medial temporal lobe and the domain-specific association cortices (Squire et al., 2007; Woloszyn and Sheinberg, 2012; Moscovitch et al., 2006; Miyashita, 2004; Hirabayashi et al., 2013b; Naya et al., 2001). Area TE (TE), which is part of the temporal association cortex, is adjacent to and interconnected with area 36 (A36) of the perirhinal cortex, which is part of the medial temporal lobe. These brain areas are known to engage in the associative representations of long-term memory of objects and to play distinct roles in memory retrieval (Miyashita, 2004; Hirabayashi et al., 2013b, 2014; Naya et al., 2001). Although the perceptual activity of visual objects emerges earlier in TE than A36, memory retrieval activity for the sought target emerges earlier in A36 than TE (Naya et al., 2001), suggesting that a backward signal flows from A36 to TE during memory retrieval. The inter-area signal, if present, would modify local signal processing implemented by laminar neuronal circuits in the target area. To test this hypothesis, we investigated (1) whether and how single-unit activity (SUA) in one area, A36, interacts with ensemble activity in the other area, TE; (2) whether and how the inter-area signal couples with translaminar information processing within TE during memory retrieval; and (3) whether this coupling was associated with monkey’s behavioral performance.

RESULTS

Two macaque monkeys were trained to perform a pair-association memory task, in which they had to retrieve a learned paired associate in response to a presented cue stimulus (Figure 1A) (Naya et al., 2001; Takeuchi et al., 2011). We recorded neuronal signals simultaneously from A36 (using tungsten electrodes) and from TE (using multi-contact linear electrodes [16 channels with 150 μm spacing]) (Figure 1B) (Takeuchi et al., 2011). A total of 68 SUAs in A36 showing the stimulus selectivity during both the cue and delay period (one-way ANOVA, p < 0.01 for both periods) were used to calculate the inter-area coherence with local field potentials (LFPs) in TE (68 data sets). Of these, 49 data sets showed significant inter-area coherence during the delay period in at least 1 channel of the TE electrode when the optimal stimulus was presented as the cue stimulus (optimal trial) (Figure 2A; see Figures S2A, S2B, and S2I–S2K for population data, Figure S1 for representative data, and Figures S2E–S2H for coherence characteristics in each animal; see also Supplemental Experimental Procedures for details). Coherence during the delay period was significantly higher than that of trial-shifted control in the alpha/beta (9–25 Hz) frequency range,
but not in the gamma (30–88 Hz) range (Figure 2B; paired t test, p < 0.01, corrected for multiple comparisons across frequencies). Coherence in the alpha/beta range was dependent on the presented cue stimulus, even after controlling for differences in both the firing rate and the number of trials (Figure 2C; one-way ANOVA, F = 39.81, p < 0.001, followed by the post hoc Tukey-Kramer test, p < 0.001; see Figure S2C for the stimulus selectivity of LFP power in TE). We found a significant correlation between the strength of the association between two visual objects in an A36 neuron (pair-coding index [PCI]) and the difference in coherence between a pair trial and other trials ("others 2") (Figure S2D; r = 0.59, p = 9.53 x 10^-10). Because neurons with large PCI values are thought to contribute to the pair-association memory system (Naya et al., 2003), these results suggest that a neuron-LFP pair contributing to the pair-association memory system showed strong coherence for the paired associate of the optimal stimulus. The distribution of \( \varphi_{\text{max}} \), which was defined as the angular phase at the frequency of maximum coherence in each data set, was significantly concentrated (Figure 2D; Rayleigh test, Z = 6.80, p < 0.001). The inter-area spike-triggered average (STA) of LFPs showed that the troughs of TE LFPs lagged A36 spikes by 17 ms (Figure 2E), and the time lag was significantly different from the corresponding value observed within A36 (paired t test, t = 2.61, p < 0.012; the intra-area STA showed that the troughs of A36 LFPs led A36 spikes by 3 ms).

Layer-Specific Inter-Area Coherence

We next examined whether coherence during the delay period was observed in all layers of TE or a specific layer of TE. The channel at the granular layer (Gr) was estimated on the basis of the current source density (CSD) calculated from the depth profile of visually evoked LFPs (Figure 3A, left; see Figure S3 for the population CSD and Figure S4 for histological evaluation of reconstruction accuracy in A36 and TE) (Takeuchi et al., 2011). A representative depth profile of coherence is shown in Figures 3A–3D. Mean coherence was significantly higher at the channels in the infragranular layer (IG) than in Gr (Figure 3C; paired t test, p < 0.01, corrected for multiple comparisons across channels) and supragranular layer (SG) (Figure 3D; paired t test, t = 149.65, p < 0.001). Figures 3E–3H show another representative depth profile of coherence. In contrast to the previous case, coherence was significantly higher in SG than in Gr and IG (Figure 3G; paired t test, p < 0.01, corrected for multiple comparisons across channels; Figure 3H; paired t test, t = 56.54, p < 0.001).

As a population, most coherence profiles at 9 to 25 Hz during the delay period showed higher coherence in either IG or SG (Figure 4A; see Figure S5A for coherence at 26–90 Hz, Figure S5B for Z scores of coherence at 9–25 Hz, and Figure S5C for coherence during the fixation period). Principal-component (PC) analysis revealed population patterns of the coherence profiles (Figure 4B). Both the first and second PCs depicted the difference in coherence between IG and SG, and up to 84% of the total variance in the data sets was explained by these two PCs. In the PC space of the data sets, we classified all data sets into clusters and found two major clusters showing distinct depth profiles of coherence (Figure 4C; see Experimental Procedures for details). In cluster 1 (n = 18 data sets), significant coherence was predominantly observed in IG (paired t test, p < 0.01, versus trial-shifted control, corrected for multiple comparisons), while coherence in cluster 2 (n = 13 data sets) was significant predominantly in SG (Figure 4D). Data sets for clusters 1 and 2 showed a significant double dissociation between the mean coherence in IG and SG (Figure 4E; two-way ANOVA, F = 50.69 for interaction between clusters and layers, p < 0.001, followed by the post hoc Tukey-Kramer test, p < 0.01; Figure 4F; one-way ANOVA, F = 105.72, p < 0.001, followed by the post hoc Tukey-Kramer test, p < 0.01; see Figure S5D for coherence values for each of the two animals and Figure S5E for difference in coherence between IG and SG for each animal). To examine whether the difference in the laminar specificity of coherence at the target area TE between clusters 1 and 2 derived from the difference in the recording depth of A36 neurons, we compared the distances from recording sites to anatomical landmarks in cluster 1 with those in cluster 2 (Koyano et al., 2011; Matsui et al., 2007). The median distance from recording sites to the
pia mater and gray matter-white matter boundary did not significantly differ between clusters 1 and 2 (distance to the pia mater 1.47 and 1.26 mm for clusters 1 and 2, respectively, Wilcoxon rank sum test, $Z = 0.78$, $p = 0.44$; distance to the gray matter-white matter boundary 0.97 and 1.11 mm for clusters 1 and 2, respectively, $Z = 0.34$, $p = 0.73$; note that the localization accuracy was within the range of 0.13 to 0.27 mm; Figure S4). These results suggest that the difference in the laminar specificity of coherence at TE is unlikely to derive from the difference in the recording depth of A36 neurons.

### Coupling of Inter-Area Coherence with Translaminar Processing

Does inter-area coherence between A36 spike and TE LFP impact on local neuronal processing in TE? To address this question, we first examined the layer specificity of LFP power in the gamma frequency range ($\gamma$ power) as an index of local neuronal processing (Singer and Gray, 1995; Singer, 1999; Hirabayashi and Miyashita, 2014; Jia et al., 2013; Liu and Newsome, 2006) in TE. Figures 5A and 5B show $\gamma$ power in the representative data set (the same data set as in Figures 3A–3D) with greater $\gamma$ power in SG than IG during the delay period. In population, the $\gamma$ power in SG during the delay period was significantly elevated compared with that during the fixation period (Figure 5C; $p < 0.01$) and was greater than that in IG (Figure 5D; $p < 0.001$) for the data sets of both clusters 1 and 2. The depth profile of $\gamma$ power was similar between animals (Figures 5E and 5F).

We then examined the coupling of the inter-area neuronal signal with the time-varying gamma activity found predominantly...
in SG. For that purpose, A36 spikes coherent with TE LFP (coherent spike; spikes firing at $\phi_{\text{max}} \pm 1/4\pi$) were extracted to calculate the STA of the TE $\gamma$ power (STA$\gamma$; see Experimental Procedures for details). STA$\gamma$ was calculated at the TE channel at which maximum $\gamma$ power was recorded during the delay period (Figure 6A). Figure 6B shows a representative STA$\gamma$ with coherent spikes (the same data set as in Figures 3A–3D, 5A, and 5B; see Figure S6A for STA$\gamma$ with non-coherent spikes), which showed a low-frequency periodic increase in $\gamma$ power compared with the STA$\gamma$ calculated with non-coherent spikes. We then examined the periodicity of the STA$\gamma$ for data sets in clusters 1 and 2 separately (Figures 6C and 6D; see Figures S6B and S6C for non-coherent spikes). In cluster 1, the low-frequency (9–14 Hz) power of the population STA$\gamma$ with coherent spikes was significantly greater than that calculated with non-coherent spikes, whereas the difference in STA$\gamma$ power between coherent and non-coherent spikes was not observed in cluster 2 (Figures 6C–6E; two-way ANOVA for factors of clusters [1 or 2] and spikes [coherent or non-coherent], F = 4.65, p = 0.035 for cluster effect, F = 11.53, p = 0.0012 for spike effect, F = 5.10, p = 0.028 for interaction; Tukey-Kramer test, p = 0.0003 for spike effect in cluster 1 and p = 0.88 for spike effect in cluster 2). STA$\gamma$ power with coherent spikes was significantly greater in cluster 1 than in cluster 2 (Figure 6E; p = 0.015), and the difference in STA$\gamma$ power between coherent and non-coherent spikes was also greater in cluster 1 than in cluster 2 (Figure 6F; t test, t = 2.34, p = 0.027). When we divided the relative phase of spikes ranging into four equispaced quadrants and calculated the STA$\gamma$ for each quadrant, the quadrant with coherent spikes exhibited the greatest STA$\gamma$ power in cluster 1 (Figures S6D and S6E). In cluster 1, a significant difference in STA$\gamma$ power was also observed between coherent and non-coherent spikes after equalizing both the number of spikes and the phase concentration of spikes (Figure S7; see Supplemental Experimental Procedures). Most data sets (83% [15 of 18]) in cluster 1 showed the maximum $\gamma$ power in SG; thus, these results suggest that the inter-area signal targeting the IG of TE (cluster 1) exerted a translaminar impact on the local ensemble activity in the SG of TE. Such impact was not detected with the other inter-area signal (cluster 2) targeting the SG of TE.

Behavioral Effect in the Coupling between Inter-Area and Translaminar Signals

Finally, we examined whether the STA$\gamma$ in cluster 1 was relevant to monkey’s behavioral performance by comparing the STA$\gamma$ in correct trials with that in error trials. In this analysis, the number of correct trials was reduced to be matched with that of error trials (see Experimental Procedures for details). The STA$\gamma$ power with coherent spikes was lower in error trials than in correct trials (Figure 7; Tukey-Kramer test, p < 0.001 after two-way ANOVA for factors of trial type [correct or error] and spikes [coherent or non-coherent], F = 8.09, p = 0.0059 for trial type effect, F = 27.27, p < 0.0001 for spike effect, F = 9.90, p = 0.0025 for interaction). Correspondingly, the difference in STA$\gamma$ power between coherent and non-coherent spikes was significantly greater in correct trials than in error trials (paired t test, t = 3.54, p = 0.0025). The firing rate of TE neurons in correct trials was also significantly larger than that in error trials (Wilcoxon signed rank test, t = 12, p = 0.034), as STA$\gamma$ was (Figure 7C), suggesting that the difference in STA$\gamma$ between correct and error trials is reflected in spiking activity in TE that differs between correct and error trials. In contrast to the STA$\gamma$ and the firing rate in TE, neither the firing rate of A36 neurons, $\gamma$ power of TE nor inter-area coherence were different between correct and error trials (Figures S8A–S8C). After equalizing both the number of spikes and the phase concentration of spikes as in Figure S7, a significant difference in STA$\gamma$ power was found between correct and error trials (Figures S8D–S8F). These results suggest that the coupling of inter-area and translaminar signal processing in cluster 1 contributes to successful retrieval of the visual object from long-term memory.

DISCUSSION

In this study, we simultaneously recorded from A36 and TE of the temporal cortex while monkeys performed a pair-association memory task (Hirabayashi et al., 2013a, 2013b, 2014; Takeuchi et al., 2011; Naya et al., 2001). Spike-field coherence analysis revealed that during the delay period, the spiking activity of A36 neurons was coherent with TE LFP in a layer-specific manner. Moreover, A36 spiking activity coherent with TE LFP in the IG was coupled with the local ensemble activity in the SG of TE, and this coupling emerged in correct trials but not in error trials. These results suggest that translaminar signal processing in TE can be recruited by way of inter-area coherent network when monkeys successfully retrieved a target visual object from long-term memory.

Coherence analysis has been widely used to estimate the coordination between two time series of neuronal signals in the frequency domain. Spike-field coherence is a measure of preferential spiking activities predominately during a specific phase range of field potential oscillations (Salazar et al., 2012; Pesaran et al., 2008). Because the spiking activity reflects output signals that generate synaptic potentials at the projection zone and the LFP reflects dendritic input (Salazar et al., 2012; Pesaran et al., 2008; Buzsáki et al., 2012; Katzner et al., 2009), it has been interpreted in previous reports that the spike-field coherence is indicative of directed synaptic influences (Salazar et al., 2012; Pesaran et al., 2008). In the present study, we calculated the STA of LFPs, which estimates the coordination between spikes and LFPs in the time domain, and found that the trough of TE LFPs exhibited significantly greater time lag from A36 spikes (17 ms) compared with the corresponding time lag between the A36 LFPs and A36 spikes (~3 ms). Thus, the present results were consistent with the interpretation of directionality of the spike-field coherence in the previous literature. The observed lag time between A36 spikes and the trough of the TE LFPs was comparable with findings in previous studies, such as that of Gregoriou et al. (2009) (inter-area time shift between FEF spikes and V4 LFPs, and vice versa; 8–13 ms). A comparable time shift was also observed between spikes in motor cortex and spikes in the substantia nigra pars reticulata (Brazhnik et al., 2012) (17 ms) (but see also Jia et al., 2013; Nowak et al.,
A Depth profile of coherence (Representative dataset 1)

B Depth x frequency dependence

C Coherence profile

D Coherence in IG and SG

E Depth profile of coherence (Representative dataset 2)

F Depth x frequency dependence

G Coherence profile

H Coherence in IG and SG

(legend on next page)
and Nowak et al., 1999, in which the inter-area time shift between V1 spikes and V2 spikes was shorter than the present results; <10 ms). It should be noted that the lag time between spikes and LFPs might involve other signal processing, such as recurrent processing within the target area (Brincat and Con-

or, 2006; Hirabayashi et al., 2013b; Tie-

singa et al., 2008) to generate field poten-
tials. We also showed that the coherence between “A36 spikes and TE LFPs” was significantly larger than that between “TE spikes and A36 LFPs” (Figure S2K).

Our finding that both directions of the spike-field coherence occur at low frequency is consistent with findings in previous studies (Pesaran et al., 2008; Salazar et al., 2012). However, it should be noted that other studies have shown recruitment of high-frequency coordination (Gregoriou et al., 2009) and
recruitment of high- and low-frequency coordination for feedforward and feedback processing, respectively (van Kerkoerle et al., 2014). Differences in task requirements and underlying information processing might constitute a possible explanation for the difference in frequency bands for inter-area coordination.

To investigate the laminar specificity of inter-area coordination in TE, we used the multi-contact linear electrodes for recording in TE. The CSD for each data set was calculated from the depth profiles of the stimulus-evoked LFPs to estimate the cortical layer for each channel (Figure S3). The resultant CSD profiles were quite similar to those in the perirhinal cortex (Takeuchi et al., 2011) and the primary sensory cortex (Fujisawa and Buzsáki, 2011; Roberts et al., 2013), showing the interlaminar “canonical” circuit, as suggested in anatomical studies (Callaway, 2004; Felleman and Van Essen, 1991). Using similar multi-contact linear electrodes, previous studies have demonstrated the layer specificity of bottom-up signal flows between distinct areas such as the flow from the lateral geniculate nucleus to the primary visual cortex in cats (Jin et al., 2008, 2011), the flow from V1 to V2 in monkeys (Roberts et al., 2013), and the flow from V1 to V4 in monkeys (van Kerkoerle et al., 2014). The laminar pattern of functional connectivity observed in these studies was in line with the well-known feedforward anatomical projection that terminates at the Gr of the target area. In the present study, by contrast, spiking activities in A36 were coherent with local field activities either at the IG or SG of TE, staying away from the Gr (Figures 3 and 4). Indeed, the segregation of inter-area signal flow targeting to the IG and SG was depicted by both the first and second PCs of coherence profiles, which explained up to 84% of the total data variance (Figure 4B). These results suggest that the observed inter-area coherence does not reflect a bottom-up
Figure 6. Inter-Area Coupling of A36 Spikes with TE Gamma Power: STAγ
(A) Schematic drawing of STAγ. In this example, A36 spike trains were coherent with TE LFP in IG. The spikes firing at \( t_{\text{max}} \pm 1/4 \pi \) (coherent spikes) were extracted to compute a STA of gamma activity at the TE channel showing maximum gamma power (in this example, in SG).
(B) Left: a representative “A36 spike-TE LFP” pair for STAγ with coherent spikes. Z score-transformed STAγ value is color coded. Small vertical lines in the color bar represent thresholds for statistical significance of STAγ values (p < 0.05 adjusted by the false discovery rate [FDR], Z = ±2.56). Right: power spectrum of STAγ with coherent spikes (black, n = 1,068) and non-coherent spikes (gray, n = 854).
(C) Left: population STAγ with coherent A36 spikes for cluster 1. Small vertical lines in the color bar represent the threshold for statistical significance of FDR adjusted 5% (t = 3.99). Right: power spectrum of STAγ in cluster 1 (mean ± SEM). Inset: difference in power spectrum of STAγ between coherent spikes and non-coherent spikes. The difference was significant at 9 to 14 Hz (p < 0.05, paired t test with Bonferroni correction across frequencies) but not significant below 8 Hz. The mean numbers of coherent and non-coherent spikes were 471.8 and 295.2, respectively.
(D) Left: population STAγ with coherent A36 spikes for cluster 2. Note that there were no threshold lines for FDR adjusted 5% in the color bar because the highest p value for STAγ did not reach FDR adjusted 5%. Right: power spectrum of STAγ in cluster 2 (mean ± SEM). Inset: Difference in power spectrum of STAγ between coherent spikes and non-coherent spikes. The mean numbers of coherent and non-coherent spikes were 320.8 and 253.0, respectively. Note that there was no significant difference in the numbers of spikes between clusters (t test, coherent spikes, t = 1.49, p = 0.145; non-coherent spikes, t = 1.82, p = 0.114).
(E) Comparison of STAγ power between coherent and non-coherent A36 spikes for clusters 1 (red) and 2 (blue). *t test, t = 2.34, p < 0.027. Errors bars show SEM.
(F) Difference in STAγ power between coherent and non-coherent A36 spikes for clusters 1 (red) and 2 (blue). *p < 0.01; *p < 0.05, Tukey-Kramer test after two-way ANOVA (F = 4.65, p = 0.035 for cluster effect, F = 5.10, p = 0.023 for interaction).
signal flow. With regard to the backward projection in the temporal cortex, four axonal arborization patterns have been morphologically identified in the target area at (1) layers 5 and 6 (infragranular), (2) layers 1 to 3 (supragranular), (3) layers 1 to 3 and 5 and 6 (both supragranular and infragranular), or (4) only layer 1 (Lavenex et al., 2002; Suzuki et al., 2000). Therefore, a fascinating possibility is that the inter-area coherence in clusters 1 and 2 reflects the functional signals that are transmitted via the aforementioned anatomical projections (1) and (2), respectively. Whether these functional signals are indeed conveyed by these anatomical projections would be an important issue for future studies.

In the present study, we identified the coupling between the inter-area signal and inter-laminar information processing by analyzing the relationship between “spiking activity around the phase of maximum inter-area coherence” and “local ensemble activity in the distant area,” (Figure 6) (see Verhoef et al., 2011; Igarashi et al., 2014; Salazar et al., 2012; Liebe et al., 2012; Fujisawa and Buzsáki, 2011; Gregoriou et al., 2009; Pesaran et al., 2008; Buschman and Miller, 2007; von Stein et al., 2000; Tallon-Baudry et al., 2001; Saalmann et al., 2012; and Fries, 2009 for previous reports on inter-area signal per se in other cognitive functions; see Godlove et al., 2014; Takeuchi et al., 2011; Sakata and Harris, 2009; Xing et al., 2012; Self et al., 2013; Kaliukhovich and Vogels, 2012; and Spaak et al., 2012 for reports on inter-laminar processing within a cortical area). Recent studies have demonstrated that low- and high-frequency synchrony are confined largely to the IG and SG, respectively (Buffalo et al., 2011; Spaak et al., 2012; Xing et al., 2012) and that the amplitude of the high-frequency LFP is modulated by the phase of low-frequency LFP (phase-amplitude coupling [PAC]) (Spaak et al., 2012; Tort et al., 2008; Canolty et al., 2006). It is likely that a similar mechanism is shared in the coupling between the inter-area signal and translaminar processing found in the present study. However, it is notable that the difference in the STA\textsubscript{g} between the coherent and non-coherent spikes still remained significant even after controlling for both the number of spikes and the phase concentration of spikes (Figure S7). This control procedure minimized the contributions of PAC (see Supplemental Experimental Procedures), suggesting that the observed coupling between the inter-area signal and inter-laminar information processing cannot be explained by PAC alone within TE. Instead, the observed coupling probably reflected precise temporal relationship between spikes in A36 and gamma activity in the SG of TE, which was mediated by slow-frequency signal in the IG of TE.

In the present study, we found that the coupling between the spiking activity in A36 and the gamma activity in the SG of TE...
was significantly greater in correct trials than in error trials. This result is consistent with previous findings that long-range interactions between distant areas were often associated with subjects’ behavioral performance in various cognitive functions, including decision making (Nächer et al., 2013), short-term memory (Liebe et al., 2012), attention (Gregoriou et al., 2014), visual discrimination (Zhang et al., 2014), and learning (Igarashi et al., 2014). In the present study, however, the amplitude of the inter-area coherence itself was not significantly different between correct and error trials, although it was higher in correct trials than in error trials. One plausible interpretation is that in correct trials, a modulatory signal from other areas enhanced the coordination between coherent A36 spikes and gamma-frequency LFP at the SG of TE, whereas such modulatory signals was reduced in error trials. Accordingly, this modulatory signal would amplify the coupling between inter-area and translaminar processing during successful memory retrieval, without affecting the inter-area coherence itself. Further studies would be required to test if this interpretation be correct and to clarify the responsible brain areas for this modulatory signal.

It is known that inter-area signal transmission is implemented not only via direct cortico-cortical pathways but also by way of thalamic nuclei such as the pulvinar nucleus (Callaway, 2004; Saalmann et al., 2012). However, the laminar specificity for an impact of the pulvino-cortical pathway has not been functionally tested so far. Elucidating the effect of other pathways, including those via sub-cortical areas, on the coordination between inter-area and translaminar signal processing in the inferior temporal cortex will be an important issue for future studies to understand the mechanistic view of the brain-wide network operation for memory retrieval.

**EXPERIMENTAL PROCEDURES**

**Subjects**

All animal procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Review Committee of the University of Tokyo School of Medicine. The subjects were two adult macaque monkeys (Macaca mulatta, weighing 7.4–8.6 kg). Detailed procedures of surgeries for head holders and recording chamber are described in Supplemental Experimental Procedures.

**Behavioral Task**

The procedure for the pair-association task was as described in detail previously (Hirabayashi et al., 2013a, 2013b; Naya et al., 2001, 2003; Takeda et al., 2005; Takeuchi et al., 2011). In each trial, following the presentation of a fixation point for 500 ms, a cue stimulus (1 of the 24 visual stimuli) was presented for 500 ms. After a delay period of 2,000 ms (or 1,000 ms for a subset of data in monkey 2), two stimuli were presented, one of which was the pair associate of the cue stimulus and the other a distractor. The monkey was cued to remember the location of the cue stimulus. The performance of each of the two animals were 80.2% ± 10.1% (monkey 1) and 78.9% ± 8.3% in monkey 2. The performance rate was significantly higher than the chance level in both monkeys (paired t test, monkey 1, t = 14.9, p = 5.86 × 10⁻¹⁶; monkey 2, t = 10.4, p = 1.10 × 10⁻⁹).

**Electrophysiological Recordings**

Extracellular recordings were conducted using glass-coated tungsten electrodes (for A36) or linear-array multi-contact electrodes (U-probe, Plexon; for TE) (Maier et al., 2010; Schroeder and Lakatos, 2009; Takeuchi et al., 2011; Godlove et al., 2014; Spaak et al., 2012; Roberts et al., 2013) containing 16 recording channels (impedance 0.3–0.5 MΩ at 1 kHz) with an intercontact spacing of 150 μm (Figure 1B).

Neuronal signals were recorded using a Plexon MAP system. Each signal was separated into two signals with different band-pass analog filters, higher frequency spiking activities (SUAs; 250 Hz to 8 kHz), and lower frequency field potentials (LFPs; 3–88 Hz) (Nelson et al., 2008).

To obtain the estimation for the cortical depth of the electrode, we conducted a CSD analysis (Maier et al., 2010; Mitzdorf, 1985; Schroeder and Lakatos, 2009; Spaak et al., 2012; Takeuchi et al., 2011; Roberts et al., 2012; Bollimunta et al., 2011; Buffalo et al., 2011; Xing et al., 2012; Kombian et al., 2014) with the same procedure used by Takeuchi et al. (2011). At the end of each recording session, the anteroposterior (AP) and anteroventral (DV) coordinates of the electrode track were measured by X-ray imaging (Hirabayashi et al., 2010, 2013a, 2013b, 2014; Koyano et al., 2011; Naya et al., 2001, 2003; Takeda et al., 2005; Takeuchi et al., 2011), and the dorso-ventral (DV) coordinates were measured by manipulator readings. In eight penetrations (six penetrations in monkey 1 and two penetrations in monkey 2), electrolytic lesions were made along the electrode track with a spacing of 1.5 or 2.0 mm by passing a direct current (5–10 μA for 15–20 s) (Koyano et al., 2011; Matsui et al., 2007; Takeuchi et al., 2011). The lesion marks were identified by histological examinations following all recordings in each monkey.

**Histological Analysis**

Histological analysis was performed using our standard protocols (Koyano et al., 2011; Matsui et al., 2007; Takeuchi et al., 2011). Each brain hemisphere was cut coronally into 40 μm cryostat sections, collected into four series, and mounted onto slides. One series of sections were stained for Nissl with cresyl violet and coverslipped with Permound (Fisher Scientific). The border between A36 and TE or area 35 was cytoarchitectonically determined according to the criteria described in previous studies (Saleem et al., 2007; Suzuki and Amaral, 2003).

**Histological Estimation of the Recording Sites**

To estimate the positions of the recording site in the histological sections, each recording site determined by the X-ray-based coordinates was manually rigid-transformed into histological space with the help of the metal deposit positions, which were measured by both X-ray imaging and histological sections as in Koyano et al. (2011). Thirty-three metal deposits (14 marks for monkey 1 and 19 marks for monkey 2) marked at the inferior temporal cortex were used to minimize errors arising from global tissue distortion. Shrinkage rates of histological sections (5.9%–9.1%) were estimated for each monkey by comparing the distances of metal-deposit marks between the histological sections and X-ray images (Koyano et al., 2011). We then evaluated the difference between the coordinates of the metal deposits on the X-ray with manipulator readings and the coordinates on the reconstructed histological sections (registration error) (Figure S4A). The distribution of registration error was −0.06 ± 0.27 mm (AP), 0.03 ± 0.17 mm (LM), and 0.07 ± 0.19 mm (DV). After correcting for the tissue shrinkage, the localization accuracy of the recording sites was calculated as the mean distance between the position of the electrolytic lesion marks estimated by the above procedure and the position of the lesion marks actually found on the histological sections (Figures S4B–S4D). The distribution of the localization accuracy was −0.01 ± 0.27 mm (AP), 0.09 ± 0.16 mm (LM), and −0.01 ± 0.13 mm (DV).

**Data Analysis**

**Analysis of Single-Unit Responses in A36**

Stimulus selectivity of SUAs recorded in A36 was examined both during the cue period (70–570 ms from cue onset) and the delay period (200–2,000 ms from cue offset or 200–1,000 ms for a subset of data in monkey 1) using one-way ANOVA. In total, 68 neurons recorded in A36 (41 and 27 neurons for monkeys 1 and 2, respectively) exhibited significant (p < 0.01) stimulus selectivity both during the cue and delay periods (Naya et al., 2001, 2003; Takeda et al., 2005). Spiking activity in the trial with the optimal stimulus (the stimulus that elicited the largest response during the delay period; 75.9 ± 29.3 trials, mean ± SD) was used for the following analysis.
Estimation of Cortical Layers by CSD Analysis

We defined the channels showing an earliest current sink in the CSD profiles as the zero point of the CSD profiles, as in Takeuchi et al. (2011). We termed this channel ‘Gr’ (see Supplemental Experimental Procedures for details; see Figure S3 for population CSD). We aligned the channel showing the earliest current sink in the CSD profiles at the center of the histological Gr to locate each electrode channel onto the histological section. Channels superficial to the earliest sink channel were estimated to be located in the SG, and channels in deeper positions than the earliest sink channel were estimated to be located in the IG. Note that channels in “SG” or “IG” did not include the neighboring (<0.3 mm) channels on either side of the channel that exhibited the earliest sink (Takeuchi et al., 2011).

Coherence Analysis between A36 Spikes and TE LFPs

Coherence between A36 SUAs and TE LFPs was calculated with a multi-taper method (Mitra and Pesaran, 1999; Pesaran et al., 2008; Bokil et al., 2010) using Chronux toolbox (http://chronux.org) for MATLAB (The MathWorks). To assess the statistical significance of population data, a coherence spectrum of each data set was calculated using both the original and trial-shifted spike trains. The trial-shifted coherence spectrum was then subtracted from the original coherence spectrum to construct the shift-predictor-subtracted-coherence. We also evaluated the dependence of the coherence on the laminar position of the TE channel. For each data set, coherence for each TE channel was sorted as a function of the distance from Gr, which was identified by CSD analysis. We calculated the maximum coherence value in the frequency range of 9 to 88 Hz during the delay period in all TE channels and then determined one TE channel in which the coherence value was the maximum between TE channels. If the maximum coherence value for a given data set did not reach 3.5 SDs above the average of the coherence values calculated by shuffling the trial order (trial-shuffled control), then that data set was not included in the coherence analysis in Figure 2. The resultant 49 data sets out of 68 were used for analysis of the phase and stimulus selectivity of coherence (29 and 20 data sets for monkeys 1 and 2, respectively). Detailed procedures for the phase and stimulus selectivity of coherence were described in Supplemental Experimental Procedures.

PC Analysis and Cluster Analysis for Coherence Profiles

To analyze the relationship between coherence and its recording depth at a population level, we performed probabilistic PC analysis (PCA) (Tipping and Bishop, 1999) for inter-area coherence values at 9 to 25 Hz (coherence profile) (Figure 4A; n = 68 data sets). We classified the coherence profiles into subsets (clusters) according to k-means clustering, which was applied to the 13-dimensional PC space (the 3D subspace for PCs 1–3 is shown in Figure 4C) (Logothetis et al., 2010; Pfeffer et al., 2013). A three-way ANOVA for factors of clusters (1 and 2), layers (IG and SG), and monkeys (1 and 2) showed that there was no significant main effect of monkeys (F = 0.01, p = 0.93, for monkey effect).

The Gamma Power of TE LFP Triggered by Coherent and Non-coherent A36 Spikes

In each data set, the gamma power (Singer and Gray, 1995; Jia et al., 2013; Hirabayashi and Miyashita, 2014) of the LFP (30–88 Hz) was calculated for each TE channel of each data set. We examined the contribution of inter-area coherence to the coupling of A36 spikes with TE gamma power during the delay period as follows. The instantaneous amplitude and phase of the LFP were extracted by convolving the raw LFP with a complex Morlet wavelet transform (5 Hz resolution) (Jie et al., 2012). The resultant time-varying amplitude of gamma LFP was used as the gamma power envelope of the LFP (Spaak et al., 2012; Tort et al., 2010; Canolty et al., 2006; Fujisawa and Buzsáki, 2011; Tort et al., 2008). The STA of the gamma power envelope (STAγ) at the channel showing maximal gamma power (gamma-power channel) was then calculated between A36 spikes and TE LFPs as follows. For trials with the optimal stimulus, the instantaneous phase value of the TE LFP at the frequency of the maximum inter-area coherence between 9 and 25 Hz was defined as the relative phase of each A36 spike. Spikes firing at the phase ranging within ±14° of θ0(gmax) were extracted as coherent spikes, and those firing at the opposite quadrant were extracted as non-coherent spikes. STAγ was then calculated for coherent and non-coherent spikes separately, as the average of the gamma envelopes within ±150 ms from each spike. We normalized the STAγ by shuffle-predictor STAγ; the resultant Z score-transformed STAγ was then calculated to evaluate its temporal periodicity, and the average power in the 9 to 14 Hz frequency range (STAγ power) was compared between STAγ values calculated for coherent and non-coherent A36 spikes. A three-way ANOVA for factors of clusters (1 and 2), spikes (coherent and non-coherent), and monkeys (1 and 2) showed that there was no significant main effect of monkeys (F = 1.78, p = 0.19).

Error Analysis

We compared the STAγ in correct trials with that in error trials as follows. To reduce any possible contribution of trial number differences between correct and error trials to the calculated STAγ, we equalized the number of trials between correct and error trials by randomly removing correct trials (error trials, 20 ± 13.9 trials, mean ± SD). We then calculated STAγ in correct and error trials separately (Figure 7). In addition to the STAγ, we compared the firing rate during the delay period between correct and error trials in 13 recording sessions in which the spiking activity was recorded in channels located within ±2 channels of the gamma-power channel. All statistical tests used in the present study were two-sided, and the Bonferroni method was used to counteract the problem of multiple comparisons, unless otherwise stated.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and eight figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.03.047.

AUTHOR CONTRIBUTIONS

M.T. and Y.M. designed the experiment. M.T. performed experiments. M.T. analyzed the data. M.T., K.W.K., T.H., Y.A., and Y.M. wrote the paper.

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