Cortical connections to area TE in monkey: hybrid modular and distributed organization. Borra E., Ichinohe N., Sato T., Tanifuji M., Rockland KS.



Supplementary Figure 1: Set of 100 complex stimuli used for the investigation of object selectivity. The stimulus set conforms to previous studies from Tanifuji

Lab, and is chosen from different categories, including faces but also fruits and vegetables, plants, tools, stuffed animals, etc. (see Sato et al., 2008). In the optical imaging experiments, only the first 25 stimuli were used, while the full set of 100 was used for the electrophysiology recording.

Injection sites were chosen first on the basis of optical imaging. Then, to further characterize the response properties of activity spots, before injection, we recorded multi-unit activity (MUA). Object selectivity was determined for the average of 48 sites within a spot previously revealed by optical imaging (3 sites at 8 depths (in a 250 μ m progression from the cortical surface), x 2 separate days = 48). The average MUAs (avgMUAs) provided a measure of through-cortical depth responses, as reported more fully elsewhere (Sato et al., 2008).

Firing rates in response to human or monkey face stimuli were often significantly greater than the spontaneous firing rate (p<0.05), but none of the spots had a best preference solely for face stimuli (see Supplementary Fig. 2). Our criteria for a spot to be face-selective were: 1) that the monkey or human face was scored as the best stimulus; and 2) that the response to monkey or human face was uniquely preferred as the best stimulus (see Supplementary Fig. 2). According to these criteria, none of the spots used for tracer injections was selective for faces.

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Supplementary Figure 2



Supplementary Figure 2: Response characteristics of the 8 injected spots. The evoked responses of avgMUAs (vertical axis) are plotted against rank ordered stimuli (dotted line, where red dots indicate the best stimulus, or the group of stimuli whose responses were not significantly different from that of the best stimulus). Evoked responses are obtained by subtracting spontaneous firing from the firing rate during the stimulus presentation (Sato et al., 2008). The box outlined in red encloses the best single stimulus (for 4 of the spots), or the best stimulus (leftmost) AND any other stimuli whose evoked responses were not significantly different from that different from the best stimulus (leftmost) AND any other stimulus. Blue line and shaded area represent the mean and standard deviation, respectively.

As an example, for Spot C (M2), the responses evoked by monkey and human faces were significantly greater than the spontaneous firing rate, but the best stimulus was "water melon". The second best stimulus was "doll", but the response to "doll" was not significantly different from that to the best stimulus. Responses to faces, however, were significantly different (p<0.05) from the response to the best stimulus. As another example, for Spot A (M2), a monkey face was the best stimulus, but the responses to "padlock", "human face", and "doll", were not significantly different from those to the best stimulus. Thus, because of the two non-face stimuli, we did not consider this as a specifically face-selective spot.

Statistical tests were done in the following way. Since MUAs used to calculate avgMUA were not recorded simultaneously, we first assumed that trialto-trial variation of each MUA follows a normal distribution. Second, we assumed that the statistical property of each MUA did not change in time. Third, we assumed that the 48 MUAs recorded from a spot were statistically independent. Based on these assumptions, we estimated mean and standard deviation of the distribution of an avgMUA, calculated from those of individual MUAs. We

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estimated the distribution of avgMUA for each stimulus, and we applied t-test to the distribution to evaluate statistical significance for evoked responses (whether firing rate during the stimulus presentation period was significantly different from the spontaneous firing rate) and to determine all the stimuli for which the evoked responses were not significantly different.



Supplementary Figure 3

Supplementary Figure 3: Two coronal sections, stained by thionin, from a brain not used in this study, to indicate the approximate AP levels of the injection sites in TE, as indicated by arrowheads. A) Level just posterior to amts corresponds to location of the green injection in M2; B) Level between amts and pmts corresponds to the green injection in M1 (and compare lateral brain schemata in Figures 3 and 1, respectively). AMTS= anterior middle temporal sulcus; Cing= cingulate sulcus; CS= central sulcus; IPS= intraparietal sulcus; LS= lateral sulcus; OTS= occipito-temporal sulcus; STS= superior temporal sulcus. Scale bar=10 mm.

Supplementary Tables

M2 hotspot C*

| slide# | | bin1 | bin2 | bin3 | bin4 | bin5 | bin6 | bin7 | bin8 | bin9 | bin10 | bin11 | bin12 | bin13 | bin14 | bin15 |
|--------|-----|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|
| 227 | s/p | - | - | - | - | 1.00 | - | 0.50 | 0.25 | - | 0.20 | 0.20 | - | - | 0.25 | - |
| | %DL | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 223 | s/p | - | - | - | 0.50 | 0.50 | - | 0.33 | 0.50 | 0.67 | 0.67 | - | - | - | - | 0.20 |
| | %DL | - | - | - | - | - | - | 8% | 8% | - | - | - | - | - | - | - |
| 219 | s/p | - | - | - | 1.00 | 0.50 | 0.17 | 0.33 | 0.60 | 0.43 | 0.40 | - | - | - | - | - |
| | %DL | - | - | - | - | - | - | 11% | 20% | - | - | - | - | - | - | 11% |
| 215 | s/p | - | - | - | 0.67 | - | 1.00 | 0.40 | 0.50 | 0.80 | 0.29 | 0.29 | 0.30 | 0.06 | - | - |
| | %DL | - | - | - | - | - | - | - | 14% | 10% | 25% | 10% | - | - | 15% | 11% |

M1 hotspot A

| slide# | | bin1 | bin2 | bin3 | bin4 | bin5 | bin6 | bin7 | bin8 | bin9 | bin10 | bin11 | bin12 | bin13 | bin14 | bin15 |
|--------|-----|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|
| 63 | s/p | - | - | - | - | 1.00 | 0.50 | 1.00 | 0.67 | - | - | - | - | - | - | - |
| | %DL | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 62 | s/p | - | - | - | - | 0.67 | 0.83 | 0.42 | 0.80 | 1.00 | 0.33 | - | - | - | 0.07 | - |
| | %DL | - | - | - | - | - | - | - | 10% | 43% | 20% | - | - | - | - | - |
| 61 | s/p | - | - | - | - | - | 0.14 | 0.17 | 0.18 | 0.20 | - | - | - | - | - | - |
| | %DL | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 60 | s/p | - | - | - | - | - | 0.20 | 0.25 | - | 0.20 | 0.40 | - | - | 0.08 | 0.08 | - |
| | %DL | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

M1 Hotspot D

| slide# | | bin1 | bin2 | bin3 | bin4 | bin5 | bin6 | bin7 | bin8 | bin9 | bin10 | bin11 | bin12 | bin13 | bin14 | bin15 | bin16 | bin17 | bin18 | bin19 | bin20 |
|--------|-----|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 59 | s/p | - | - | - | - | - | - | - | 0.33 | 0.33 | 0.44 | 0.10 | - | 0.11 | - | - | - | - | - | - | - |
| | %DL | - | - | - | - | - | 25% | - | - | 11% | - | - | 20% | 6% | - | 7% | 14% | 8% | - | - | - |
| 58 | s/p | - | - | - | 0.20 | - | - | 0.30 | 0.43 | 0.38 | 0.07 | 0.07 | 0.56 | 0.70 | 0.50 | 0.15 | 0.09 | - | - | - | - |
| | %DL | 7% | 17% | - | - | - | - | 13% | 9% | 21% | 16% | 6% | 13% | 15% | 20% | 6% | 8% | - | 5% | - | - |
| 57 | s/p | - | - | - | - | 0.33 | - | 0.60 | 0.71 | 0.29 | 0.09 | 0.17 | 0.22 | 0.07 | 0.08 | 0.20 | 0.31 | - | 0.17 | 0.15 | 0.09 |
| | %DL | - | - | - | - | - | - | 11% | 8% | 10% | - | 13% | 21% | 20% | - | 14% | - | - | - | - | 8% |
| 56 | s/p | - | - | - | - | 1.00 | - | 0.40 | 0.25 | 0.11 | 0.30 | 0.60 | 0.86 | 0.38 | 0.57 | 0.10 | 0.18 | 0.13 | - | - | 0.14 |
| | %DL | - | - | - | - | - | 17% | 13% | 9% | 17% | 13% | 11% | 24% | 15% | - | 21% | 13% | 10% | - | - | - |
| 55 | s/p | - | - | - | - | 1.00 | 0.67 | 0.08 | 1.00 | 1.00 | 0.57 | 0.57 | 0.14 | 0.17 | 0.20 | 0.33 | 0.33 | - | 0.08 | 0.06 | - |
| | %DL | - | - | - | - | - | 17% | 7% | 11% | 9% | 27% | - | - | - | 10% | 11% | 20% | 8% | 7% | - | 5% |
| 54 | s/p | - | - | - | 0.25 | - | 0.50 | 0.67 | 0.80 | 0.50 | 0.33 | 0.17 | - | 0.13 | - | - | 0.33 | - | 0.11 | - | - |
| | %DL | - | - | 100% | - | 33% | 14% | - | - | 14% | - | 22% | - | 18% | - | - | - | - | - | - | - |
| 53 | s/p | - | - | - | - | 0.14 | 0.17 | - | 0.08 | 0.08 | - | 0.15 | 0.20 | 0.08 | - | - | - | - | - | - | - |
| | %DL | - | - | - | - | - | - | - | 7% | 7% | - | - | - | 13% | 25% | 8% | 22% | 14% | - | - | - |
| 52 | s/p | - | - | - | 0.17 | - | 0.13 | - | - | 0.07 | - | - | - | 0.20 | - | - | - | - | - | - | - |
| | %DL | - | - | - | - | 14% | - | - | - | - | - | - | - | - | - | 14% | - | - | - | - | - |

Supplementary Tables: Quantitative analysis of 3 selected hotspots, obtained by counts of red, green, and DL neurons through bins of 100 μ m width. Tables show results for three hotspots: A and D from M1 and C (C^{*} caudal part only) from M2. Bins were through the supragranular layers, perpendicular to the pial surface, and continued through several sequential sections (spaced 200 μ m apart).

For all the bins (columns within the Tables), we show the s/p ratio ("secondary" to "primary" color) of red and green cells, and the percentage of DL cells for individual sections (labeled in rows, at left). Results for bins that satisfied all 3 criteria (co-occurrence of red and green neurons, $s/p \ge 0.4$, DL $\ge 6\%$) are highlighted in red, while blue indicates bins where 2 of the 3 hotspot criteria were satisfied (i.e., bins where red and green were balanced, but there were no DL cells, or bins where DL, red, green cell were all present, but s/p < 0.4). Dashes: for s/p= only one color of cell was present in that bin; dashes for DL= 0%.

Supplementary Figure 4



Supplementary Figure 4: Quantitative analysis of 3 hotspots. Bar graphs show the number of green, red and DL cells (respectively green-, red-, or yellow-colored bars) plotted in each bin (100 μ m wide) in each section, for the same three hotspots as in the Supplementary Tables. Asterisks by specific bins and sections (as well as the whitened areas on the floor of the graphs) indicate where all the 3 criteria were satisfied (i.e., red highlight in the Tables). For M1 hotspot D, we used "red bins" to establish the outmost boundary. Thus, the whitened area includes some "blue bins" in the table (see the corresponding Supplementary Table).

Supplementary Figure 5



Supplementary Figure 5: Drawings of two representative coronal sections showing the distribution of retrogradely labeled neurons in the IPS of M3. The small coronal section drawing at the right is for orientation, and the dashed box indicates the zone (in the lower bank of the IPS) that corresponds to the two higher magnification chartings (a, b). The dorsolateral view of the injected hemisphere is repeated at the left, for further orientation. IAS= inferior arcuate sulcus; IOS= inferior occipital sulcus; IPD= infraprincipal dimple; LuS= lunate sulcus; PMTS= posterior middle temporal sulcus; PS= principal sulcus; SAS= superior arcuate sulcus. Other abbreviations as in Supplementary Fig. 3.