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Extension of corticocortical afferents into the anterior bank of the intraparietal sulcus by tool-use training in adult monkeys

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Abstract

When humans use a tool, it becomes an extension of the hand physically and perceptually. Common introspection might occur in monkeys trained in tool-use, which should depend on brain operations that constantly update and automatically integrate information about the current intrinsic (somatosensory) and the extrinsic (visual) status of the body parts and the tools. The parietal cortex plays an important role in using tools. Intraparietal neurones of naïve monkeys mostly respond unimodally to somatosensory stimuli; however, after training these neurones become bimodally active and respond to visual stimuli. The response properties of these neurones change to code the body images modified by assimilation of the tool to the hand holding it. In this study, we compared the projection patterns between visually related areas and the intraparietal cortex in trained and naïve monkeys using tracer techniques. Light microscopy analyses revealed the emergence of novel projections from the higher visual centres in the vicinity of the temporo-parietal junction and the ventrolateral prefrontal areas to the intraparietal area in monkeys trained in tool-use, but not in naïve monkeys. Functionally active synapses of intracortical afferents arising from higher visual centres to the intraparietal cortex of the trained monkeys were confirmed by electron microscopy. These results provide the first concrete evidence for the induction of novel neural connections in the adult monkey cerebral cortex, which accompanies a process of demanding behaviour in these animals.

Keywords: Intraparietal cortex; Temporo-parietal junction; Corticocortical connections; Neuronsal labelling; Monkey

1. Introduction

The human brain constantly updates and automatically integrates information about the ongoing intrinsic status of the body parts (such as body shape and posture) and their extrinsic status (forms of actions in space, interactions with external objects, etc.) and thereby creates representations of the current bodily state in the space, or the body schema (Head & Holmes, 1911). Using external objects as tools is a complex motor skill that requires integration of visuospatial information about the body and the tools, to match their ever-changing relationships, with cognitive components such as problem solving and planning for forthcoming actions. Thus, repeated manipulation of a tool leads humans to form an experience-dependent psychological image to incorporate the tool into the original body schema as an extension of the body parts holding it (Head & Holmes, 1911; Paillard, 1993). For example, when we touch something with the tip of a probe (tool) held by the hand, we feel the contact at its tip, although it is actually sensed by somatosensory receptors on the hand holding the tool. Such psychological phenomena should be attributable to the existence and modification of body representation elsewhere in the brain. The parietal cortex is a strong candidate for the storage of such representations, because patients with parietal lesions exhibit disrupted body representation and a variety of cognitive disorders, including extinction and asomotognosia. Indeed, the posterior parietal cortex of monkeys is likely to be the site that integrates somatosensory and spatial vision information. That is, cortical processing of somatosensory information is processed starting from the postcentral somatosensory cortex and going

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caudally towards the posterior parietal cortex (Iwamura, 1998; Iwamura, Iriki, & Tanaka, 1994; Iwamura, Tanaka, Sakamoto, & Hikosaka, 1993). Conversely, visual information related to spatial components is processed from the occipital visual cortex anteriorly through a dorsal pathway to reach the posterior parietal cortex (Ungerleider & Mishkin, 1982). Thus, information related to the body parts from various sensory areas should be integrated in this vicinity to subserve the formation of subjective bodily representations. Once these representations are formed on tool-use, neurones in the above-described cortical area would be expected to change their mode of integrating bodily information to incorporate the tools into neuronal representations of the body schema.

In our previous studies, 2 weeks of training enabled monkeys to use a stick to rake in pieces of food placed out of reach (Iriki, Tanaka, & Iwamura, 1996; Ishibashi, Hihara, & Iriki, 2000; Maravita & Iriki, 2004). By combining such behavioural studies with single neurones recording, we have suggested that monkeys develop a perspective, as with humans, that the tool is incorporated into their body schema as an extension of the forearm and hand. In monkeys trained in tool-use, a group of bimodal neurones (that respond both to somatosensory and visual stimuli related to the hand) in the anterior bank of the intraparietal sulcus dynamically altered their visual receptive field properties (the region where a neurone responds to certain visual stimuli) in accordance with the characteristics of the tool at hand. That is, only after tool-use, were the visual receptive fields of intraparietal neurones, originally covering the space only around their hand, extended to include the space at the tip of the tool. These newly acquired multisensory (visual, tactile and proprioceptive) integration properties would match each monkey's introspection, estimated from their behaviour, in regarding the tools as extensions of their own body parts.

In contrast, in naïve or untrained monkeys, there are very few conspicuous bimodal neurones in this area. That is, most of the intraparietal neurones of naïve monkeys usually responded unimodally (somatosensory mode). Visual input is weak, if at all, and at most, it modified the intensity of somatosensory responses when combined with visual stimuli (Iwamura et al., 1993). However, in those monkeys in which tool-use learning was complete, neurones in this area became easily activated by both somatosensory and visual stimuli, and were able to adaptively change their response characteristics in accordance with the properties of the tool at hand. Thus, the mode of visual inputs onto the intraparietal area might be modified by 2 weeks of rather demanding training for monkeys to use tools, enabling this area to code the subjective image of the hand explicitly. As a result, the response properties of the neurones can be modified to match an ongoing behavioural situation of whether a tool should be incorporated into their body schema.

Besides these neurophysiological findings, we have shown that the expression of immediate-early-genes, such as c-Fos, Zif-268, brain-derived-neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), increased significantly during the training period, but not after the training was completed. This was found at a specific location at the anterior bank of the intraparietal sulcus (IPS) behind the SI-shoulder to forearm representation, which exactly corresponds to the area where the above-described bimodal neurones were found in monkeys after training (Ishibashi et al., 2002a, 2002b). Based on these results, we hypothesized that this cortical reorganization (reformation and reintegration) might be induced during this demanding task for the monkeys in this particular cortical region to cause electrophysiological changes and induce molecular processes (e.g. protein synthesis). In particular, a projection pattern arising from visual-related areas onto the intraparietal cortex is strongly suggested. In normally behaving adult animals, variant experiences (including acquisition of new motor skills and sensory experience) trigger physiological or neurochemical changes in the nervous system. However, changes to axonal projection patterns have rarely been reported, except for those induced during recovery processes after cortical (Dancause et al., 2002) or peripheral (Florence, Taub, & Kaas, 1998) injuries. Therefore, in this study, we attempted to confirm that demanding training in normal adult monkeys induces such morphological changes.

To clarify the changes of neural circuitry that might be induced by learning how to use tools, ideally one should compare changes in connections in the same animal. However, this is impractical using tracer technique. Instead, it is necessary to compare labelled connections between two different groups of animals: one group not training and one after the training. This might lead to errors in the placement of injection sites and variations in the amount of injected tracers between different groups of animals. To overcome these difficulties, we attempted reciprocal labelling to establish, as clearly as possible, the sprouting of novel neuronal connections. First, to search for the source of visual information from the dorsal stream to the somatosensory-related anterior bank of the IPS, we injected a retrograde tracer into this region and compared the distribution of labelled cell bodies between tool-use trained monkeys and control monkeys across the entire cortical hemisphere. Second, to visualize the reformation of axons in the IPS, we used anterograde tracer injections in the area in which retrogradely labelled cells were found in the trained but not the naïve animals in the first experiment. We reconstructed single axons in the anterior bank of the IPS by light microscopy, and further observed their terminal fields to search for anterogradely labelled active synapses using electron microscopy.

2. Methods

2.1. Monkey training and tracer injection schedules

Nine young adult Japanese monkeys (*Macaca fuscata*, weighing 3.2–7.0 kg) were used in this study. For retrograde mapping, five monkeys were injected with a fluorescent tracer in the anterior bank of the IPS posterior to the somatosensory forearm regions. Four monkeys were injected with an anterograde tracer in the temporo-parietal junction (TPJ) areas that displayed distinct retrograde labelling only after training (see Section 2.3 for exact location of injections and Section 3.2 for labelling). In each experiment, animals were classified into a tool-use trained group or an untrained (control) group; injections were made to only one hemisphere of the brain of each monkey. Five of the monkeys (three for retrograde tracer studies and two for anterograde tracer studies) were trained to

use a rake-shaped tool to retrieve a piece of food placed out of reach, as described previously (Iriki et al., 1996; Ishibashi et al., 2000). After 3 weeks of training, monkeys became over-trained for the task, and surgery for the tracer injections was performed. Injections were made into the hemisphere contralateral to the hand trained to use the tool. After 1-week of recovery, the training was continued over another 3 weeks, thereby allowing the tracer to be transported through axons under conditions that permitted the monkeys to maintain their skill in using the tool. The monkeys were then anaesthetized and perfused for neurohistochemical analyses. Four monkeys were used as controls. These monkeys underwent the same procedures, however, they were not trained before or after surgery. The Animal Care and Use Committee of the Tokyo Medical and Dental University and the Experimental Animal Committee of the Riken Institute approved this study.

2.2. Retrograde labelling by intraparietal injection

2.2.1. Retrograde tracer injection

Two trained (FT1 and FT2) and three untrained monkeys (FC1, FC2 and FC3) were injected with a retrograde tracer, Fast Blue (FB; 3.3% in saline; Sigma catalogue F-5756; Sigma Chemical Co., St. Louis, MO, USA). With the monkeys under pentobarbital anaesthesia (50 mg/kg, i.v.), the tracer was injected through a Hamilton microsyringe into two locations at 7 and 9 mm medioposterior from the rostrolateral tip of the IPS (Fig. 1A, blue dots). The injection points corresponded to the region posterior to the postcentral forearm representation, which is at the centre of the distribution of bimodal neurones that change their visual receptive field properties in accordance with the characteristics of the tool at hand and where modified body images are found (Iriki et al., 1996; Iriki, Tanaka, Obayashi, & Iwamura, 2001). In the cytoarchitectonic map of the parietal cortex by Lewis and Van Essen (2000b), this area corresponds to the anterolateral portion of area 5 V, anterolateral to the medial intraparietal area (MIP) and ventral intraparietal area (VIP), and anteromedial to the anterior intraparietal area (AIP). Although the corticocortical connections of the surrounding intraparietal area have been extensively analysed (Lewis & Van Essen, 2000a; Luppino, 2005; Rizzolatti, Luppino, & Matelli, 1998), detailed examinations of the connections in this particular area have not been conducted. For each injection, the tip of the needle was inserted into the anterior bank of the IPS 1 mm from the sulcus, aiming at a depth 6 mm from the lip of the sulcus, and 0.5 μL of FB solution was gradually delivered over 30 min (Fig. 1B). The injection needle was maintained in this position for 30 min before retracting. No electrophysiological recording of these bimodal neurones was attempted to verify the points of injection because these bimodal neurones in trained animals are sparsely scattered in the vicinity of this area without distinct boundaries. In addition, the neurones could not be recorded in control animals. Later histological examination showed that the injection sites were between 5.6 and 10.8 mm below the surface of the pia, and were confirmed to be restricted to the grey matter with sites 1.2–2 mm in diameter. No leakage of the retrograde tracer into the white matter was detected (data from two animals in which leakage into the white matter was suspected were discarded from the present analysis and thus not included in the final number of animals used). Injection sites also corresponded to the area where the expression of BDNF is selectively induced in trained monkeys (Ishibashi et al., 2002a, 2002b).

2.2.2. Histochemistry

After a 3-week period of training or rest (controls), two trained monkeys and three control monkeys were euthanized with an overdose of sodium pentobarbital (50 mg/kg i.v.). Under artificial respiration with 100% oxygen, the monkeys were transcardially perfused with 1 L of saline (with heparin-HCl as anticoagulant) bubbled with oxygen, followed by 4 L of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Each whole brain was removed from the skull and stored in 10% (for 24 h), then 20% (48 h), and finally 30% (48 h) sucrose solution at 4 °C to prepare for cutting frozen sections. The entire hemispheres ipsilateral to the injection site were cut into serial 50 μm thick coronal sections and mounted on gelatin-coated slides. The other sections were saved for Nissl staining for later fluorescent study. Locations of the retrogradely labelled cell bodies and injection sites were plotted and reconstructed using an MD-Plot system (Minnesota Instruments, MN, USA) mounted on the fluorescent microscope (excitation wavelength 360 nm).

2.3. Anterograde labelling by injection of the temporo-parietal junction

The retrograde labelling results (see Section 3.2) suggested that novel corticocortical connections were formed between the injection site (intraparietal cortex) and the cortical region around the caudal end of the superior temporal sulcus (STS) at the zone of junction between the temporal and parietal cortices, namely the temporo-parietal junction. However, because the TPJ and IPS are about 15 mm apart, it is unlikely that axons grew through the entire distance from the cell body to the target IPS area during only a few weeks of training. Instead, it is reasonable to assume that the projections existed close to the IPS before the training and that their branches grew during training, possibly over a rather short distance, to form new synapses in the intraparietal cortex. To test this assumption, anterograde tracer was injected into the TPJ area to explore the possibility of axonal sprouting in the intraparietal cortex using light microscopy. Possible synapse formation was confirmed by electron microscopy.

2.3.1. Anterograde tracer injection

Two trained monkeys (BT1 and BT2) and two control monkeys (BC1 and BC2) were anaesthetized with pentobarbital sodium (50 mg/kg, i.v.). Biotinylated dextran amine solution (BDA; 10% in distilled water; Molecular Probes catalogue D-7135, 3000 MW; Molecular Probes, OR, USA) was microinjected into the TPJ area, which showed different to retrograde labelling patterns in trained and control animals. To ensure the injection sites matched the location of the unique retrograde labelling in trained animals, the characteristic morphology of the STS at its caudal end (Fig. 2A, arrow) was used as a guide, although no electrophysiological recording was employed (refer to Fig. 1 in Fujita, 1997). That is, in many, but not all, Japanese macaque monkeys, there tends to be a short 'branch' of sulcus towards the parietal cortex (Fig. 2A, arrow), which can be a reliable landmark of this cortical location. Only monkeys having this evident branch were used for anterograde injection studies. After identifying this 'branch', the injection needle of a Hamilton syringe was held perpendicular to the brain surface and penetrated 1 mm anterior to this 'branch' at two locations, one 1.5 mm distant from the bifurcation point and the other 2 mm further from the first location (Fig. 2A, red dots). Injections were made 4 mm below the surface. For each injection, 1 μL of BDA solution was gradually delivered over 30 min and the needle was maintained in the same position for 30 min before retracting. Later histochemical examination showed that all injection sites were restricted within the grey matter and formed patches 3.8–4.5 mm below the surface of the hemisphere (Fig. 2B). According to the occipital cytoarchitectonic map of Lewis and Van Essen (2000b), this region corresponds to the area in between 7a, MSTda and TPOc.

2.3.2. Light microscope histochemistry

During the 4-week period after surgery, trained monkeys continued to perform tool-use tasks as they did before the injection, whereas control animals were not trained. Monkeys were then anaesthetized and perfused as described above. After perfusion, the blocks containing the IPS were sectioned with a vibrating microtome, and the block including the injection site was sectioned using a freezing microtome. For visualizing BDA, sections were left for 4 h in ABC solution (ABC *Elite*, 1:50 dilution in phosphate buffered saline (PBS) Vector Laboratories, CA, USA) at room temperature and then reacted for 10 min in a solution containing 0.05 M Tris–HCL (pH 7.6), 0.04% diaminobenzidine tetrahydrochloride, 0.04% $NiCl_2$ and 0.003% H_2O_2 (Ojima & Takayanagi, 2004). The reaction was stopped by repeated washes in PBS and sections were mounted on glass slides for light microscopy examination. Sections that contained BDA-positive terminals were further processed for electron microscopy (see below). Serial section reconstruction of individual axons was performed by camera lucida drawings.

2.3.3. Electron microscopy

Selected portions of sections in the anterior bank of IPS of a trained monkey containing BDA-positive nerve terminals and boutons confirmed by light microscopy were cut into 2 mm^2 square pieces. In the control monkey, because no anterograde fibres were detected in this area, pieces of tissue from a corresponding portion of the IPS bank were taken. Sections dissected from both animals were post-fixed in 1% osmium tetroxide in 0.01 M phosphate buffer

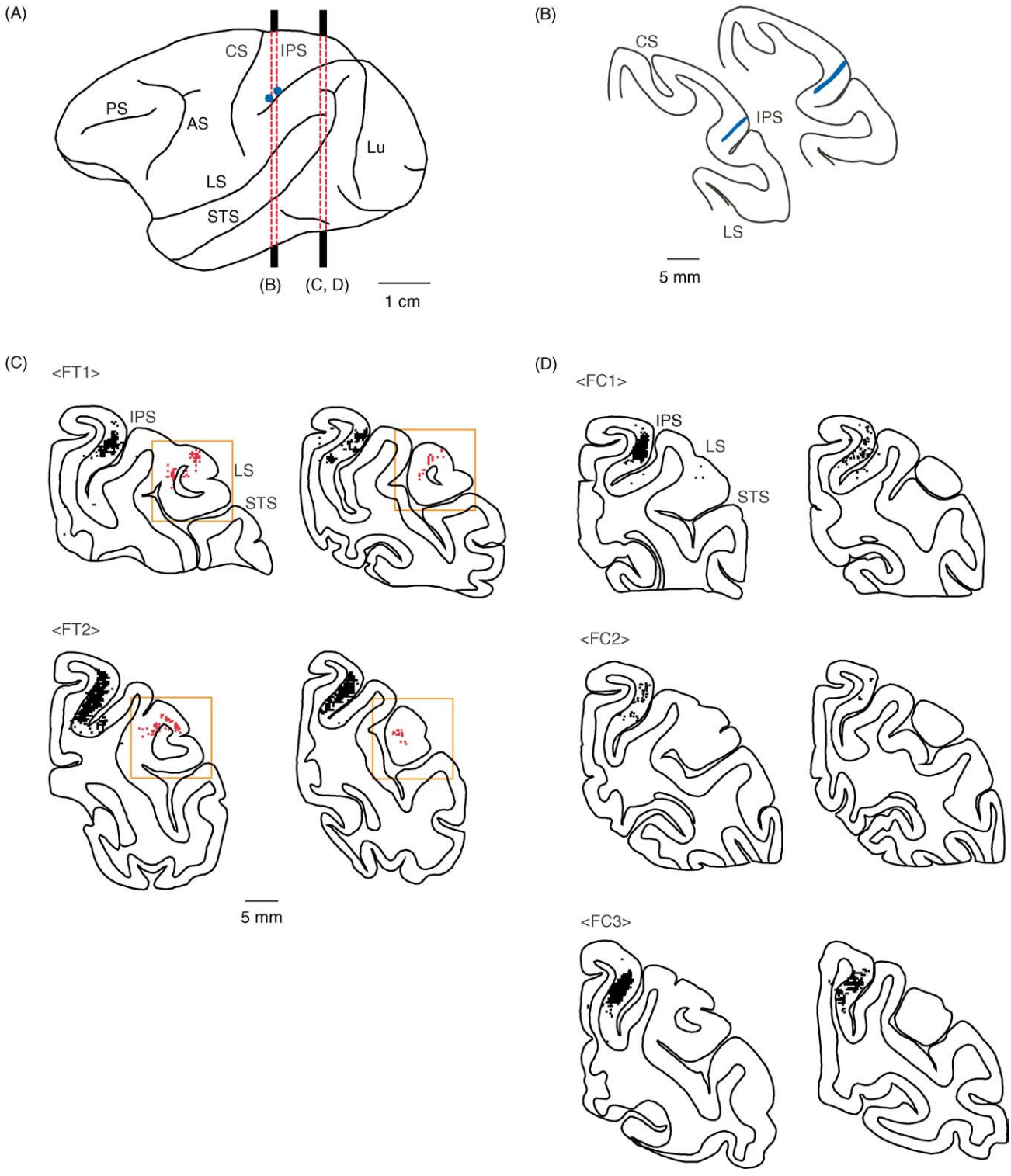

Fig. 1. Injection sites of Fast Blue (FB) and the distribution of retrogradely labelled cells at the temporo-parietal junction area. (A) Injection sites of FB (blue dots). Two injections were made into the anterior bank of the IPS corresponding to the posterior representation of the forearm in the primary somatosensory area. The bars indicate the coronal planes shown in B (left bar) and in C and D (right). *Abbreviation* of sulci: AS, arcuate sulcus; CS, central sulcus; IPS, intraparietal sulcus; Lu, lunate sulcus; LS, lateral sulcus; PS, principal sulcus; STS, superior temporal sulcus. (B) Coronal sections illustrating a representative example of the centres of two injection sites (monkey FT1). All injections were found within the bank of the IPS and restricted to the grey matter. (C and D) Coronal sections showing the temporo-parietal junction area of trained (C; monkeys FT1 and FT2) and control monkeys (D; monkeys FC1, FC2 and FC3). Each red dot represents one labelled cell, and plots from adjacent sections covering a thickness of 1.25 mm are superimposed. Note that distinctly labelled cells were consistently found among the temporo-parietal junction in trained monkeys (squares), but not (except for rare ambiguous labelling; FC1 left section) in untrained controls.

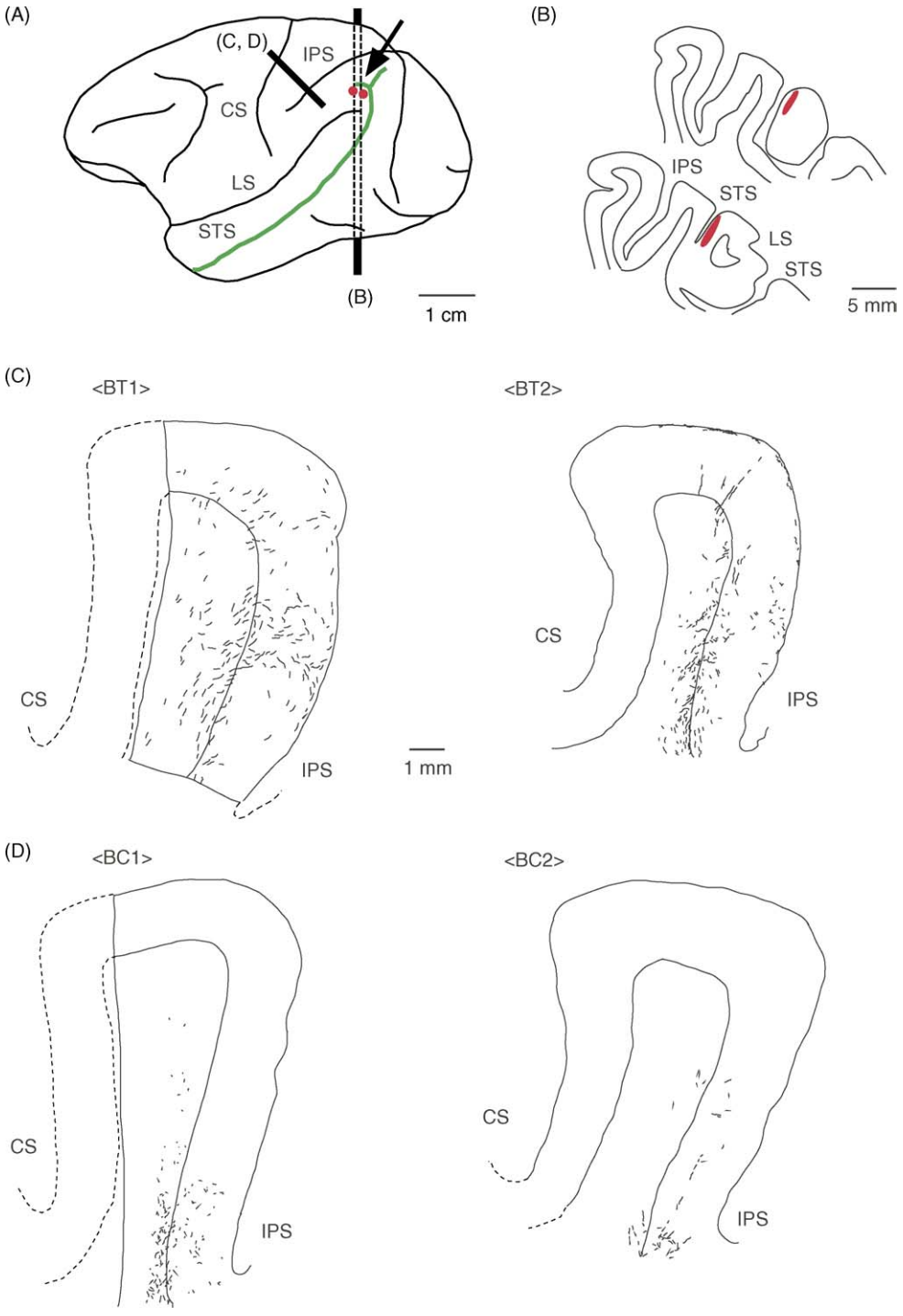

Fig. 2. Sites of biotinylated dextran amine (BDA) injections and distribution of anterogradely labelled fibres. (A) Schematic drawing of the macaque brain from a lateral view. Red dots indicate injection sites. STS is highlighted in green, and the characteristic branch, which was used as landmark for identifying injection sites (see text), is at the posterior end indicated by an oblique arrow. The vertical bar indicates the coronal section shown in B and the oblique bar indicates the sections of the postcentral gyrus orthogonal to the IPS shown in C and D. (B) Coronal sections of injection sites in the TPJ area, in the most caudal part of the upper bank of the posterior portion of the STS where it branches. (C) Distribution of anterogradely labelled fibres in the anterior bank of the intraparietal sulcus, posterior to the SI forearm regions. Camera lucida-based drawings from serial sections have been superimposed. BT1 and BT2 are tissues from trained monkeys on which sections covering a thickness of 250 μm have been superimposed. (D) Distribution of anterogradely labelled fibres in control monkeys (BC1 and BC2) on which sections covering a thickness of 500 μm have been superimposed. Note the difference in thickness of sections between C and D, indicating a much denser labelling of fibres in the trained animals. In the left panel of C and D (BT1 and BC1), anterior portions of the postcentral gyrus, which were cut-off during preparation of tissues, have been supplemented by dashed lines to give better morphological understanding.

(pH 7.4). They were then dehydrated in a graded series of alcohol and propylene oxide and embedded in epoxy resin (TAAB, Epon 812). Ultrathin sections were cut on a Reichert microtome (Ultracut OmU-4, Reichert AG, Austria) and double-stained with uranyl acetate and lead citrate. About 7 mm^3 of tissue blocks from trained monkey and about 4 mm^3 of tissue blocks from control monkey were processed for ultrastructure examination. Sliced sections were observed with an electron microscope (JEM-1010, JEOL, Tokyo, Japan), from which digital images were taken at a resolution of 756 × 581 pixels.

3. Results

3.1. Retrograde labelling by intraparietal injection

Among the forebrain areas, there was denser labelling in trained monkeys compared with untrained monkeys in the lateral and mesial motor-related cortical areas (regions F1–7, BA23 and 24). However, there was always some sparse labelling in control animals. Among the cortical areas where visual inputs were expected, the overall labelling patterns appeared qualitatively similar between trained and control monkeys, we found prominent levels of labelling in somatosensory-related regions, that is, the postcentral gyrus (occupied by BA3, 1 and 2), the bank of the IPS around the injection site, and the secondary somatosensory area and insula (granular and dysgranular insula). Labelling was also found in the posterior parietal cortices as follows: (1) in the adjacent bank of the IPS, including the VIP, lateral intraparietal area (LIP), MIP and AIP and (2) the inferior posterior parietal lobule, which corresponded to the 7a, 7b and 7ab areas, according to the cytoarchitectonic map of the parietal cortex by Lewis and Van Essen (2000b). Quantitative comparisons were not drawn for these regions at this point because the aforementioned technical limitations required the animals to be different.

3.2. Unique retrograde labelling of cells in trained monkeys

The distribution of labelled cells in the TPJ area differed between the two groups of monkeys: no labelling, except for very rare ambiguous and vague labelling in one case, was found in the untrained monkeys (FC1–3; Fig. 1D). However, consistent labelling was found in the trained monkeys (FT1 and FT2) in the upper bank of the posterior STS at its caudal end (Fig. 1C, red dots in the area shown by squares). The peculiar 'branch' of the STS, as described earlier, enabled this area to be reliably compared between animals because frontal sections across this area form a characteristic isolated piece of cortex, as illustrated in Fig. 1C and D. According to the occipital cytoarchitectonic map of Lewis and Van Essen (2000b), this region corresponds to the area in between 7a, MSTda and TPOc, which is considered to be a part of the visual motion processing areas in monkeys. Thus, projections from this area might be a source of visual information feeding into the intraparietal area after training to use tools.

In addition to the above-described TPJ area, we found a few consistent regions of labelling in the ventral prefrontal cortex of trained animals FT1 and FT2 (Fig. 3B), whereas no labelling was found in control animals FC1–3 (Fig. 3C). These areas were focused in the ventral portion of the principal sulcus, across a 2.5–3.0 mm area of region BA46.

3.3. Anterograde labelling of axonal branches

Many anterogradely BDA-labelled fibres and terminal boutons were found in trained and untrained animals, at the fundus of the anterior bank of the IPS. In contrast, no retrogradely BDA-labelled cell bodies were found in these areas, whereas many anterogradely labelled fragments of fibres and boutons, as well as sparse distribution of retrogradely BDA-labelled cell bodies, were found in the posterior bank of the IPS among the anterior portion of the 7b area in all monkeys. Fig. 2C and D illustrate the distribution of labelled fibres in the anterior bank of the IPS. The control monkey brains (Fig. 2D, monkeys BC1 and BC2) have been illustrated by superimposing serial sections extending across a thickness of 500 μm. The brains of trained monkeys (Fig. 2C, monkeys BT1 and BT2) are illustrated at a thickness of 250 μm because much denser labelling was evident in the trained animals. Labelled intracortical fibres and terminals of control animals were mainly found among deeper layers (layers 4–6) at the fundus of the IPS, whereas those of trained animals were found invading the superficial layers (layers 2–3) of the fundus and also towards the lip and the crown of the postcentral gyrus. Thus, the overall distribution patterns of labelled fibres differed between the two groups. Differences were evident in the region corresponding to the area where bimodal neurones that code for modified body image by tool-use were recorded, and where BDNF production was found in our previous studies (Iriki et al., 1996, 2001; Ishibashi et al., 2002a, 2002b).

3.4. Distribution of synaptic terminals

We performed camera lucida reconstructions of individual single axons from serial sections of the brains of one trained (BT2) and one control monkey (BC2). From the trained monkey, three single axons were almost completely reconstructed after their entry from the white matter to the grey matter (Fig. 4A–C). Their terminal fields spread among the superficial layers. All axons of the trained monkey had several collaterals and ascended to the lip of the sulcus or close to the crown. They branched among various layers and decreased in diameter as they ascended through the cortical layers. Although arbours of deep layers were thick with a small number of boutons, those of the superficial layers ramified into increasingly thinner axons and formed a large numbers of *en passant* boutons. Most of these axons terminated in layer 1 with small boutons arranged at irregular intervals.

In contrast, only one axon was identified in the control animal (Fig. 4D), which ascended to one-third of the IPS depth at most; it terminated in the deep layers, had some branches and very few boutons at the bottom of the fundus. We could not reconstruct other axons in the anterior bank of IPS of the control animal because they were confined to the fundus of the sulcus, as described above.

In parallel with single axon reconstruction, we attempted to detect labelled boutons with electron microscopy in the superficial layers of the IPS of trained (BT1) and control (BC1) monkeys. We confirmed reaction end products that formed asymmetric synapses (Grey's type 1) with the spine and were packed with synaptic vesicles together with postsynaptic densities (Fig. 5B),

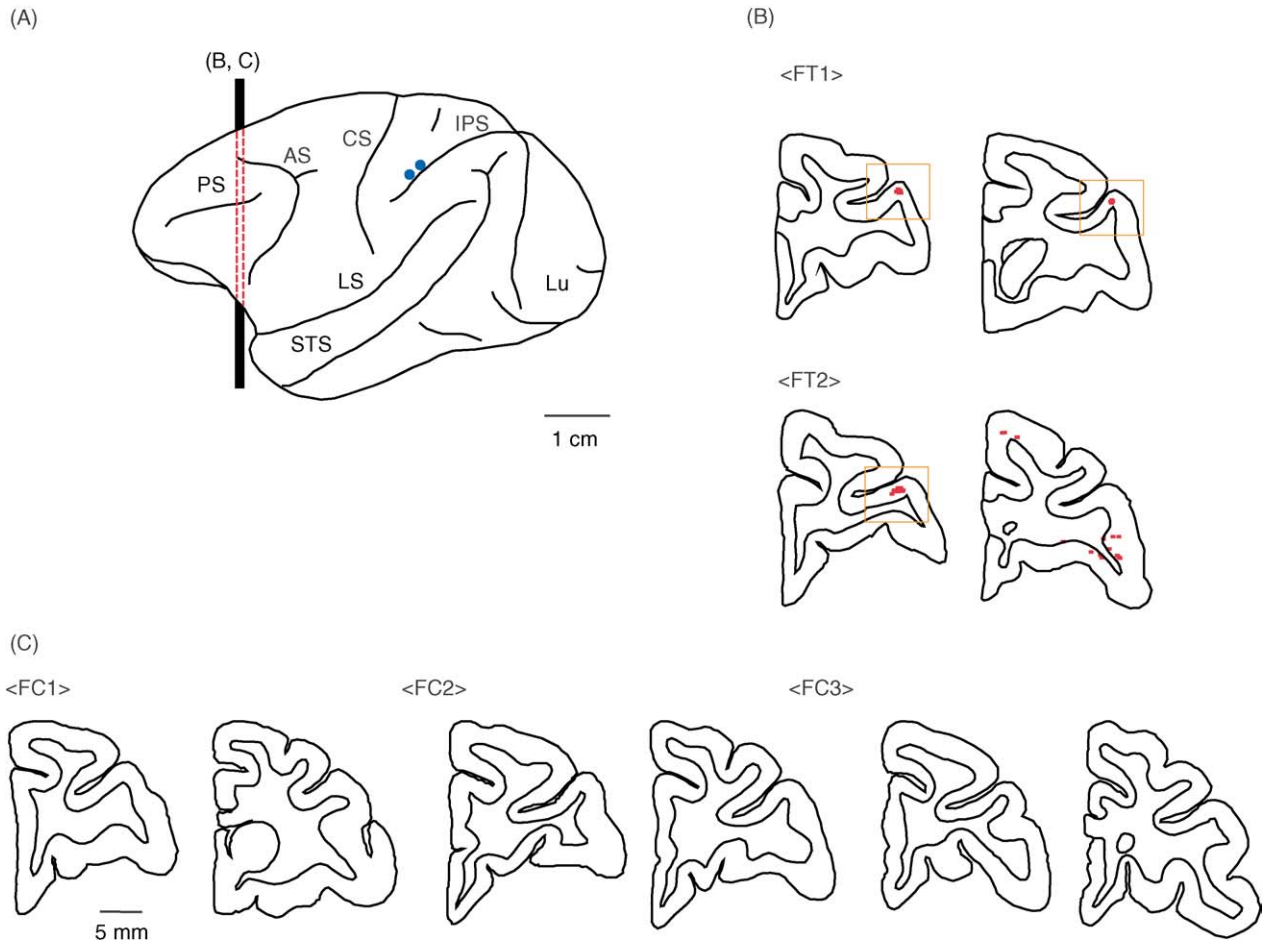

Fig. 3. Distribution of retrogradely labelled cells in the prefrontal cortex. (A) Blue dots indicate two FB injections, as shown in Fig. 1A. The bar indicates the coronal planes shown in B and C. (B and C) Coronal sections including the prefrontal cortex of trained (B) and control monkeys (C). Each red dot represents one labelled cell, on which plots from adjacent sections of 1.25 mm thickness have been superimposed. Note that labelled cells were consistently found in the prefrontal cortex in trained monkeys (squares), but not in controls.

corresponding to the boutons detected by light microscopy as described above. No labelled synapses were detected in this portion of cortex in the control monkey, in which boutons were not observed by light microscopy. This confirmed the formation of active excitatory synapses after tool-use learning.

4. Discussion

4.1. Large-scale sprouting of corticocortical afferents in adulthood

In the adult primate brain, large-scale reorganization of axonal projections has long been believed not to occur in natural conditions once development is complete. Reorganization of axonal projections in the adult monkey cerebral cortex is reportedly induced only during the processes of recovery, or compensation after injury elsewhere in the brain (Dancause et al., 2002) or the periphery (Florence et al., 1998). Thus, the adult monkey cortex has a hidden ability for additional axonal growth and sprouting during emergency situations; however, such large-scale reorganizations of neural circuitry in the intact brains of animals undertaking normal behaviour have not been shown.

In the barn owl midbrain, learning auditory localization induces axonal remodelling related to visuo-auditory integration (Knudsen, 2002). Thus, as these authors have suggested, during natural behaviour, at least in birds, those actions that involve multiple senses for skilful execution can induce axonal remodelling. This suggests that a novel mode of multisensory integration that could participate in the mechanisms of learning processes. The present results provide the first concrete evidence for the induction of novel neural connections in the adult monkey cerebral cortex, which accompany a process of demanding behaviour for the subject.

In the studies reported here, there is a possibility that novel labelling occurred because axonal transport (both retrograde and anterograde) was activated by training in axons that existed before the training but were not active or functioning. At this point, we cannot completely exclude this possibility without demonstrating anatomical signs of on-going growth of axons (such as growth cones), which we could not identify in the trained monkey brains. However, we can assert that connections, at least functionally, were newly induced by tool-use learning, which enables interaction between these areas. However, judging from our previous molecular biology data that these changes

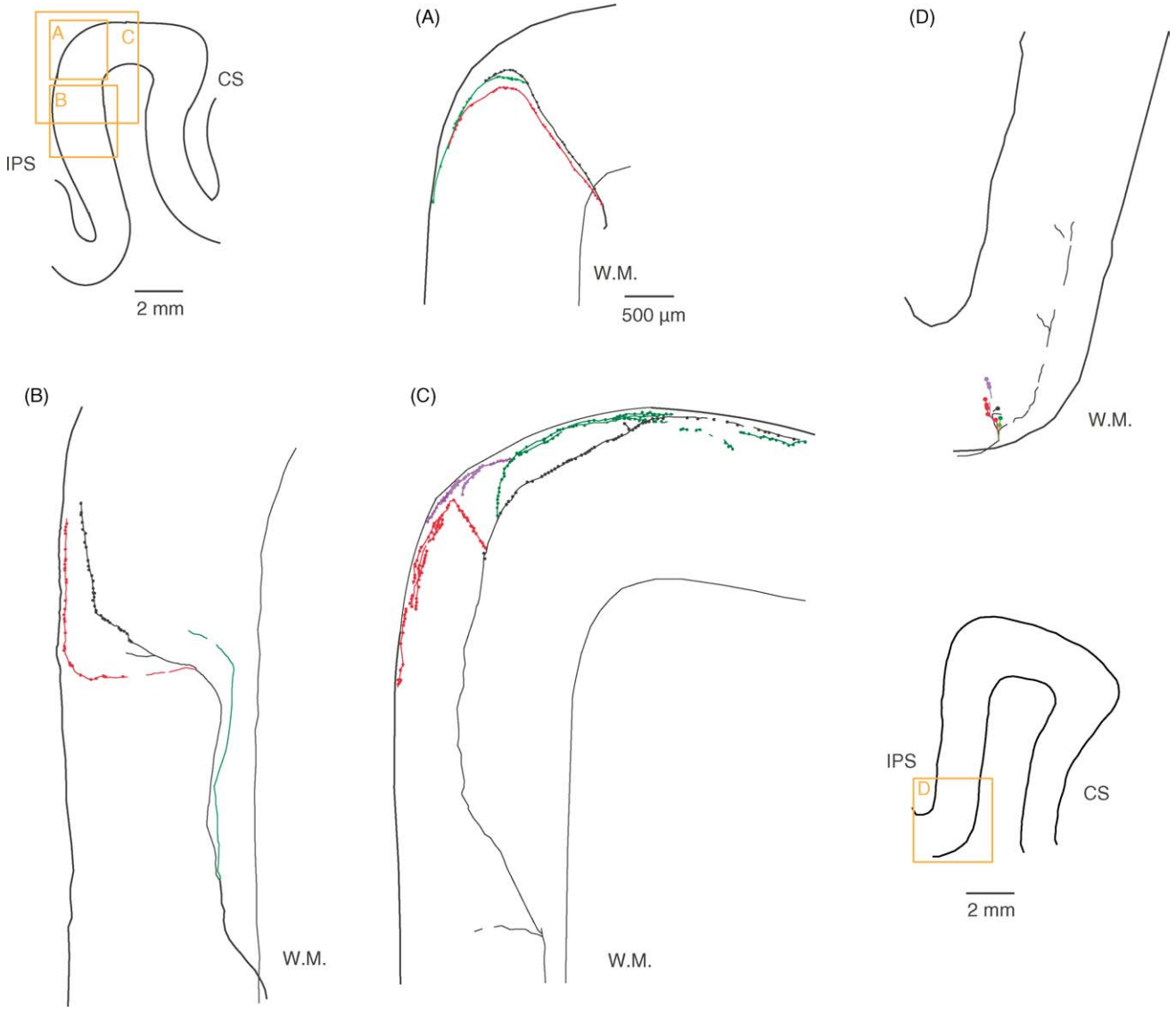

Fig. 4. Camera lucida reconstructions of anterogradely labelled single axons in trained (A, B and C) and control (D) monkeys. Top left inset: representative section orthogonal to the intraparietal sulcus, with squares illustrating the location of axons shown in A, B and C. (A) A single axon at the lip of the intraparietal sulcus in a trained monkey (BT2) reconstructed over 1300 μm of thickness. Different colours indicate different branches and each dot represents one bouton (common throughout B–D). (B) A single axon in the bank of the intraparietal sulcus from a trained monkey (BT2) reconstructed across 2250 μm. (C) A single axon in the area 2/5 from a trained monkey (BT2) reconstructed across 4550 μm. These axons (A–C) had large terminal fields that spread among the superficial layers. (D) A single axon in the fundus of the intraparietal sulcus from a control monkey (BC2) reconstructed for 850 μm. Compared with the three axons from trained animals, this axon had fewer branches and boutons and ended within the deeper layers at the fundus of the sulcus. No boutons could be identified on the branch that extended up through the deeper layers. Bottom inset illustrates the location of the axon shown in D.

are accompanied by expression of neurotrophic factors, which very well be an indicator of reformation of neural network wirings, we should like to suggest that concrete morphological changes (including growth and sprouting of new branches and synapses) could be potentially be induced by the training and learning processes of the normal adult brain.

4.2. The temporo-parietal junction region as a source of visual input to the intraparietal area

The present reciprocal study shows that corticocortical afferents arising from the area in the vicinity of the TPJ, which might originally project towards the fundus of the IPS extend their axons into the shallower portion of the anterior bank of the IPS behind the SI forearm representation regions. This portion of the IPS corresponds to the area in which we have reported bimodal neurones that code for extended images of the hand/arm towards the tool after rather demanding tool training (Iriki et al., 1996, 2001; Maravita & Iriki, 2004). This area corresponds to PEip (Luppino, 2005; Rizzolatti et al., 1998) or 5 V (Lewis & Van Essen, 2000b) in the anteromedial bank of the IPS, posteriorly adjacent to the shoulder-to-forearm representation region of the postcentral somatosensory cortex. Because this area is considered part of the higher somatosensory centres, and thus its visual response is rarely observed in naïve monkeys (Iwamura, 1998; Iwamura et al., 1993), less attention has

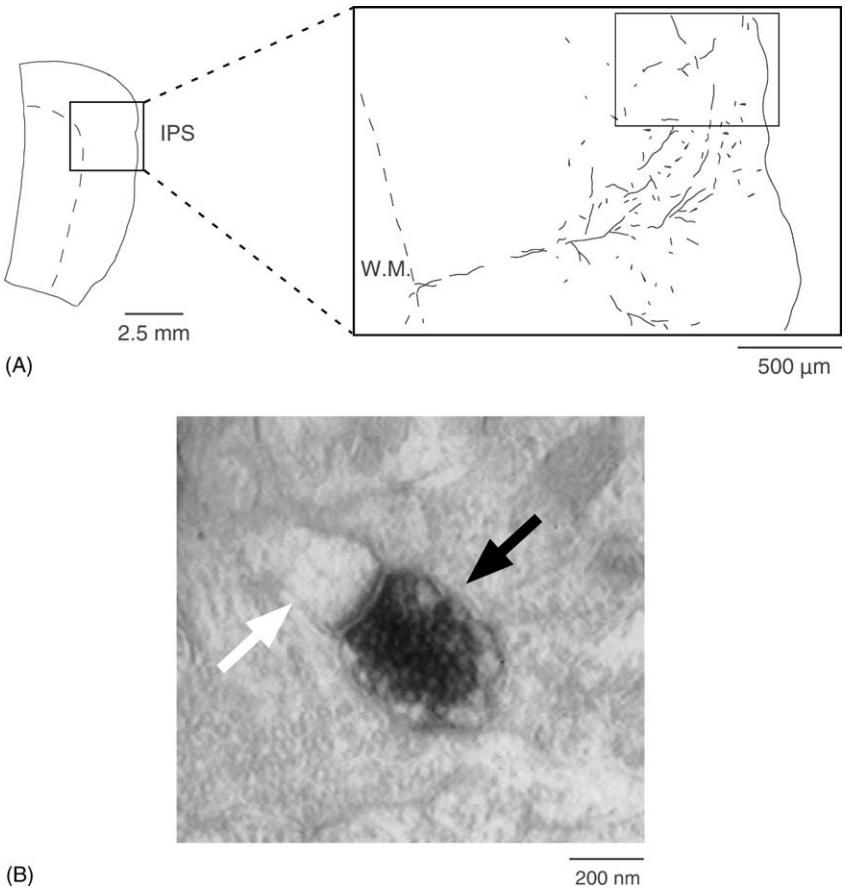

Fig. 5. Ultrastructure of the BDA-positive boutons identified in a trained monkey. (A) Reconstruction of an anterogradely labelled (presumably single) axon through 1050 μm in the anterior bank of the intraparietal sulcus. The square in the right enlarged inset indicates the portion of the tissue that was dissected and processed for electron microscopy. (B) Ultramicrograph of an anterogradely labelled terminal (filled arrow) in layer II, making an asymmetric synapse with the dendritic spine (open arrow) of a postsynaptic neurones.

been paid to the corticocortical connection of this area with visual-related cortical centres. The present study provides evidence that a some extension of axonal branches may occur with demanding tool-use training, which has been shown to induce expression of neurotrophic factors, particularly in this cortical area during the period when monkeys acquire the ability to use the tools (Ishibashi et al., 2002a, 2002b). The present study also shows that these axons form extensive terminal fields, with active synapses over superficial layers in the monkey brain after tool training, and would be a source of visual information that codes body image and its modification when using tools.

The above-described corticocortical afferents arise from the TPJ area. We could not identify this cortical area by cytoarchitectonic analyses because the characteristic branch of the STS, which enables reliable orientation of brain sections, as described earlier, is cut in frontal sections of the brain, thus making laminar analyses ambiguous. In addition, because electrophysiological recordings were not attempted on these neurones, it was difficult to identify to which higher visual centres this area corresponds. However, the characteristic branch of the STS reliably suggests that this area corresponds to the region caudally adjacent to the MSTda and TPOc, and laterally adjacent to area 7a in the map of Lewis and Van Essen (2000b). From data shown in the connection study by Lewis and Van Essen (2000a), cortical areas adjacent to the presently identified TPJ region appear to project to the VIPm and LIPv, which are located at the fundus and posterior bank of IPS. This is in agreement with the anterograde labelling results of naïve monkeys in the present study. By training monkeys to use tools, these axons would extend into the anterior bank of the IPS at the anteromedially neighbouring structure. The neuronal characteristics of bimodal neurones in the intraparietal areas of naïve monkeys, as well as the IPS anterior bank of trained monkeys, can be explained by this new projection. That is, visual information provided to this IPS area is related to location and motion covering a wide range of the space around the body. Although, represented information in the VIP is heavily related to the face, mouth and head, and in the MIP to the arm (Colby & Duhamel, 1991), whereas in the anterior bank of the IPS in tool-use trained monkeys, information is related to the spatial extension of the hand/arm and tool (Maravita & Iriki, 2004). These visual response properties may correspond to those represented in the higher visual centres located in the vicinity of the TPJ area, which are shown as a source of visual input in the present study. That is, assuming this area is located close to the MST complex as describe above, neurones that extend their projections to the anterior IPS after tool-use training should have large receptive fields and convey visual information related to location and motion in space, which would eventually contribute

to the guidance of movements of the body in space (Orban, 1997; Zeki, 1993).

Because this connectivity does not exist in naïve monkeys and appears after tool-use training, this may represent a function in addition to those necessary in the wild environment. Furthermore, in humans, this area of the cortex appears to be particularly expanded compared with monkeys (Orban, Van Essen, & Vanduffel, 2004). Thus, the present source of input in monkeys might be a precursor to some additional function that has evolved in humans. Human fMRI data suggests, although not directly, a potential role for the TPJ. A study on the human cortex by Orban et al. (2004), suggests that this area in the human cortex corresponds to the posterior portion of angular gyrus (BA39) at the TPJ. Although the TPJ is not well defined, human fMRI studies suggest that this area is related to self monitoring and monitoring of other agents in comparison with the self (Decety, Chaminade, Grezes, & Meltzoff, 2002; Gallagher & Frith, 2003; Saxe & Kanwisher, 2003). When the TPJ is damaged or stimulated in human patients, they occasionally experience unusual feelings of 'autoscopy': seemingly observing themselves from outside their own body (Blanke, Landis, Spinelli, & Seeck, 2004; Blanke et al., 2005), thus 'objectifying' the bodily structures of the self (Mitchell, 1993). Tool-use might require this input to 'objectify' that part of the body, regarding it as equivalent or to assimilate the tool (object) into the body image. Thus, how can tool-use learning drive interactions between the TPJ and the intraparietal cortex? A potential implication would be that the tool is seen as an extension of innate body parts and induces a temporary mismatch with an existing body image stored in the intraparietal region, and thus requires recalibration driven by the monkey's own intention to incorporate the external object (tool) into the internal representation of its body. These functions may not be established simply through bottom–up processes, rather, they may require top–down control, suggesting that combination with other sources of information may occur, possibly that from the prefrontal area. The present study suggests this possibility, which will be argued in the next section.

4.3. Role of projections from the prefrontal cortex in tool-use

Although connection between the prefrontal cortex and the intraparietal cortex in trained monkeys has been suggested only by retrograde labelling in the present study, we can speculate on its functional roles. Disruptions to the human prefrontal cortex cause various behavioural deficits, including those for short-term memory, decision-making and social interactions (Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994; Fuster, 2001; Pascual-Leone, Grafman, & Hallett, 1995; Passingham & Sakai, 2004). Similar symptoms have been reported in monkeys (Funahashi, 2001; Tanji & Hoshi, 2001), and the activities of the prefrontal neurones of monkeys include temporal memory (Funahashi, Chafee, & Goldman-Rakic, 1993), categorizing visual objects (Miller, Nieder, Freedman, & Wallis, 2003), monitoring ongoing behaviour (Fujii & Graybiel, 2003), deciding future acts (Matsumoto, Suzuki, & Tanaka, 2003) and predicting behavioural results (Leon & Shadlen, 1999; Tsujimoto & Sawaguchi, 2005). These data suggest that the prefrontal cortex is able to process various forms of contextual parameters that might be essential for the completion of immediate behaviours.

Therefore, what could be expected to happen when the prefrontal cortex acquires access to the intraparietal cortex, where body image modifiable with tool-use is stored? Our recent studies suggest that monkeys benefit from prior motor memory of tool-use in situations where flexible modifications are required. In addition, previous tool-use learning could develop into more complicated and abstract skills, such as the combination of two different tools (Hihara, Obayashi, Tanaka, & Iriki, 2003; Obayashi et al., 2002). This task could be learned within a day, if a basic single-tool-use task has been previously acquired. A series of positron emission tomography studies in monkeys performing the above tasks detected brain activation patterns that involved the prefrontal and intraparietal cortices. Thus, prefrontal activities may relate to the degree of cognitive demand for complex tasks. The strength of prefronto-intraparietal connectivity during the original tool-use learning, as shown in this paper, may be a prerequisite for monkeys to produce such cognitive flexibility.

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