Journal of Neuroscience Methods, 20 (1987) 115–129
Elsevier

NSM 00685

Deoxyglucose mapping in the cat visual cortex following carotid artery injection and cortical flat-mounting

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(Received 3 March 1986)
(Revised 29 December 1986)
(Accepted 30 December 1986)

Key words: Deoxyglucose; Arterial injection; Cortex; Visual; Flat-mount; Cat

Two techniques are described for improving the efficiency of the deoxyglucose metabolic mapping procedure for studies on the cat visual cortex. The first technique involves the bilateral cannulation of the lingual arteries and the symmetrical injection of 2-deoxy-D-[U-^{14}C]glucose in amounts significantly smaller than required with systemic intravenous administration. The second technique is carried out at the end of the stimulation period and involves unfolding the grey matter of the occipital region of the unfixed cortex by blunt dissection (defibrillation) and cutting of the white matter to make a cortical flat-mount: this permits the preparation of large sections parallel to the cortical laminae and thus the interpretation of deoxyglucose uptake patterns in any one lamina over a large area of the visual cortex. The experiments are relatively cheap and the time required to flat-mount the cortices does not seem to produce any significant decrease in spatial resolution of the autoradiograms. In appropriate experiments (published elsewhere) the techniques allow a comparative analysis of the deoxyglucose patterns between hemispheres receiving different visual stimulation

Introduction

When applied to studies on the cat visual cortex, the major limitations of the 2-deoxyglucose (2-DG) mapping technique are due to the high cost of the ^{14}C-labeled 2-DG and the time required to reconstruct the patterns of 2-DG uptake over extensive regions of the visual cortex. This is particularly true when the patterns of activity in the left and right visual cortices are to be compared. In order to improve the 2-DG mapping technique for studies on the cat visual cortex we developed procedures so that: (i) less radioactive material was required per animal and, (ii) the

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total pattern of the orientation columns in each hemisphere could be reconstructed and compared efficiently.

We attempted the first aim by slowly injecting small amounts of 2-deoxy-D-[U-^{14}C]glucose (2-DG) bilaterally into the external carotid arteries thereby reducing loss of the tracer to other organs as occurs with systemic or intravenous bolus injection (Oldendorf, 1971; Oldendorf et al., 1982). Since it was important in these experiments to compare the patterns of labeling seen in the two visual cortices, the tracer was injected symmetrically via two identical systems of cannulae, 3-way taps and syringes.

The second aim was approached by developing a method for unfolding and flattening the unfixed cat visual cortex prior to freezing the tissue for sectioning in a cryostat. In contrast to earlier studies in the cat in which the 2-DG patterns were reconstructed from many serial sections (Albus, 1979; Flood and Coleman, 1979; Singer, 1981; Singer et al., 1981; Albus and Sieber, 1984), our flat-mount technique allows the demonstration of 2-DG patterns in at least areas 17, 18 and 19 on single autoradiographs from cryostat sections in which most of the section is cut parallel to the cortical surface and lies within a particular lamina. Compared to the previously published flat-mounts of cat striate cortex (Tootell et al., 1981), our unfolding protocol allows the visualization of substantially larger cortical areas including some extrastriate regions. Recently, Tootell and Silverman (1985) and Olavarria and Van Sluyters (1985) have independently developed techniques for flat-mounting the monkey and cat cortex, that are — in several aspects — similar to our procedures. The different approaches will be compared in the Discussion.

Methods

Surgery

For bilateral arterial injection of the deoxyglucose tracer, adult cats from the Institute's colony were anaesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (Ketanest 50 mg/ml, Parke-Davis; dose 0.2 ml/kg) and xylazine hydrochloride (Rompun 5%, Bayer; dose 0.15 ml/kg). Neosynephrine (Ursapharm) and atropine (Dispersa) were instilled into the conjunctival sacs and the corneas were covered by clear contact lenses. A T-junction tracheal cannula was inserted into a mid-level tracheostomy and a cephalic vein was catheterized by venesection. A rectal thermistor was inserted and the cat was laid upon an electric heating blanket. Following resection of the ventral neck musculature and sectioning of the transverse (facial) vein (Crouch and Lackey, 1969) the lingual arteries were mobilized for about 1 cm by ligating and cutting finer branches to the hyoid and pharyngeal musculature. Each lingual artery was cannulated with a tapering cannula prepared by stretching 1.0 mm outside diameter polyethylene tubing (Portex) over a gentle Bunsen flame and cutting it at a slightly acute angle.

Each cannula was cut to the same length and connected to a standard plastic 3-way tap (B. Braun, Melsungen) which in turn was joined to a second 3-way tap as shown in Fig. 1. Each lingual artery was cannulated, the tube being tied around this artery so that the tip just protruded into the lumen of the external carotid artery.

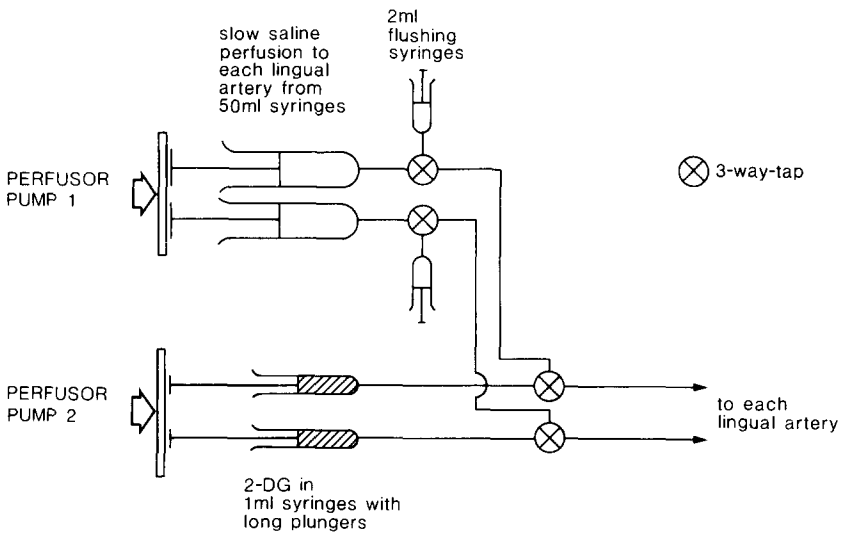


Fig. 1. Schematic diagram showing connections of syringes and taps required for lingual artery cannulation and injection of 2-DG.

Bubbles and clotting were avoided during insertion by rinsing the cannula with a solution of 100 IU Heparin in 1 ml saline and by connecting each cannula to a 50 ml syringe reservoir of non-heparinized saline in a dual perfusor pump (B. Braun, pump 1, Fig. 1) so that saline was continually delivered from the cannulae at a rate of 0.6 ml/h. Smaller flushing syringes (2 ml) were mounted on each of two 3-way taps (Fig. 1); these also facilitated the removal of bubbles from the injection system.

During surgery, anaesthesia was maintained with intravenous injections of a mixture of 3 parts ketamine to 7 parts Ringer solution. All wound margins were injected with xylocaine (Astra) and xylocaine aerosol was periodically sprayed on the wounds during the experiment. The cat was initially mounted in a stereotaxic frame in the usual way. After alignment of the head, a metal bar was attached to the skull with dental cement (Paladur, Grill&Grill) so that the eye, ear and mouth bars could be removed thus avoiding pain at pressure points.

The cat was then paralysed by an intravenous injection of a bolus (2–5 ml) of hexcarbacholinbromide muscle relaxant (Imbretil, Hormon-Chemie München GmbH; dose 5 ml in 45 ml of Ringer) and artificially respired with a mixture of 30% oxygen and 70% nitrous oxide. The muscle relaxant was then infused slowly at a rate of 3 ml/kg/h and the expired pCO_2 level was maintained in the range 3.5–4.5%. The rectal temperature was maintained at 38° C. The lung pressure was monitored on the inspiratory inlet (typically 4–10 mbar) and if the pressure was excessive (over 20 mbar) the cat was "sighed" or mucus was sucked out of the trachea with a small plastic catheter.

In some experiments cats were artificially fed overnight via an intragastric catheter with a glucose–Ringer solution (Laevulose-5%; dose 4 ml/h) and the

electroencephalogram from the occipital region of the brain was monitored with silver-ball electrodes inserted through dental burr holes in the posterior part of the skull and cemented in position with the ball touching the dural surface. Any pronounced decrease in the heart rate during the experiment was treated with an intravenous injection of orciprenalinsulphate (Alupent, Boehringer; dose 0.1 ml/kg).

Tracer injection

Prior to injection of 2-DG, the axes of the refracted eyes were aligned with wedge prisms which conjugated the projection points of the two areae centrales on the tangent screen 57 cm from the cat and equalized the position angles of the two blind spots (Bishop et al., 1962). The cat was dark-adapted while two 1 ml syringes with long plungers were each loaded with 0.4 ml of 2-deoxy-D-[U-^{14}C]glucose (Amersham, spec. act. 310 mCi/mmol) which had been previously evaporated overnight under a slow stream of nitrogen gas and redissolved in Ringer solution. The two syringes were attached to the 3-way taps closest to the animal (Fig. 1) and were mounted in a second dual perfusor pump. In initial experiments we used high doses of 2-DG at 50 μCi/kg (Fig. 5), but by gradually lowering the dose we were able to achieve excellent cortical labeling with 17.4 μCi/kg in a 2.3 kg animal (Fig. 6).

About 5–10 min, before the injection of the 2-DG, the state of the animal was checked (EEG, ECG, expiratory pCO_2, absence of pressure points, etc.) and all wounds were again treated with xylocaine. Then the nitrous oxide/oxygen mixture was turned off and the locally anaesthetised animal was ventilated on room air.

Visual stimulation by back projection onto the tangent screen was commenced and the pump driving the 2-DG syringe plungers was started to provide a positive system pressure at the moment of switching the two 3-way taps to commence the tracer injection. The symmetrical injection of the deoxyglucose was at the rate of 1.7 ml/h and was therefore completed after about 14 min. During this time the slow Ringer injection was diverted into the 2 ml flushing syringes.

At the end of the 1 h period of stimulation (i.e. 46 min after the slow 2-DG injection) the cat was killed by an overdose of Nembutal injected intravenously. In most experiments the fresh brain was removed for flat-mounting as soon as possible after repositioning the ear, eye and mouth bars, pulling off the skull bar and opening the cranium with small rongeurs. In some experiments the dead cat was perfused transcardially with 600 ml of saline and then 600 ml of 1.25% glutaraldehyde in 0.1 M phosphate buffer prior to removal of the brain.

Cortical flat-mounting

The diencephalon was blocked and frozen immediately on a cryostat chuck mounted in a block of dry ice while the two occipital regions of the hemispheres were blocked and dissected to make flat-mounts of the visual cortices as described below. The time taken to completely freeze the flattened cortices from the death of the animal was usually in the range 20–30 min.

The cortices are blocked and separated after a coronal section is made at about AP + 10 (Figs. 2A and 3A). Each block is placed on a sheet of parafilm on a 50 × 75 mm glass slide and the two cortices are flat-mounted simultaneously by two people

working separately. Drops of saline are used sparingly to prevent tissue dehydration during the dissection. Stages in the flat-mounting procedure are illustrated in Fig. 2 which is based upon the dissection of the glutaraldehyde-fixed brain of a cat used in a different experiment (made available to us by H. Luhmann), rather than the fresh tissue obtained in most 2-DG experiments. This is because it was thought unwise to let the taking of the photographs lengthen the flat-mounting procedure in an actual experiment and because no major difference was noted between the 2-DG uptake patterns of the cortices of non-perfused versus perfused animals (unpublished observations of the authors; L. Martinez-Millan, personal communication).

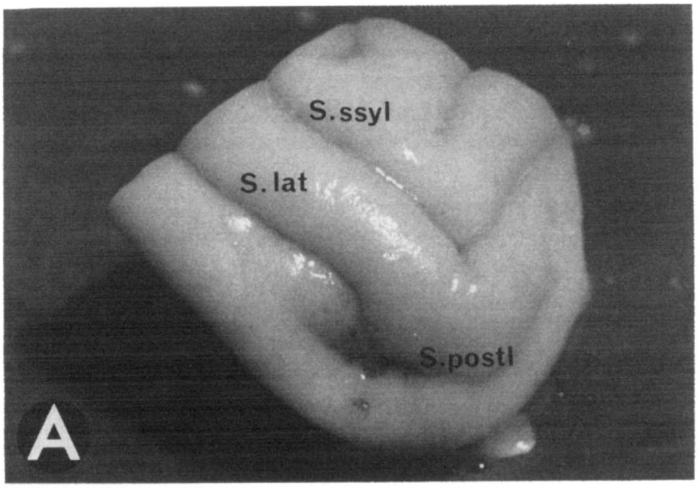
In the first step of the unfolding procedure the arachnoid and pia mater is cut with small scissors along the lateral, posterolateral, suprasplenial, splenial, suprasylvian and posterosuprasylvian sulci (Fig. 2A, B). All the strands of pia are gently pulled from the gyral surfaces using No. 7 jeweller's forceps. Thereafter all adjacent gyri are carefully separated from each other by gradually probing the sulci and splaying their walls with small metal spatulas (Fig. 2C). Any remaining pia is then removed from the exposed walls of the sulci.

For the next step, the block is turned over so that the cortical surface lies on the parafilm and the white matter is exposed for dissection. Using combinations of spatulas, jeweller's forceps, scissors and sharpened wooden sticks, the white matter is incised parallel to the fibre bundles and bluntly dissected by defibrillation in the manner described by Hultkrantz (1935), until the deepest layer of the cortical grey matter is just appearing under the remaining white matter (Fig. 2D). By continuing this dissection along the planes of the fibres in all the white matter, the subcortical bracing of the neopallium is cut and the grey matter can be deconvoluted (Fig. 2E), even in the most curved regions of the cortex without the need for perpendicular scalpel cuts across the cortical surface.

A second sheet of parafilm and a second slide is placed over the specimen and after removing any excess saline the tissue is flat-frozen by placing the "sandwich" of the two glass slides and cortex onto a smooth block of dry ice and applying gentle pressure to the top slide. The whole procedure is summarized in Fig. 3. The cortical flat-mount is usually 1.5–2.0 mm thick. In the case of tissue perfused with glutaraldehyde (e.g. Fig. 2), it was necessary to apply slightly greater pressure to overcome the increased rigidity of the tissue and to achieve the same degree of flattening as obtained with fresh tissue.

The "flatness" and gyral pattern of such an unfixed flat-mount is illustrated in Fig. 4. The entire brain of this cat (made available to us by M. Schmidt) was dipped in 1% Cresyl violet solution for 3 min prior to unfolding (modified after Olavarria and Van Sluyters, 1985), a method that selectively stains the surfaces of the gyri. Fig. 4D demonstrates that on the surface of the unfrozen flat-mount only minor undulations occur: the cortex being slightly thicker in stained regions compared to unstained regions.

Immediately after freezing, the sheets of parafilm are peeled off the flat-mount which is then frozen to a rapidly cooling pool of water on the face of a large-diameter cryostat chuck mounted in a hole in a second block of dry ice. It is critical during this mounting to avoid any air bubbles being trapped between the large flat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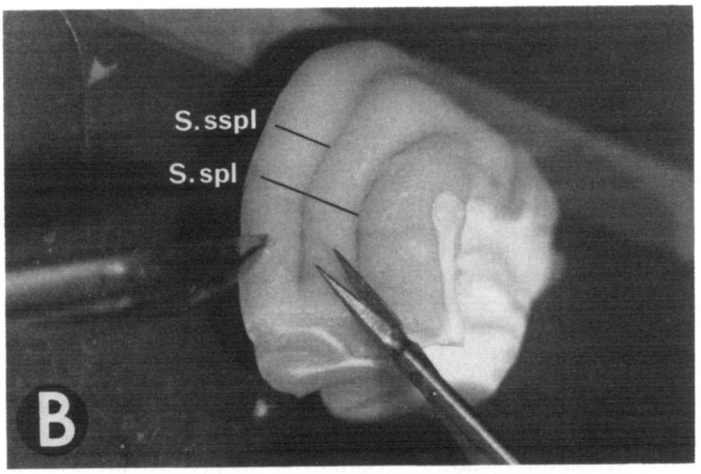
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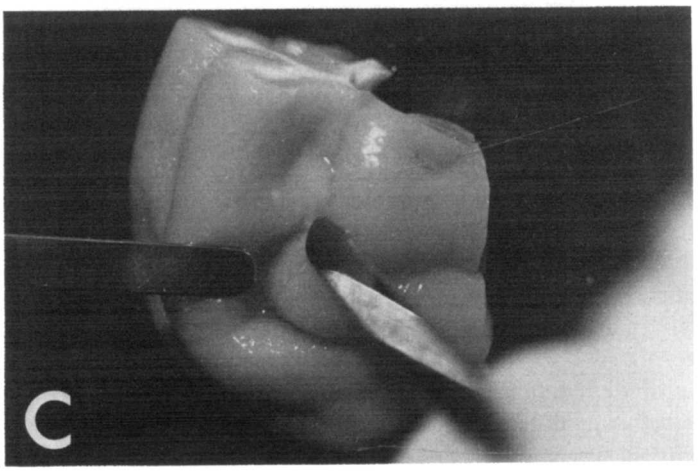
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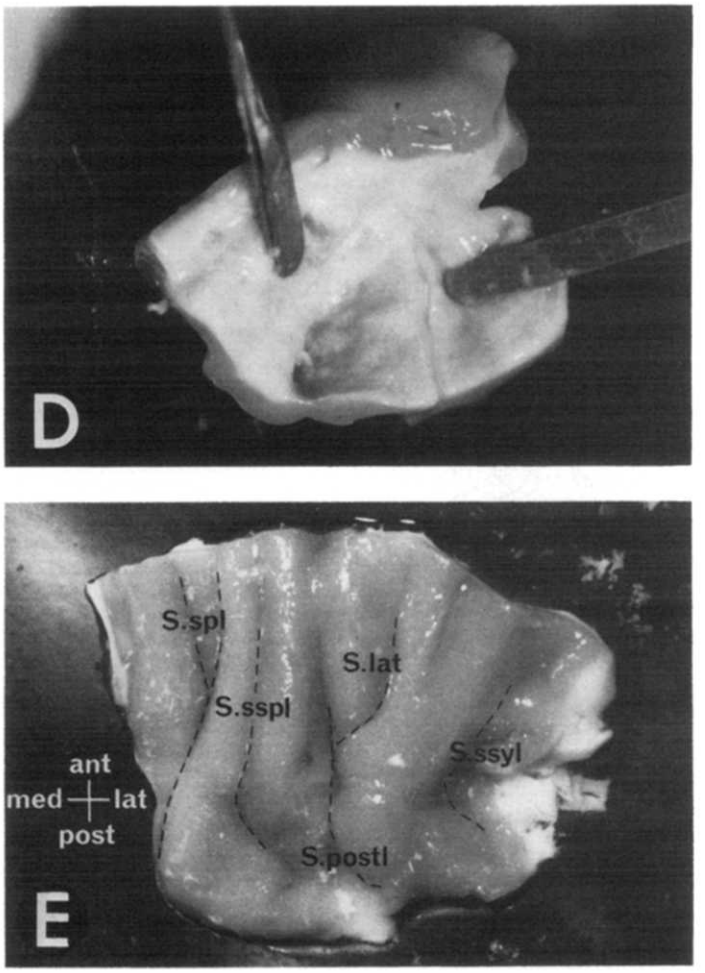

Fig. 2. Photographs of the cat brain at 5 stages in the flat-mounting procedure. A: occipital region of blocked right hemisphere in dorsal view showing the lateral (S.lat), suprasylvian (S.ssyl) and posterolateral (S.postl) sulci. B: right cortex in medial view showing the cutting of the arachnoid and pia mater along the suprasplenial sulcus (S.sspl) and splenial sulcus (S.spl). The cut splenium of the corpus callosum is also visible to the right. C: separation of the walls of the suprasylvian sulcus with two spatulas; the lateral gyrus is under the left-hand spatula. The approximate magnification may be gauged from the width of the spatula (3 mm). D: blunt dissection of the visual radiation from the inferior aspect of the same specimen—the posterior pole is in the upper part of the photograph and the anterior pole, at the bottom of the photograph, is already unfolded. E: preparation as seen from the external surface before slight compression and flat-freezing. Note that all photographs were taken from the brain of a cat used in another experiment and perfused with glutaraldehyde and not from the unfixed brain in the acute 2-DG experiment (because there is no time for the flat-mounting to be interrupted by photography). The buckling visible in E is never seen with unfixed tissue (for comparison see Fig. 4D) and, in any case, vanishes after compression and freezing. The approximate locations of the floors of the sulci are indicated by the interrupted lines; labeling as for A and B above. It should be noted that the exact sulcal pattern strongly depends on the type of cortex (see classifications in Otsuka and Hassler, 1962) and is also subject to individual variations.

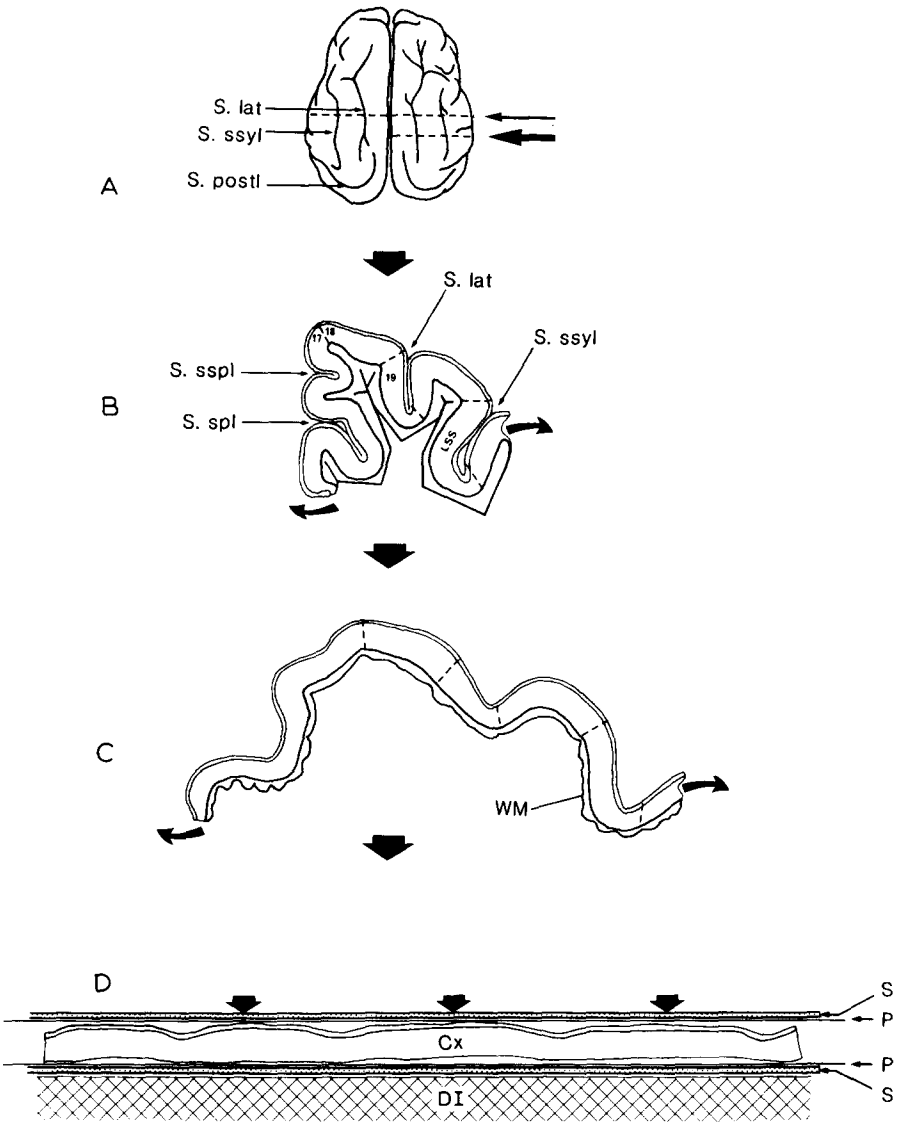

Fig. 3. Schematic summary diagram of the main steps in flat-mounting (adapted with permission from Löwel et al.,1987, Fig. 2). Sulci labelled as for Fig. 2. A: dorsal view of cat brain showing (thin arrow) the coronal blocking plane. B: coronal section of right hemisphere at level of thick arrow in A showing incision planes for white matter and boundaries (dotted lines) for areas 17, 18 and 19 and lateral suprasylvian (LSS). C: coronal section after separation of white matter (WM) and unfolding of the cortex. D: sandwich of cortex (Cx) between sheets of parafilm (P) and glass slides (S) on block of dry ice (DI). Arrows indicate even pressure to top of specimen during freezing.

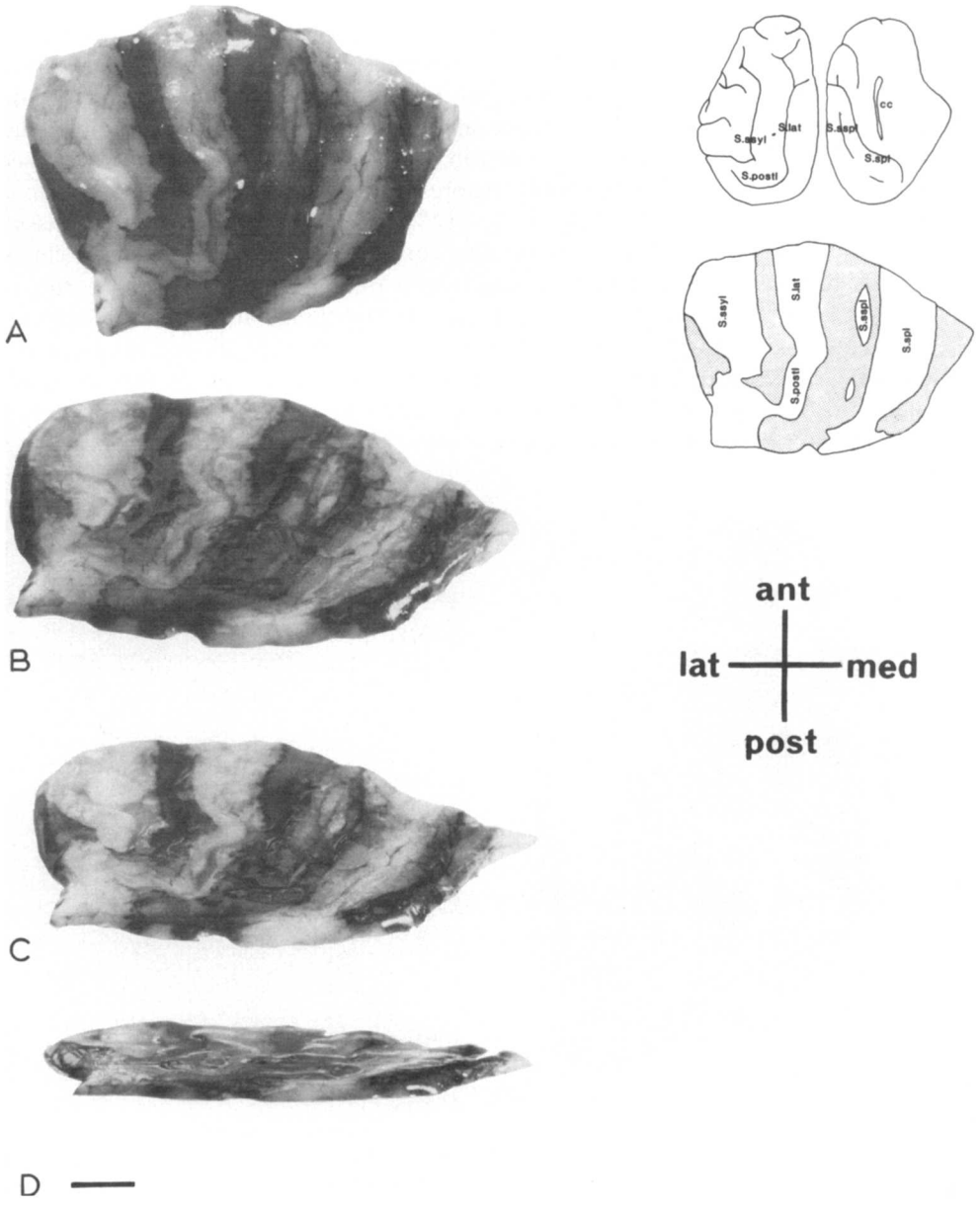

Fig. 4. Unfrozen, unsectioned flat-mount of a left visual cortex photographed from different angles to illustrate the "flatness" and gyral pattern seen in an unfixed preparation. The surfaces of the gyri were stained before flat-mounting with Cresyl violet (modified from Olavarria and Van Sluyters, 1985). Dark and light areas correspond to gyri and sulci, respectively. A: top view of specimen. B, C: oblique photographs. D: profile, demonstrating that only minor undulations occur on the surface of the flat-mount. Insets represent drawings of the intact cortex in dorsal (upper left) and medial (upper right) aspect, and of the flat-mount in A. Sulci labelled as for Fig. 2. The scale bar indicates 10 mm.

specimen and the mounting medium, and of course, to avoid any significant thawing and refreezing of the specimen. To protect the frozen specimen from dehydration by sublimation of superficial water, the flat-mount is painted with room temperature egg white while the chuck is still mounted in the block of dry ice. The egg white freezes instantly and several layers are applied evenly to provide an ample, flat "tissue" layer for preliminary sectioning in the cryostat so that the specimen face can be aligned parallel to the knife before slicing the cortex. The specimen is sectioned at a thickness of 24–28 μm at $-12°$C to $-15°$C. If necessary the tissue can be stored in a $-70°$C deep freeze after first wrapping the specimen and chuck in cold aluminium foil and then sealing it in a plastic bag. The second cortex is stored at $-70°$C while the first is being sectioned. Storage at higher temperatures (e.g. in a cryostat at $-12°$C for 12 h) was found to degrade the resolution of the autoradiograms significantly. Prior to sectioning, 3 perpendicular marker holes are bored into the specimen with a fine syringe needle to facilitate superimposition of adjacent autoradiograms for enhanced contrast (Fig. 5).

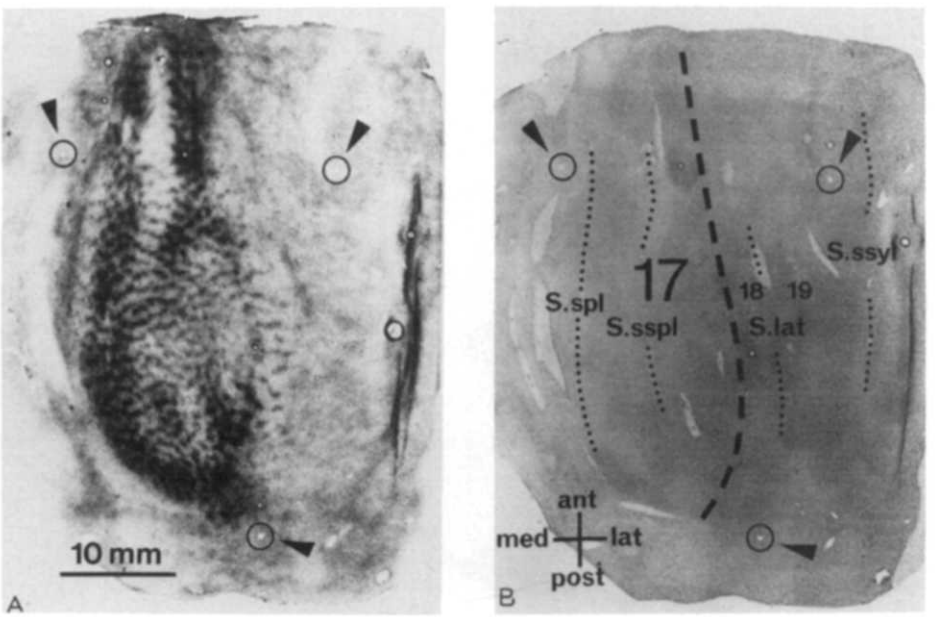

Fig. 5. A: a representative autoradiogram showing iso-orientation bands in the flat-mounted, unfixed right visual cortex of a cat which received 50 μCi/kg 2-DG intra-arterially. Visual stimulation consisted of a moving square wave grating. Exposure time 6 weeks. Contrast enhanced for photographic reproduction by the superimposition of the original autoradiograms from two adjacent sections taken from layers 3–4. Adapted with permission from Löwel et al. (1987). B: corresponding Nissl-stained section with some cortical landmarks indicated. The approximate location of the 17/18 border is marked with the interrupted line and was inferred from the trajectory of the lateral sulcus and its relationship to area boundaries as demonstrated in previously published cortical maps (e.g. Otsuka and Hassler, 1962; Tusa et al., 1979). The floors of the sulci are indicated by dotted lines. Sulci labelled as for Fig. 2. The arrowheads in A and B point to the 3 needle holes used for the alignment of adjacent sections.

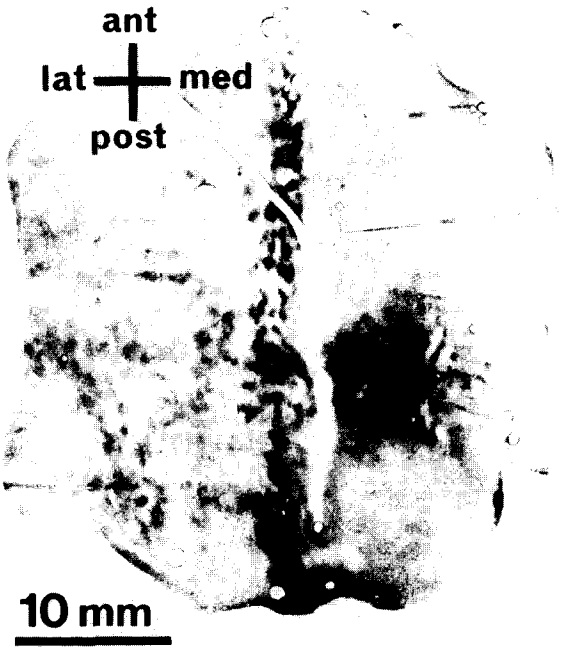

Fig. 6. A representative autoradiogram showing the pattern of increased 2-DG uptake in the flat-mounted, unfixed left visual cortex of a cat which received a total of 17.4 μCi/kg 2-DG intra-arterially. Visual stimulation consisted of a horizontally moving, vertically oriented grating restricted to the horizontal and vertical meridian in a "Maltese cross" pattern. Exposure time 9 weeks. Contrast enhanced by the superimposition of three autoradiograms from adjacent sections through layers 2–3. Adapted with permission from Löwel et al. (1987).

The sections are collected on large, cold slides precoated with chrome-alum gelatine, flattened a little with a cold paint-brush in the cryostat and immediately thawed on a hot plate at +85 °C. The sections are further dried on the hot plate for about 5 s before being stored in an ordinary slide box containing silica gel. After mounting the slides in two rows (left and right hemispheres) on rubberized aluminium plates (2–5 mm thick plates covered with a 2–4 mm thick layer of black, non-slip rubber; alternatively one can use normal X-ray cassettes) and covering them with Agfa Mammo-ray T3 film in a dark room at room temperature, the compressed stack of plates, slides and films is exposed for 6–9 weeks in a cold room at +4 °C. After exposure, the stack is removed from the cold room, thawed to room temperature and the films are developed in Kodak D19B (1 + 4 dilution) for 4 min at 20 °C. One example of an autoradiogram from a flat-mount is shown in Fig. 5: this cat received 50 μCi/kg 2-DG intra-arterially and the films were exposed for 6 weeks. In the case of the cat which received 17.4 μCi/kg (Fig. 6), the films were exposed for 9 weeks. The resulting autoradiograms are described in detail in Löwel et al. (1987).

Discussion

In these experiments the deoxyglucose tracer in the arterial blood reaches the visual cortex primarily via the internal maxillary artery which continues on from the external carotid artery to the circle of Willis via anastomotic vessels forming the extracranial rete (Davis and Story, 1943; Holmes et al., 1958). Using dye injection techniques in living cats, Holmes et al. (1958) determined that carotid arterial blood supplies the cerebral hemispheres and thalamic regions and that vertebral arterial blood supplies medullary, pontine and cerebellar tissue. However they could not exclude the possibility that in some cats vertebral arterial blood could extend as far as the rostral end of the basilar artery or to the posterior communicating arteries of the circle of Willis and thereby supply the visual cortex. This question was addressed by Reneman et al. (1974) who injected radioactive microspheres into the vertebral and/or carotid arteries and showed that in some cats, vertebral arterial blood does supply the posterior cerebral cortex but that the main cerebral supply is via the carotid system; in particular, for those cats which received simultaneous injections into the left common carotid and left vertebral arteries, the maximum relative contribution of vertebral arterial blood to the posterior cortex was only about 7% (Table 2 in Reneman et al., 1974). Because of this small, inconstant contribution of vertebral arterial blood to the vascular supply of the cat visual cortex, we developed the injection system using only the carotid arteries.

In fact, the injection of relatively small amounts (17.4 μCi/kg) of the deoxyglucose tracer into the external carotids via the lingual artery cannulae resulted in successful labeling of the visual cortex (see Fig. 6). However, in the absence of a patent internal carotid artery in the cat, there are two other routes carrying arterial blood to the circle of Willis, these being the ascending pharyngeal artery branching from the external carotid below the lingual artery and the anastomotic branch artery from the internal maxillary, which arises above the lingual artery (Davis and Story, 1943; Chungcharoen et al., 1952). Therefore it is possible that improved cortical labeling might be achieved by cannulation of the arterial supply system beneath the origin of the ascending pharyngeal artery, perhaps via the superior thyroid artery, together with ligation of the carotid muscular branches.

In contrast to microelectrode recordings that give insights into the fine-grain organization of circumscribed cortical regions, the 2-DG method provides a more global view of the topographic organization of functional systems. The flat-mount technique described here, similar to that used in deoxyglucose studies on the cat and primate striate cortex (Tootell et al., 1981; Tootell et al., 1982) and recently described in methodological papers (Olavarria and Van Sluyters, 1985; Tootell and Silverman, 1985), represents a further improvement with respect to the demonstration of stimulation-induced metabolic changes in large regions of the cat brain. In all but one of the previously published 2-DG studies about orientation columns in the cat, only relatively small regions of the visual cortex could be reconstructed from serial horizontal sections (see for example Albus, 1979; Singer, 1981; Singer et al., 1981; Albus and Sieber, 1984). The exception is the study of Tootell et al. (1981) where two autoradiograms of flat-mounted cat striate cortex are reproduced.

However, these autoradiograms do not cover the full extent of area 17 and in contrast to all earlier studies, autoradiograms obtained from our flat-mount sections reveal activity patterns over substantially larger areas (Figs. 5 and 6 and Löwel et al.,1987). The directly visible 2-DG patterns are up to 10 times as large as those from horizontal sections and cover at least the visual areas 17, 18 and 19.

Following the demonstration of iso-orientation bands in larger regions of the cortex it is possible to make more reliable statements about the topography and orderliness of this system in the cat brain (Löwel et al., 1985; Löwel et al. 1987).

As Olavarria and Van Sluyters (1985) claim it is difficult to completely flatten the cortex. One possible approach (to solve this problem) is to introduce some radial cuts adjacent to tightly convoluted cortical areas (Olavarria and Van Sluyters, 1985), thereby sacrificing the continuity of area 17. Complementary to their protocol by thorough cutting of the white matter under the gyri and sulci, we have demonstrated a flat-mounting technique which is applicable to unfixed tissue and which also retains the integrity of area 17 without causing major deformation of the tissue (see Fig. 4). With respect to its complexity, our preparation lies somewhere in between that of Tootell and Silverman's methods I and II, is applicable to 2-DG experiments as is their method I but enables the "production" of sections nearly as large as their more complex method II.

The distortions of the tissue produced during unfolding have not been studied in detail. We expect them to be small, at least in the anterior part of area 17, because the suprasylvian, lateral, suprasplenial and splenial sulci run nearly parallel in an anterior–posterior direction (Fig. 2E and 4D). Therefore any slight distortions should be restricted to the posterior part of area 17 where the posterolateral gyrus is more strongly curved. We have no reason to believe that the linear distortions occurring in our work are greater than those suggested by Tootell and Silverman (1985) for highly curved regions of the monkey cortex. This is because the average centre-to-centre spacing of orientation columns obtained with the present flat-mount technique from measurements over the whole of area 17 (Löwel et al., 1985; Löwel et al., 1987) as well as their general organization, agrees with the spacing and topography previously obtained under similar stimulation conditions from serial horizontal section reconstruction (Singer, 1981). In addition the relatively long duration of the flat-mounting procedure (up to 30 min) does not seem to produce any significant decrease in spatial resolution of the autoradiograms, their contrast being at least as good as in experiments before the flat-mount era in the same laboratory (Singer, 1981; Singer et al., 1981).

Olavarria and Van Sluyters (1985) have commented on the intrinsic undulations in the thicknesses of individual cortical laminae, which variations are not obliterated by flat-mounting. These variations, occurring at the crests of the gyri and the floors of sulci (Fig. 4D), as well as incomplete unfolding, may account for local inhomogeneities in our 2-DG patterns as can be seen in the unlabeled oval lacuna in the anterior part of area 17 in Fig. 5. However by means of superposition of 2–4 adjacent autoradiograms showing label in this same region, it is possible in principle to reconstruct the 2-DG pattern for any one lamina over the entire expanse of area 17.

In summary, for 2-DG studies on the cat visual cortex, the combination of intra-arterial injections and cortical flat-mounting provides a relatively inexpensive method of studying neural activity patterns over wide regions of cortex with reasonable exposure times for the X-ray films. In particular, by increasing the exposure duration from 3–4 weeks (exposure time needed after intra-venous injection of 120–200 μCi/kg 2-DG (Singer et al., 1981)) to 9 weeks (17.4 μCi/kg, see Fig. 6), it is possible to carry out between six and eleven 2-DG experiments using intra-arterial injection with the same amount of 2-DG as was originally required for one animal using intravenous injection (Singer, 1981; Singer et al., 1981).

Though specifically designed for studies on the cat visual cortex and developed with particular emphasis on an intact area 17, slight modifications of the technique described above allow its application to other cortical regions. As pointed out by previous flat-mounters, the technique is also compatible with standard histological or histochemical protocols. The special advantages of this flat-mounting protocol compared to the recently published techniques, are its simplicity and applicability to 2-DG experiments requiring the mapping of activity patterns in large cortical areas.

Acknowledgements

We wish to thank Sigrid Thel for help with these experiments, Hedwig Thomas and Margitt Ehms-Sommer for the photographs, Gisela Knott for processing the manuscript and Matthias Schmidt and Heiko Luhmann for supplying brains from their experimental cats. B.F. is a research fellow of the von Humboldt Stiftung. The research in this work was supported by a grant from this foundation.

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