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A stereotaxic method of anatomical localization by means of $H_2{}^{15}O$ positron emission tomography applicable to the brain activation study in cats: Registration of images of cerebral blood flow to brain atlas

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In the neuronal activation study of normal animals, precise anatomical correlation, preferentially to a detailed brain atlas, is required for the activation foci co-registration. To obtain precise regional correlation between $H_2{}^{15}O$-PET images and the brain atlas, a method of stereotaxic image reorientation was applied to an activation study with vibrotactile stimulation. Cats anesthetized with halothane underwent repeated measurements of regional cerebral blood flow (rCBF) in the resting condition and during vibration of the right forepaw. The image set was adjusted three-dimensionally to the atlas. The postmortem brain was sectioned according to the atlas planes. The activated areas were determined by the stimulus-minus-resting subtraction images, and the areas were projected to the atlas. The PET images of the cat brain were compatible both to the postmortem brain slices and to the brain atlas. The activation foci obtained from the subtraction images corresponded to the area around the coronal sulcus, which is electrophysiologically known as the primary sensory area as described in the atlas. There were precise regional correlations between the PET image and anatomy in a PET activation study of the cat by means of stereotaxic image reorientation.

Key words: PET; $H_2{}^{15}O$; cerebral blood flow; atlas, cats; vibrotactile activation

INTRODUCTION

ANATOMICAL LOCALIZATION of human brain PET images has been performed by referring to the CT or MRI image of the same individual,[1–3] or anatomical standardization to the Tarailach's brain atlas.[4,5] These methods have been applied to the PET activation study for anatomical identification of the activation foci in the stimulus-minus-resting subtraction images. Anatomical localization has also been studied in recent animal PET studies following middle cerebral artery (MCA) occlusion in baboons and cats,[6,7] by comparing PET images with tissue slices, but no work has been reported concerning the anatomical localization for PET activation study of normal animals. The anatomical interpretation of activation foci is quite difficult with rCBF images alone because of insufficient spatial resolution of the apparatus for a small animal brain. Since high resolution MRI imaging of individual animals is hardly available for an ordinary laboratory, it is not feasible to register the PET image to the MRI of the same animal.

To realize anatomical localization of the PET images of cats, we used the atlas of Reinoso-Suârez.[8] This atlas was prepared as a map for physiological experiments, representing 43 coronal cross-sections of a cat brain with 1 mm interval in free-hand drawings. The topography is based on a three-dimensional coordinate system, in which each cross-section was vertical to the line connecting the olfactory bulb and the center of the cerebellum (Bulbo-Cerebellar or BC line). The BC line at zero represents the interaural plane of reference, from which the distance is described anteriorly (plus) and posteriorly (minus) on a millimeter scale.

In this paper, we devised a method of reorienting the

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PET image to the atlas, which is applicable to a PET activation study on cats in which physiological conditions are constantly maintained under halothane anesthesia. We also utilized this method for anatomical localization of a focally activated areas by vibrotactile stimulation.

MATERIALS AND METHODS

Animals

Three normal male cats 7-8 years old with a body weight of 3.6–4.0 kg were used for the present experiment. The cats were anesthetized with halothane from a conventional anesthesia apparatus (Metran Compos β-EA). During the experiment the halothane concentration was kept at 1.0%. The animal was immobilized with galamine triethiodide and artificially ventilated to maintain the end-tidal carbon dioxide at 3.0%. The body temperature was maintained at 37.5°C with a heating pad and lamp with a thermocontroller (Nihon Koden, Japan), and the systemic arterial blood pressure was maintained at 90–120 mmHg. These physiological conditions were continuously monitored on a polygraph recorder (NEC San-ei, Japan).

The animal study was approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

Positioning

Prior to the measurement of rCBF, the anesthetized animal was positioned in a stereotaxic holder, which had been designed for physiological experiments and was modified for a PET study (Narishige, Tokyo, Japan). The animal was attached to the holder by the two bony landmarks on each side, the external auditory meatus and the lower margin of the bony orbit. Ear bars were inserted into the external auditory meatus, and the lower margin of the bony orbit was fixed with a clamp.

PET scanning

The cat underwent repeated measurement of rCBF while resting and under vibrotactile stimulation once or twice for each condition, in which one measurement in each condition was used for the data analysis. The vibrotactile stimulation was applied to the right forepaw during the scanning period (3 min) with an electric vibrator (Daito model MD9100, Osaka, Japan).

As a radioactive tracer, oxygen-15-labeled water ($H_2{}^{15}O$) was mixed with 7 m*l* of physiological saline solution. Following intravenous injection of 600 MBq of $H_2{}^{15}O$, PET scanning was performed for 3 min starting at the time of injection with an SHR-2000 PET camera (Hamamatsu Photonics, Shizuoka, Japan) providing a 14-slice image set at 3.25-mm intervals with image spatial resolution of 3.5-mm full width at half maximum (FWHM).[9] Correction of photon attenuation was carried out with transmission data obtained by rotating the ^{68}Ge rod source. The tomographic images were reconstructed by using a Shepp & Logan filter with a cutoff frequency of 90 cycles/cm. The arterial blood radioactivity was measured by continuous sampling of arterial blood and using a peristaltic pump and a beta-detector loaded with plastic scintillator with delay and dispersion correction.[10] The count rate was expressed as Bq/m*l* using blood samples collected by a fraction collector (at 9 sec/sample).

The functional images of rCBF were created by the PET-autoradiographic method,[11,12] with a look-up table derived from the data for blood radioactivity, the blood/brain partition coefficient being set at 1.0.[13] The rCBF values were normalized by the global CBF (gCBF) which was set at 50 m*l*/min/100 m*l*.[14]

Image reorientation and data analysis

After the experiment, the animals were killed with an overdose of sodium pentobarbital, decapitated, and subsequently immersed in formalin for 24 hours. Then the head was cut along the midsagittal line into two pieces, one of which was positioned in the holding apparatus of the PET camera by the two external bony landmarks in the same way as the intact animal was positioned for CBF measurement. The deviation angle (X°) between the horizontal axis of the scanner and the BC line of the animal was measured as shown in Fig. 1.

The original image volume of 14 slices was resliced at the deviation angle (X°) to obtain PET images perpendicular to the BC line so that the reoriented image could be compared with the stereotaxic atlas on the same plane. Axial rotation and lateral tilt, if any, was adjusted on the image volume. Axial shift was adjusted according to both inspection of the images and the bed position information in the scanner. No scaling was carried out on the images.

Subtraction images were created as vibration minus rest from the images transformed by the reorientation procedure. All analysis procedures were carried out on SGI workstations (Indigo 2, Indy) with image analysis software system Dr. View (Asahi Kasei Joho System, Tokyo, Japan).

Tissue slice preparation

To validate the above image reorientation technique and to determine structural difference between the atlas and the individual brain anatomy, the brains of the three cats were dissected, and 5 mm interval slices were obtained perpendicular to the BC line according to the atlas and stained by the Klüver-Barrera staining technique.[15]

RESULTS

The comparison between the standard atlas, brain tissue slices and the normalized rCBF images of three cats after stereotaxic image reorientation is shown in Fig. 2 (anterior slices) and Fig. 3 (posterior slices). The tissue slices from each cat resembled the corresponding atlas, indicating a small morphological variation between individuals.

The PET images were all very noisy and did not give any intracerebral structural information by themselves, but the contour of the brain discernible in the PET images agreed with the contour of the corresponding tissue slice and the atlas. When the PET images were compared carefully with the corresponding tissue slices and atlas, the gray matter area tended to have higher blood flow than the white matter area, although obscured by noise.

A comparison of the atlas, rCBF image at rest, rCBF image at vibratory stimulation and stimulus-minus-resting subtraction image in Cat #3 is shown in Fig. 4. In the subtraction images, hot spots were observed in various regions scattered across the brain, and activation foci could not be distinguished from noise, but one of the hot spots was located in the left coronal sulcus, which is an anatomical marker of the primary sensory cortex, at 20 mm anterior to the interaural line. The other cats had similar scattered hot spots in the subtraction images. Cat #1 had a hot spot between the left coronal sulcus and the left anterior ectosylvian sulcus at 15 mm, and Cat #2 had a hot spot on the left coronal sulcus at 20 mm almost corresponding to the area of the hot spot seen in Cat #3. The amount of rCBF increase in the activation foci was 25.0% in Cat #1, 46.5% in Cat #2 and 24.3% in Cat #3 (% resting rCBF).

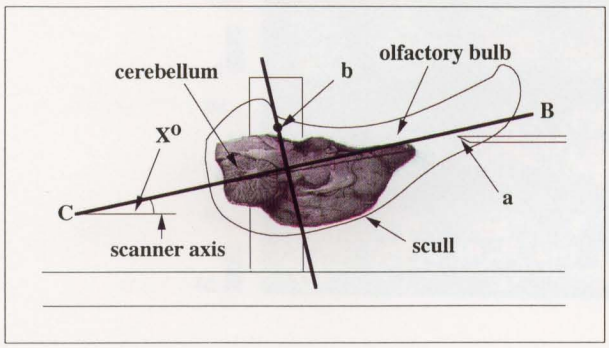

Fig. 1 Schematic representation of the right half of a midsagittally sectioned postmortem cat head in supine position attached to the stereotaxic holder by two bony landmarks (a, b) as in the PET scanning. a: lower margin of the bony orbit. b: external auditory meatus. The angular deviation (X°) was measured between the scanner axis and the BC line (line connecting the center of the olfactory Bulb and Cerebellum) as defined in the atlas.

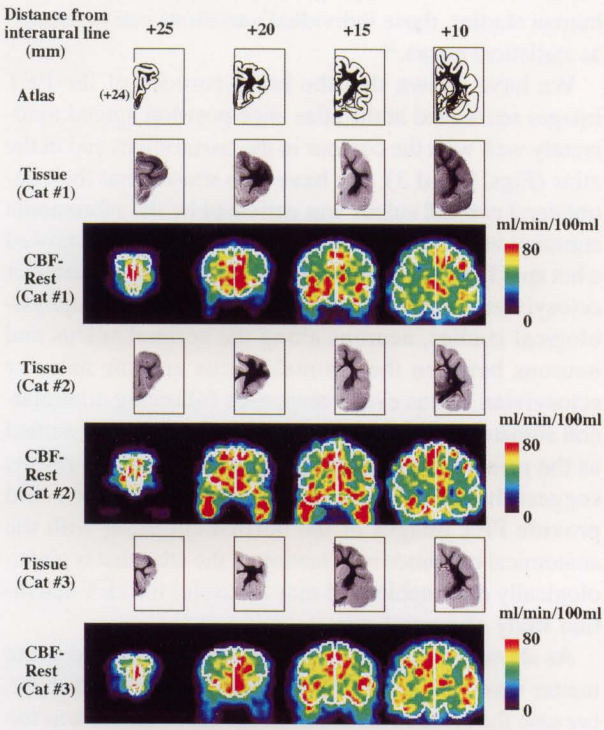

Fig. 2 Comparison of the brain configuration between the standard atlas, tissue slices and the normalized resting rCBF images of three cats after stereotaxic image reorientation as described in the text. Each slice was +25, +20, +15 and +10 mm anterior from interaural line. White lines superimposed on the PET images represent the contour obtained from the atlas.

DISCUSSION

Although PET is a promising tool for the research on the

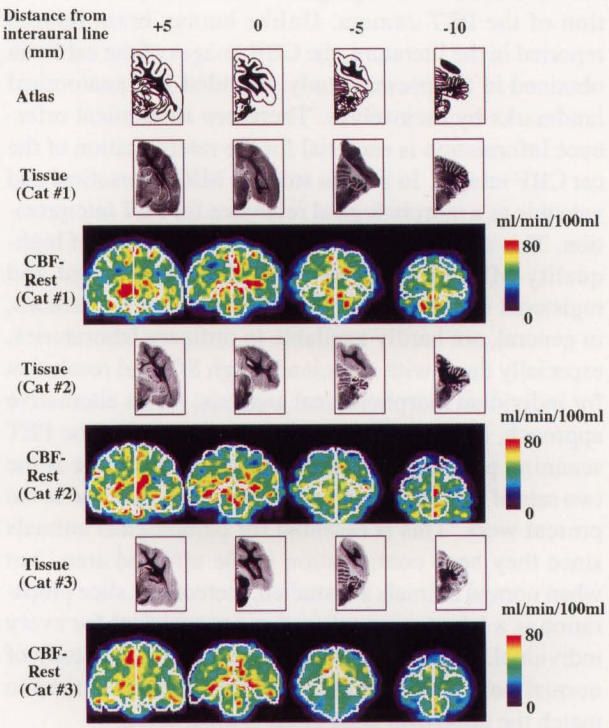

Fig. 3 Comparison of the brain configuration between the standard atlas, tissue slices and the normalized resting rCBF images of three cats after stereotaxic image reorientation as described in the text. Each slice was +5 anterior and 0, −5 and −10 mm posterior from interaural line. White lines superimposed on the PET images represent the contour obtained from the atlas.

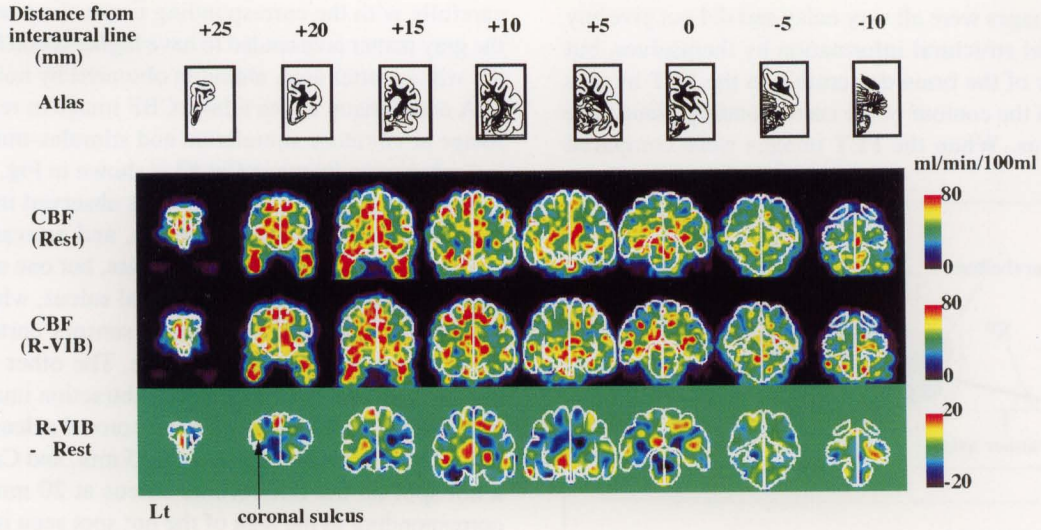

Fig. 4 The comparison of the atlas, rCBF images (at rest and during vibratory stimulation of the right forepaw) and stimulus-minus-resting subtraction images of Cat #3. White lines superimposed on the PET images represent the contour obtained from the atlas. Although hot spots were scattered across the brain, one of them was located on the left coronal sulcus, which is an anatomical marker of the primary sensory cortex (indicated by arrow).

animal brain as well as the human brain, imaging an animal brain is challenging due to limited spatial resolution of the PET camera. Unlike human brain images reported in the literature, the CBF images of the cat brain obtained in the present study provided few anatomical landmarks by themselves. Therefore anatomical reference information is essential for the interpretation of the cat CBF images. In human studies, MRI is practical and valuable as a morphological reference for PET interpretation. This approach is valuable for animal study if high-quality MRI of the same individual is obtained and registered with the PET,[16] but MRI scanners for animals, in general, are hardly available to ordinary laboratories, especially those with sufficiently high S/N and resolution for individual morphological analysis. As an alternative approach, the brain slices cut and stained after the PET scanning provide exact morphological reference if the two sets of slices are matched together, as was done in the present work. This is essential for pathological animals since they need confirmation of the affected area,[7] but when normal animals are studied, stereotaxic slice preparation is a laborious work and is not practical for every individual. It would be very convenient for the study of normal animals if the PET images are transformed to match the published atlas of the animal brain.

In the present study, we reoriented the CBF images of three cat brains to match the cat brain atlas, which is based on the BC line. We also prepared postmortem tissue slices of the cats cut at the same positions. When the tissue slices of the three cats were compared with the atlas, we found individual morphological variation small enough to permit anatomical interpretation of the PET images, allowing for the resolution of the PET (Figs. 2 and 3). If inter-individual averaging analysis is performed as in human studies, those individual variations can be treated as statistical errors.[2,5]

We have shown that the brain contour of the PET images reoriented at the atlas slice position agreed moderately well with the contour in the tissue slices and in the atlas (Figs. 2 and 3). We have also shown that the contralateral coronal sulcus was activated by the vibrotactile stimulation in two of the three cats. The other cat showed a hot spot between the coronal sulcus and the left anterior ectosylvian sulcus. According to previous electrophysiological studies, neurons along the coronal sulcus and neurons between the coronal sulcus and the anterior ectosylvian sulcus evoke responses following contralateral somatosensory stimulation, and the area is classified as the primary somatosensory cortex.[17,18] Those results suggest that the method presented in this paper could provide PET images of the normal cat brain with the anatomical reference information of the atlas that is physiologically reasonable, and may be useful for PET activation study of normal cats.

As shown in Figs. 2–4, the contrast of grey and white matter was unclear on CBF images of the present study, because the spatial resolution of the PET camera was too low to discriminate 2–3 mm of the grey matter in the cat brain. Recently Heiss et al. reported that CBF images obtained enabled identification of the main anatomic structures of the cat brain and distinction between grey and white matter with the best resolution obtained in the CMRglc images.[19] FDG images provide higher contrast between gray and white matter than $H_2{}^{15}O$-CBF images.

It may be easier to extract anatomical landmarks from the FDG images and match them into the atlas.

A number of human studies reported a substantial increase in blood flow over the contralateral primary somatosensory cortex induced by the vibrotactile stimulation of the hand.[14,20,21] One of them estimated the amount of CBF increase to be 30% of the resting value.[22] In the present cat study, the CBF increase in the vibrotactile activation foci was smaller than the human data and was variable: 25.0% in Cat #1, 46.5% in Cat #2, and 24.3% in Cat #3. This may be the effect of the anesthetics or due to species difference. A similar study on barbiturated cats showed an increase of 15% in $H_2{}^{15}O$ radioactivity caused by vibrotactile stimulation of the forepaw, although CBF was not quantified and no subtraction images were presented.[16]

In conclusion, the stereotaxic reorientation method presented in this paper can provide precise correlation between PET and the anatomy of normal cats and will be useful for activation studies in physiological research.

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