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Air embolism may cause unrecognized ischemia of the gray-white junction

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Dutka AJ, Kochanek P, Hallenbeck JM, Storey JR. Air embolism may cause unrecognized ischemia of the gray-white junction. Undersea Biomed Res 1988; 15(2):99–106. The border between the gray and white matter is defined by an abrupt change in average blood flow. This difference allows one to distinguish structure with [14C]iodoantipyrine autoradiography. The angioarchitecture of the cortical gray-white junction suggests that an air embolism might preferentially lodge in this border zone, and thus ischemia of the border might go unrecognized if one depended only on the difference in average blood flow to define the gray-white junction. Accordingly, a computerized image processing technique was applied to compare the area of the cortex measured on an autoradiogram to the area measured on a histologic section after staining for myelin. In dogs that had received air embolism, the autoradiogram underestimated the thickness of the cortical mantle even in sections that did not seem to have an obvious focal zone of low blood flow. This suggests that the deep cortical layers are especially vulnerable to air embolism.

air embolism	blood flow
computers	cerebral arteries
	image processing

INTRODUCTION

Air embolism from diving or surgical accidents can cause a variety of neurologic signs, including mental confusion suggestive of diffuse cortical damage. Brierly et al. (1) have commented on the difficulty of pathologically determining whether ischemic changes after cardiac bypass are due to hypotension or air. This difficulty is partially due to the similar distribution of the pathologic changes in the two conditions. The similarity to hypotensive injury suggests that the deep cortical layers might be particularly vulnerable to air embolism. Inasmuch as this area is supplied by long arterioles penetrating from the cortical surface, it occupies a microwatershed that could be very vulnerable to arteriolar obstruction (2). With the use of [14C]iodoantipyrine

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autoradiography, we have previously noted multiple areas of low flow surrounded by areas of high flow after air embolism, but did not find any area with increased vulnerability (3).

The detection of the concentration of a diffusable radioactive tracer by autoradiography is the only presently available technique that allows simultaneous determination of blood flow differences within and between brain structures (4, 5). The visual identification of various brain regions on an autoradiogram depends on the assumption that average blood flow in gray matter is 3 to 4 times that in the white matter; in a mid-coronal section of brain, for instance, the cortex, basal ganglia, and thalamus appear dark in contrast to the relatively light centrum semiovale and internal capsule. Thus, we can clearly discern areas of low flow only when the ischemic zone is well within the region of a known structure. The boundaries of gray matter areas are a particular problem. Autoradiography may indicate that the area of high tracer concentration defining the cortical mantle is, for instance, 7-mm wide at some point. The width at the same point measured by an anatomic indicator of the extent of cortex might be 9 mm; the 2-mm difference is due to low flow at the boundary, which goes unrecognized if only the autoradiogram is considered.

Our hypothesis, then, was that the gray-white junction was particularly vulnerable to air embolism, and that this vulnerability had been obscured by the nature of the method used to detect blood flow. We tested this hypothesis by calculating the autoradiographically determined cortical area and the area determined by staining the section for myelin. We used a digital image analyzer to accomplish this, and compared the results in animals that received air embolism to animals that did not.

MATERIALS AND METHODS

We used autoradiograms that were prepared for a study of white cell accumulation after air embolism. The details of the experimental procedure and the results of that investigation are published elsewhere (6). In 5 chloralose anesthetized animals (Canis familiaris), we repeatedly injected 20–50 μl of air into the internal carotid over the course of 1 h to maintain the somatosensory evoked response amplitude at 10–20% of its preischemic control values. Four hours after the end of the ischemic period, we infused 60 μCi/kg of [^{14}C]iodoantipyrine for determination of regional blood flow according to the method of Sakurada et al. (4). We stopped the heart within 10 s by injecting KCl into the right ventricle, removed the brain, and froze it at $-65°C$ in Freon over liquid N_2. The time interval between ending circulation and brain freezing was about 10 min. Six animals received no air embolism and served as controls.

We prepared 20-μm sections using an American Optical Company freezing microtome at $-15°C$; the sections were dried on glass slides and incubated in contact with Kodak SB 5 film in a sealed cassette. After incubation, we stained the sections with 0.1% eosin B in 50% methanol and water. We used luxol fast blue to restain some sections to ensure that the eosin technique, which was very convenient, accurately outlined white matter. We selected a single coronal section from the mid-portion of the brain from each animal for image processing, and matched the autoradiogram with the dried 20-μm tissue section which had produced it.

The EYECOM II digital image analysis system (Spatial Data Systems, Goleta, CA) was used to determine the areas of gray matter in the selected region. This system

allowed precise superimposition of the autoradiographic image and the image of the stained section. It also permitted conversion of the video input to numerical arrays that can be manipulated for contrast enhancement, calculation of the area in an irregular shape, and superimposition of precisely defined masks to ensure that identical areas on the image are analyzed. The image of the autoradiogram is stored as a 640-column, 480-row array, with the row and column number forming an address in the array. Each of the elements (called pixels) in the 640 × 480 array is a number that represents the amount of light reaching the video camera at that point. This number (gray value) is set at 0 for the darkest area on the image, and at 255 for the whitest area.

If one calculates a histogram of the relative frequency of each gray value in the picture, the result is a bimodal distribution of values. In the image of the stained section, the lower of the two peaks (darker areas) represents the white matter, whereas in the autoradiogram the lower peak is the distribution of values in the gray matter. We selected several sample sections that are clearly within the white matter (such as the centrum semiovale and corpus callosum), using a cursor to define the sample. We then obtained the frequency distribution of the gray values within these white-matter samples. The samples were defined so that at least 2500 pixels were sampled for each image. We then simplified the original image by replacing the gray values that were within ±2 SD of the mean white-matter gray value within a gray value of zero (black). All other gray values were replaced with gray = 255 (white). The number of pixels within the specified region that had this 255 gray value represented the cortical area. Enough pixels were sampled to make the frequency distribution of gray values a normal distribution; thus, inclusion of 2 SD represents inclusion of 95% of gray values within a sample of white matter. Any gray value that we counted as belonging to the cortex had a 5% chance of having been falsely assigned to the cortex on the basis of its relative optical density.

This method is relatively free of observer bias. It includes some pixels that are clearly deep within the cortex, but are counted as part of the white matter area, and vice versa. Therefore, we also asked two observers to outline what each felt was the cortex on every image, and we calculated the area from this outline. An observer with experience in reading autoradiograms could accurately define the cortical mantle as well as easily discern which animal had received air. We therefore did not reveal the experimental group to a second observer, who had no experience in autoradiography, and was told only to outline the lighter or darker area of the image with as continuous a line as possible. We could not present one image in each group to the "blind" observer because they had been clearly marked with the experimental group.

We were interested to know if there were areas of unsuspected ischemia at the border of the cortex; we therefore selected a region of the cortex that appeared normal in animals that had received air embolism. We avoided any obvious low-flow areas and drew a box around the region of interest. In control animals, we selected a similar region in the dorsolateral cortical mantle. The calculated cortical areas are thus derived only from a region in which one would not find ischemia when inspecting the autoradiogram. We could exactly superimpose the same box on the section to ensure analysis of the same region. Each image contained a millimeter scale that allowed conversion of the area in pixels to square millimeters.

To emphasize the poor match between the cortical areas on the stained section and the autoradiogram, we outlined the white matter on the section and superimposed
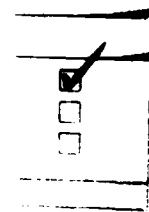
this boundary on the autoradiogram. Examples of this image are shown in Figs. 1 and 2.

RESULTS

We have tabulated the difference between the area measured on the autoradiogram and that measured on the myelin-stained section in Table 1. The results are classified by experimental group (ischemia vs. control) and by the method of area determination (computer method vs. blinded or unblinded observer). The quantity presented is the autoradiogram area minus the section error; a positive number indicates that the autoradiogram overestimated the cortical area, and a negative number indicates that the autoradiogram underestimated the cortical area. Although there is a wide variation in the absolute value of these differences, the general trend is clear: the autoradiogram underestimated the cortical area in all ischemic animals, and overestimated the area in all but one of the control animals, regardless of the method of area determination used.

The ANOVA table presents the results of the two-way ANOVA. There is a highly significant effect ($P < 0.001$) of the experimental group (ischemia vs. control) but no significant effect of the measurement method nor any interactions between the two tested classifications ($0.10 > P < 0.25$).

Autoradiograms with an outline of the extent of the white matter superimposed are shown in Figs. 1 and 2 to contrast the ischemic situation with the control. In both the control and the ischemic animals, there are areas cf poor correlation of the boundary of the white matter defined by eosin staining and that defined by the concentration of [^{14}C]iodoantipyrine. In the ischemic animals there is often a zone of ischemia at

Fig. 1.   An image of an autoradiogram from a control (no air embolism) animal. *Heavy black line* denotes the limit of the myelin stain on the section from which the autoradiogram was prepared. *Note* several areas where the extent of the high flow extends over this line.

Fig. 2. This image is taken from an air embolized animal and shows areas of high flow with extension of this flow into the myelin, and areas, especially in the cortex just to the right of the midline, where there is a much narrower cortical mantle defined by the autoradiogram.

the gray-white junction; in control animals the radioactivity overlaps into the white matter.

DISCUSSION

Regardless of the method used to estimate the cortical area, the autoradiogram underrepresents the extent of the cortex in ischemic animals and overrepresents the extent in control animals. This is not simply a result of the presence of an obvious focal ischemic area; we purposely selected for our analysis areas that did not appear to have ischemia. The poor numerical correlation between the computer method and the observer method is a result of the difference in the method of information processing. A human can extract a continuous border from very noisy information much more easily than can the computer. The computer method included many points in its area calculation that one would usually decide were clearly in the cortex. This is because the computer considers only relative brightness of each point, not its location relative to other points of similar brightness. However, if one relies only on the visual inspection of the autoradiogram, without reference to some anatomic marker of brain structures, one may not notice areas of ischemia that occur at the borders of gray matter regions.

Our method of defining the gray-white junction used a myelin stain; because myelinated fibers extend into the deep cortical layers, this would slightly underestimate the true thickness of the cortical mantle. This fact can partially explain the overestimation of the thickness of the cortex derived from the autoradiogram in control animals. However, it is more likely that most of this overestimation is due to diffusion of radioactive tracer from areas of high concentration to areas of low

TABLE 1

AUTORADIOGRAM AREA MINUS SECTION AREA, IN SQUARE MILLIMETER,
CLASSIFIED BY METHOD OF AREA MEASUREMENT AND BY EXPERIMENTAL GROUP

Two-way analysis of variance

Method of Determination

Experimental Group	Computer Determined	Unblinded Observer	Blinded Observer
Control	10	13.9	0.7
	29	−4.1	−3.5
	12	7.6	N.A.
	21	6.9	9.7
	11	15.3	23.2
	−3	16.6	84.9
Ischemia	−11	−8.6	N.A.
	−12	−5.9	−0.5
	−39	−5.9	−4.8
	−43	−4.8	−4.3
	−102	−7.2	−4.0

ANOVA Table

Source of variation	Degrees of Freedom	Mean Square	F
Method of determination	2	1,200	2.43
Ischemia vs. control	1	7,761	15.77
Interaction	2	1,100	2.23
Within subgroups (error)	25	492	

N.A. = not applicable;   these sections were labeled with the experimental group and therefore were not presented to the blinded observer.

concentration. Diffusion occurs during the time needed to remove the brain and freeze it (approximately 10 min in this study). Sakurada et al. (4) showed that the diffusability of iodoantipyrine was such that all areas of the brain were uniformly stained 2 h after the injection of the tracer. This clearly suggests that 10 min would be sufficient to account for the amount of overestimation seen here.

Both of these errors in the precise definition of the deep cortical layers would bias this study against finding low flows at the gray-white junction. Despite this bias, our findings suggest that these low flows exist. One possible explanation for this vulnerability of the deep cortical areas to microembolization is the potential for emboli to become lodged in the arcade of vessels that supply the deep cortical zones. The cortex receives its blood supply from pial arteries that penetrate the surface; there are no arterial-arterial anastomoses within the parenchyma, although there are arteriovenous channels (2, 7). Short- and medium-length arteries supply cortical layers 1–4; arterioles leave the trunks at right angles and often form complex spirals of

vessels extending horizontally and vertically (8). The arterioles have ring-shaped compressions at the point where they branch to become capillaries (9). Layers 5 and 6 are supplied by long, penetrating vessels that terminate in a fan of vessels, which turn upward to form a structure resembling a candelabra (10). Tumor metastases and bacterial abscesses are most commonly found at the gray-white junction, which suggests that this candelabra structure may predispose to the trapping of microemboli.

The gray-white junction occupies a watershed between the penetrating arteries of the cortex and the deep penetrators nourishing the white matter. Oligemic hypoxia that is neither sufficiently prolonged nor profound enough to cause a classic watershed infarction can give rise to necrosis in the deep cortical layers alone (11). Network analysis of flows in the microcirculation would predict that the resistance of this complex series of small vessels would rise precipitously at low-flow rates, thus further increasing the vulnerability of deep cortical layers (12). Our results support the concept that the deep cortical layers are particularly vulnerable to air embolism.

Dutka AJ, Kochanek P, Hallenbeck JM, Storey JR. L'embolie gazeuse peut provoquer une ischémie non reconnue de la jonction de la matière grise-blanche.—Undersea Biomed Res 1988; 15(2):99–106. La démarcation entre la matière grise et blanche est définie par un changement brusque dans le débit sanguin moyen. Cette différence permet de distinguer les structures par autoradiographie avec la [¹⁴C]iodoantipyrine. L'architecture angiographique de la jonction corticale grise-blanche suggère qu'une embolie gazeuse pourrait se loger préfer-entiellement dans cette zone limite de démarcation, et que l'ischémie de la bande de démar-cation pourrait demeurer inconnue, si quelqu'un dépendait seulement sur la différence dans le débit sanguin moyen pour définir la jonction grise-blanche. Par conséquent, une technique de traitement automatisé de l'image fut appliquée pour comparer la région du cortex mesurée sur une section histologique après la coloration de la myéline. L'autoradiogramme sous-estima l'épaisseur de la gaine corticale même dans les sections qui n'apparaissaient pas avoir de zone focale évidente de débit sanguin faible chez des chiens qui avaient subit une embolie gazeuse.

Dutka AJ, Kochanek P, Hallenbeck JM, Storey JR. La aeroembolia puede ocasionar isquemia de la unión de la materia blanca-gris que puede pasar desapercibida.—Undersea Biomed Res 1988; 15(2):99–106. El límite de la sustancia gris y blanca se define por un cambio abrupto en el flujo sanguíneo promedio. Esta diferencia nos permite distinguir la estructura con autora-diografía con [¹⁴C]yodoantipirina. La arquitectura angiológica de la unión gris-blanca cortical sugiere que una aeroembolia se puede alojar preferentemente en esta zona limítrofe. Por lo tanto, si sólo se basa en la diferencia del flujo sanguíneo promedio para definir la unión gris-blanca, la isquemia del límite puede pasar desapercibida. De esta manera, se empleó una técnica procesadora de imágenes computarizada, para comparar el área de la corteza medida

con autoradiograma y la obtenida por sección histológica, después de haber realizado la tinción para mielina. El autoradiograma de perros con aeroembolia, subestimó el espesor del manto cortical, aún en secciónes que no parecían tener una zona focal clara de bajo flujo sanguíneo. Esto sugiere que las capas corticales profundas pueden ser especialmente vulnerables a la aeroembolia.

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