Journal of Cerebral Blood Flow and Metabolism
14:7–11 © 1994 The International Society of Cerebral Blood Flow and Metabolism
Published by Raven Press, Ltd., New York

Rapid Communication

Magnetic Resonance Imaging of Propagating Waves of Spreading Depression in the Anaesthetised Rat

A. R. Gardner-Medwin, *N. van Bruggen, *S. R. Williams, and †R. G. Ahier

*Department of Physiology, University College, London; *The Royal College of Surgeons Unit of Biophysics, Institute of Child Health; and †MRC Cyclotron Unit, Hammersmith Hospital, London, England*

Summary: Gradient echo magnetic resonance (MR) imaging was used to demonstrate propagating waves of cortical spreading depression (SD) in the anaesthetised rat. SD was initiated by remote perfusion with 150 m*M* KCl applied for 0.5–2 min to the left parietal cortex. Gradient echo MR images were obtained every 12–30 s in either a vertical coronal section or a horizontal section including the superficial cortex in plan view. Within 2 min of application of KCl, we observed a zone of increased signal intensity (3–15%) on the MR image, up to 2 mm across, lasting approximately 1 min and propagating away from the site of initiation. The mean velocity for 27 of such waves seen in seven animals was calculated to be 2.79 mm/min, with means (±SD) in individual animals averaging 2.90 ± 0.46 mm/min (n = 7). Increased signal intensity in gradient echo images has been attributed to an increased level of oxygenation within the venous blood. Our results are consistent with this interpretation although other physiological changes during SD may also contribute to the signal changes. **Key Words:** Cortical spreading depression—Magnetic resonance imaging—Rat.

Spreading depression (SD) in susceptible tissue occurs following interventions such as trauma, ischemia (Nedergaard, 1988), raised extracellular potassium ($[K^+]_e$), and neuronal hyperactivity (Leão, 1944*a*; Bureš et al., 1974). It propagates reliably in rat cortex, producing reduced $[Ca^{2+}]_e$, a nondecrementing wave of increased $[K^+]_e$, release of transmitters, depression of neural activity, cell swelling, and extracellular negativity. SD is known to occur in cortical tissue surrounding an acute ischemic lesion (Nedergaard and Astrup, 1986), and although its involvement in the infarction process is not clear, there is some evidence to suggest that the suppression of SD results in reduced infarct volume (Gill et al., 1992; Iijima et al., 1992). SD is readily initiated in the cerebral cortex of the rat by a number of stimuli, including K^+. The wave of SD spreads from the site of origin and eventually invades the entire ipsilateral cortex, affecting each zone profoundly for approximately 1 min. A second wave can usually be elicited by an adequate stimulus within 5 min, and multiple waves can occur with continuous stimulation or in some special circumstances with recirculating wave patterns (Bureš et al., 1974). It is thought possible that a similar phenomenon may occur in humans and contribute to the pathophysiology of migraine (Leão and Morison, 1945; Milner, 1958; Lauritzen and Olesen, 1984), trauma (Gardner-Medwin, 1992), and stroke (Hansen and Lauritzen, 1984). Clearly, a noninvasive technique capable of demonstrating propagating waves of SD could be of great value in characterising the pathophysiology of these conditions.

Magnetic resonance (MR) is a valuable noninvasive tool for localising structural and chemical abnormalities in brain tissue. Recently it has been shown that MR imaging can demonstrate physiological changes in human brain tissue, on a time scale of seconds, during activation of visual or motor cortex (Kwong et al., 1992; Ogawa et al., 1992; Bandettini et al., 1992). The mechanisms responsible for these changes have been attributed primarily to alterations in the deoxyhaemoglobin content of ve-

Received July 26, 1993; final revision received September 9, 1993; accepted September 14, 1993.

Address correspondence and reprint requests to Dr. N. van Bruggen, the Royal College of Surgeons Unit of Biophysics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, England.

Abbreviations used: FOV, field of view; MR, magnetic resonance; NEX, number of excitations; SD, spreading depression; TE, echo time; TR, repetition time.

nous blood. The purpose of this study was to determine whether similar imaging techniques could be used to demonstrate propagating waves of SD in the anaesthetised rat. Preliminary findings have been presented elsewhere (van Bruggen et al., 1993).

MATERIALS AND METHODS

Rats (male, Sprague–Dawley, weighing 230–300 g) were anaesthetised with halothane in oxygen (1.5–2%) for all surgical procedures. The skin above the skull was removed to expose the skull. The dura was excised, and a region of left parietal cortex was exposed through a 4.5-mm-diameter craniotomy centred ~4 mm lateral and 5 mm behind the bregma. A silicone rubber chamber equipped with inlet and outflow tubing for perfusion was placed over the hole. Spreading depression was initiated with 150 m*M* KCl applied to the left parietal cortex and washed out with NaCl (150 m*M*). In most experiments, NaCl solution was constantly perfused through the chamber at a rate of ~1 ml/min and was switched to 150 m*M* KCl for 1–3 min for initiation of SD. In two experiments, including that for Fig. 1, fluid was static except for 1 ml flushing with 150 m*M* NaCl or KCl. Flushing with fresh NaCl, which does not elicit SD, did not produce either

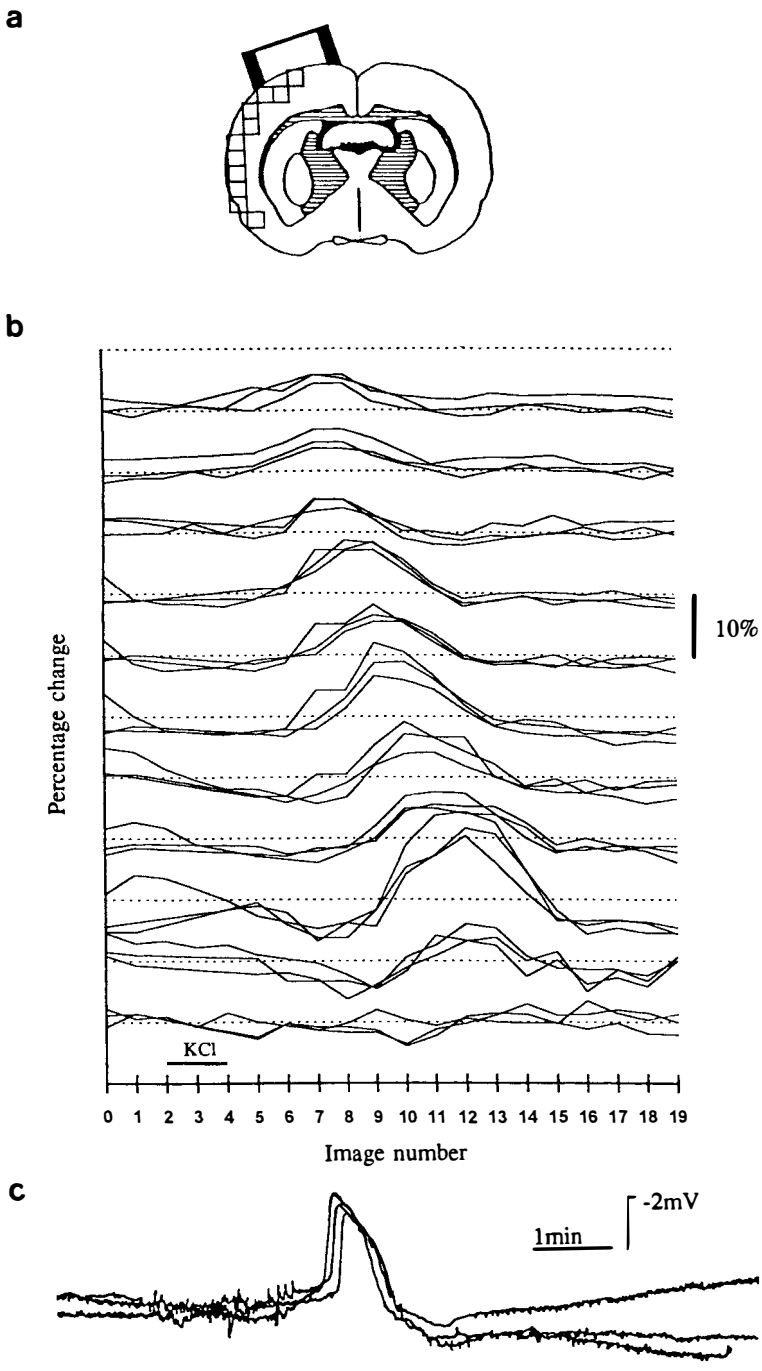

FIG. 1. Changes of MR image signal intensity recorded from regions of interest in a coronal slice through the brain of an anaesthetised rat **(a)** are shown **(b).** KCl was applied during image 2 and washed out during image 4. Images were collected at 21-s intervals. Regions of interest are individual pixels (0.8 mm^2) shown superimposed on a standard atlas diagram (Paxinos and Watson, 1986) corresponding to the slice (to scale). The slice was 2 mm thick and 3 mm anterior to the centre of the chamber, which is drawn in projection. Data are superimposed from three successive KCl applications, producing three waves of spreading depression as shown by simultaneous voltage records **(c).** MR signal changes **(b)** are in the same order top to bottom as shown in **(a)** and are expressed as percentage differences from the mean for the same pixel throughout the records shown. Data are omitted for clarity from images 2–4 on two of the runs and from image 6 on the third run, since these images contained artifacts due to movement or interference.

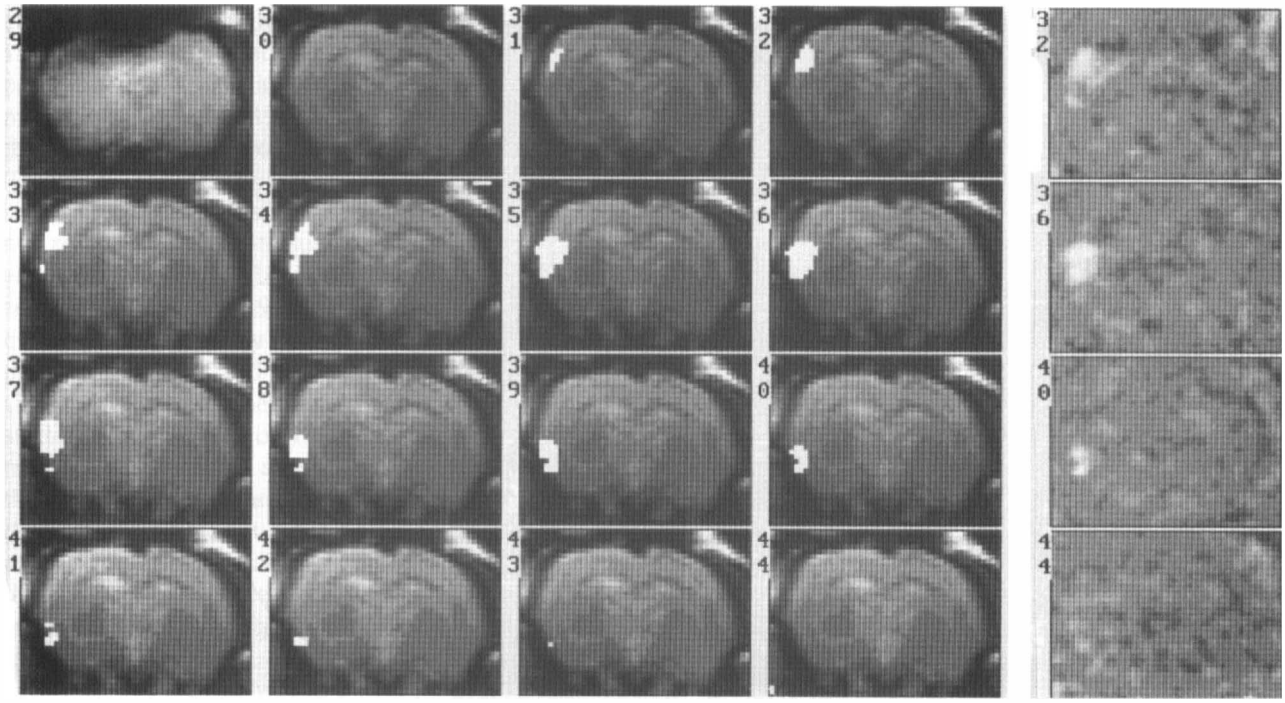

FIG. 2. Distribution of zones of increased MR signal intensity in successive images (at 12.2-s intervals) following induction of spreading depression as in Fig. 1. White zones are those in which each image exceeds a reference image (no. 27), taken prior to the sequence shown, by more than 5.5% of the average intensity over the whole brain. A single raw gradient echo image (no. 29) is shown in the top left panel. Regions above threshold for images 30–44 are shown in the remainder of the left-hand four columns, superimposed on a conventional (spin-echo) image to reveal the relationship to brain structure. Grey-level maps of the difference between images 32, 36, 40, 44, and the reference image are shown in the right-hand column. KCl was applied during images 12–15 (finishing 3 min before the start of the illustrated sequence), and the electrically monitored SD wave began during image 30. Images were smoothed in time and space before subtraction, using a kernel (0.25, 0.5, 0.25) in t, x, y; i.e., each pixel value is replaced by the sum of its own value times 0.5 plus the values of its immediate neighbours times 0.25, repeating the operation for the temporal sequence of images and for each spatial dimension. MR imaging parameters are as follow: TR, 80 ms; TE, 40 ms; 0.3-mm pixels, NEX, 2; 1-mm slice thickness; 64 phase-encoding steps; imaging time, 12 s per image.

electrical changes or propagating MR imaging changes, though image artifacts due to the movement were seen in the image taken during the flushing. Propagation of SD waves away from the chamber was verified with voltage recordings between a 2-mm-diameter subdural Ag-AgCl ball electrode 2 mm anterior to the bregma and a s.c. reference electrode in the neck. An even layer of Agar gel (3% in 0.9% NaCl) was placed around the apparatus on the skull to reduce magnetic susceptibility artifacts. The level of anaesthesia was reduced to 1–1.5% halothane in oxygen and maintained at this level for the duration of the MR imaging experiments. MR imaging data were acquired using a 2.35-T horizontal magnet (Oxford Instruments, Oxford, United Kingdom) interfaced to an SMIS console (Surrey Medical Imaging Systems, Surrey, United Kingdom). MR imaging experiments were performed with the rats held rigid in a plastic frame so that the parietal surface of the skull was horizontal within the magnet. Core temperature and respiratory rate were monitored throughout, and the rats were kept warm (33–38°C) with water or air circulation. Radio-frequency pulses were delivered by a cylindrical whole-body transmitter coil and received with a 2.5-cm-diameter surface coil placed ~2 mm above the skull surface. Gradient echo MR images were obtained every 12–30 s in either a vertical coronal section (Figs. 1 and 2), a parasagittal section, or a horizontal section including the superficial cortex in plan view (Fig. 3). Imaging parameters were typically: repetition time (TR), 80–100 ms; echo time (TE), 30–40 ms; either 128 or 64 phase-encoding steps and two averages per step (NEX); field of view (FOV), 40 mm; slice thickness, 1 or 2 mm. The pulse angle was adjusted to give maximum signal on control images. A pilot series of spin-echo images was acquired to locate the slice of interest.

RESULTS AND DISCUSSION

Within 2 min of application of KCl, we observed a zone of increased signal intensity (3–15%) on the MR image, up to 2 mm across, lasting ~1 min and propagating away from the site of SD initiation for distances up to 9 mm. With coronal sections close to the initiation chamber, the zone of increased signal intensity was restricted to the superficial 1–2 mm of the brain and moved in a curve through temporal cortex (Figs. 1 and 2), usually as far as the rhinal fissue (~9 mm from the centre of the chamber). The time courses of three successive waves in one animal are shown in Fig. 1. Sometimes the zone propagated part of this distance and then subsided, varying sometimes between successive waves in the same animal. We saw no consistent indications

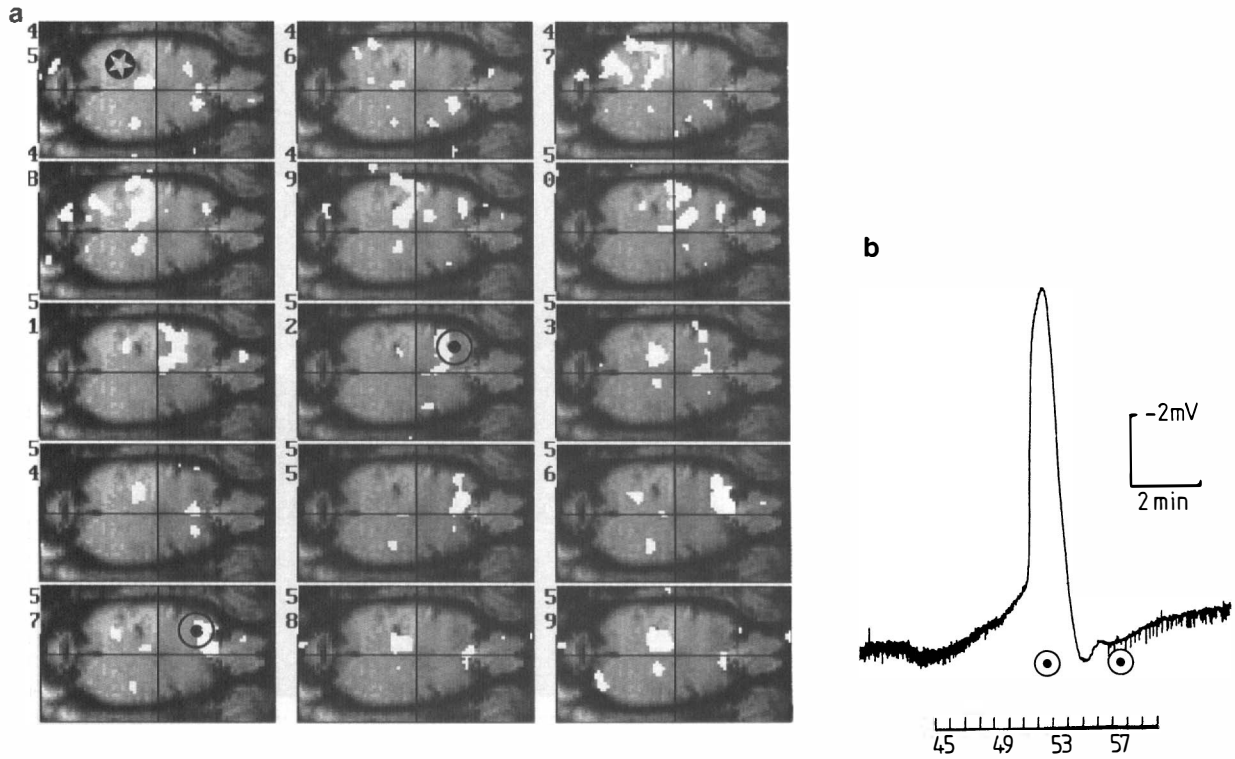

FIG. 3. a: Threshold gradient echo MR images from a horizontal slice through the superficial cortex, superimposed on a high-resolution gradient echo image of the same slice. Images were acquired every 25 s. KCl was applied at the starred region during images 40–42, ~2 min before the start of the sequence shown. **b:** Voltage record shows SD recorded at the monitoring electrode. The approximate times at which the zone of enhanced MR signal reached and passed beyond the electrode site are shown by circled dots, and the site of the electrode is marked on the corresponding images by the same symbol. Signal-to-noise ratio was improved by smoothing images four times in time and three times in space with the kernel given for Fig. 2 and thresholding the second time derivative of the resulting sequence. Thresholds correspond approximately to a 2% signal increase. MR imaging parameters are as follow: TR, 90 ms; TE, 30 ms; 0.3-mm pixels; NEX, 2; 1-mm slice thickness; 128 phase-encoding steps; imaging time, 25 s per image.

of zones of increased intensity entering the entorhinal cortex at the base of the brain, hippocampus, thalamus, or the contralateral side of the brain. We occasionally saw large and variable signals close to the midline in small zones around the sagittal sinus or third ventricle, more prolonged than the changes propagating through the cortex, with both increases and decreases of intensity; these changes may have been due to tissue displacement, due to edema, or blood flow changes.

The mean propagation velocity for 27 of these waves of increased signal intensity in seven animals, corrected for temperature variation to 38°C with a Q_{10} of 1.7 (Bureš et al., 1974), was 2.79 mm/min, with the means (±SD) in individual animals averaging 2.90 ± 0.46 mm/min. The duration of the changes varied from site to site in the range of 1–2 min, longer than the negativity recorded at the monitoring electrode (0.5–1 min).

Signals with the best signal-to-noise ratio during SD were obtained for the temporal cortex seen in coronal sections (Figs. 1 and 2). This tissue is normally inaccessible for study of SD with invasive techniques. The poorer quality of data from superficial cortex is at least in part due to artifactual distortion of the images in the regions of the electrode and chamber (Fig. 2, top left panel), with the loss of gradient echo MR signal due to the magnetic susceptibility effects of neighbouring boundaries. This limitation prevented us from obtaining data from cortical regions immediately under the chamber and lessened the accuracy with which we could study the time relationship between the voltage changes and the propagating wave, as seen on the MR image, in the neighbourhood of the electrode. We were able to reduce but not eliminate these artifacts by using an even layer of Agar gel (3% in 0.9% NaCl) surrounding the apparatus on the skull. We could then see waves of SD in plan view propagating away from the chamber, restricted to the ipsilateral cortex, past the anterior superficial monitoring site (Fig. 3). The increase of MR signal in the neighbourhood of the electrode site occurred within about 30 s of the onset of negativity (Fig. 3b).

Gradient echo MR imaging sequences have been shown to be sensitive to alterations in the oxygen-

ation state of haemoglobin within the local cerebral vasculature (Ogawa et al., 1990*a*). The presence of paramagnetic deoxyhaemoglobin causes loss of signal (Thulborn et al., 1982) due to spin dephasing in the resulting local field gradients near blood vessels (Ogawa et al., 1990*b*). The increase in signal intensity that we have observed with SD could thus be due to an increase of tissue oxygenation and arterialization of venous blood, which have been observed in SD (Leão, 1944*b;* Back et al., 1993). However, there are many profound changes of tissue physiology in SD, and we cannot rule out other possible mechanisms. The pulse sequence used in these studies has a degree of T1-weighting and, as such, may be sensitive to alterations in cerebral blood flow due to inflow of unsaturated spins from outside the image volume. It remains to be seen to what extent alterations in deoxyhaemoglobin concentration of the tissue, cerebral blood flow, or some other aspect of SD contribute to signal changes seen in these experiments.

If SD can occur in human cortex, it would readily explain some symptoms (fortification spectra, scotomata, etc.) in the aura of classical migraine (Leão and Morison, 1945; Milner, 1958). A common pattern of symptoms relates to profound cortical disturbance that propagates at a steady rate of ~3 mm/min through limited regions of human cortex (Lashley, 1941). If this disturbance is indeed SD, we must suppose that much of the cortex in humans is not susceptible to SD propagation, as has been shown for other primates (Marshall, 1959). It is therefore perhaps not surprising that recordings during neurosurgery rarely provide evidence of SD propagation (Blau, 1992; Gloor, 1986). SD-like events may occur in particular circumstances and particular brain regions and may, even in humans, contribute to secondary insults causing damage following stroke or trauma (Iijima et al., 1992). If so, adequate study of such transient disturbances would benefit from noninvasive techniques such as we have employed here.

Acknowledgment: Supported by the Wellcome Trust, Imperial Cancer Research Fund, Maxime Hanss Prize, the Medical Research Council, and the Harold Bridges Foundation.

REFERENCES

Back T, Kohno K, Radermacher B, Fischer M, Hossman K-A (1993) Characterisation of tissue oxygenation and spreading depression-like DC shifts in the penumbra of focal ischemic rats. *J Cereb Blood Flow Metab* 13(suppl 1):S83

Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS (1992) Time course EPI of human brain function during task activation. *Magn Reson Med* 25:390–397

Blau JN (1992). Migraine: Theories and pathogenesis. *Lancet* 339:1202–1207

Bureš J, Burešová O, Křivánek J (1974) *The Mechanisms and Applications of Leão's Spreading Depression of Electroencephalographic Activity*. Prague, Academia

Gardner-Medwin AR (1992) Magnetic and impedance measurements for detection of spreading depression at a distance. *Exp Brain Res* 23:63–74

Gill R, Andiné P, Hillered L, Persson L, Hagberg H (1992) The effect of MK-801 on cortical spreading depression in the penumbral zone following focal ischaemia in the rat. *J Cereb Blood Flow Metab* 12:371–379

Gloor P (1986) Migraine and regional cerebral blood flow. *Trends Neurosci* 9:21

Hansen AJ, Lauritzen M (1984) The role of spreading depression in acute brain disorders. *An Acad Bras Cienc* 56:457–479

Iijima T, Mies G, Hossman K-A (1992) Repeated negative DC deflections in the rat cortex following middle cerebral artery occlusion are abolished by MK-801: Effect on volume of ischemic injury. *J Cereb Blood Flow Metab* 12:727–733

Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R, Cheng H-M, Brady TJ, Rosen BR (1992) Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci USA* 89: 5675–5679

Lashley KS (1941) Patterns of cerebral integration indicated by the scotomas of migraine. *Archs Neurol Psychiat Chicago* 46:331–339

Lauritzen M, Olesen J (1984) Regional cerebral blood flow during migraine attacks by xenon-133 inhalation and emission tomography. *Brain* 107:447–461

Leão AAP (1944*a*) Spreading depression of activity in the cerebral cortex. *J Neurophysiol* 7:359–390

Leão AAP (1944*b*) Pial circulation and spreading depression of activity in the cerebral cortex. *J Neurophysiol* 7:391–396

Leão AAP, Morison RS (1945) Propagation of spreading cortical depression. *J Neurophysiol* 8:33–45

Marshall WH (1959) Spreading cortical depression of Leão. *Physiol Rev* 39:239–279

Milner PM (1958) Note on a possible correspondence between the scotomas of migraine and spreading depression of Leão. *EEG Clin Neurophysiol* 10:705

Nedergaard M (1988) Mechanisms of brain damage in focal cerebral ischemia. *Acta Neurol Scand* 77:81–101

Nedergaard M, Astrup J (1986) Infarct rim—effect of hyperglycaemia on direct-current potential and [14c]2-deoxyglucose phosphorylation. *J Cereb Blood Flow Metab* 6:607–615

Ogawa S, Lee T-M, Kay AR, Tank DW (1990*a*) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci USA* 87:9868–9872

Ogawa S, Lee T-M, Nayak AS, Glynn P (1990*b*) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magn Reson Med* 14:68–78

Ogawa S, Tank DW, Menon R, Ellermann JM, Kim S-C, Merkle H, Ugurbil K (1992) Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci USA* 89:5951–5955

Paxinos G, Watson C (1986) *The rat brain in stereotaxic coordinates* (2nd ed). London, Academic Press

Thulborn KR, Waterton JC, Matthews PM, Radda GK (1982) Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochim Biophys Acta* 714:265–270

van Bruggen N, Gardner-Medwin AR, Ahier R (1993) Propagating waves of spreading depression demonstrated by magnetic resonance imaging in the anaesthetised rat. *J Physiol (Lond)* 467:318