Cortical spreading depression in the feline brain following sustained and transient stimuli studied using diffusion-weighted imaging

Daniel P. Bradley*†, Justin M. Smith*†, Martin I. Smith‡, Kurt H.-J. Bockhorst*, Nikolas G. Papadakis*, Laurance D. Hall†, Andrew A. Parsons‡, Michael F. James‡ and Christopher L.-H. Huang*

*Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG, †Hercul Smith Laboratory for Medicinal Chemistry, University of Cambridge, Robinson Way, Cambridge CB2 2PZ and ‡Neurology Centre of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, Harlow, Essex CM19 5AW, UK

Cortical spreading depression (CSD) was induced by transient (10 min) applications of KCl in agar upon the cortical surface of α-chloralose anaesthetised cats. Its features were compared with CSD resulting from sustained applications of crystalline KCl through a mapping of the apparent diffusion coefficient (ADC) using diffusion-weighted echo planar imaging (DWI) over a poststimulus period of 60–100 min. Individual CSD events were computationally detected with the aid of Savitzky–Golay smoothing applied to critically sampled data derived from regions of interest (ROIs) made up of $2 \times 2$ pixel matrices. The latter were consistently placed at three selected sites on the suprasylvian gyrus (SG) and six sites on the marginal gyrus (MG). The CSD events thus detected were then quantitatively characterised for each ROI using the original time series. Both stimuli consistently elicited similar spreading patterns of initial, primary CSD events that propagated over the SG and marginal MG and were restricted to the hemispheres on which the stimuli were applied. There followed secondary events over smaller extents of cortical surface. Sustained stimuli elicited primary and secondary CSD events with similar amplitudes of ADC deflection that were distributed around a single mean. The ADC deflections were also conserved in peak amplitude throughout the course of their propagation. The initial primary event showed a poststimulus latency of 1.1 ± 0.1 min. Successive secondary events followed at longer, but uniform, time intervals of around 10 min. Primary and secondary CSDs showed significantly different velocities of conduction (3.32 ± 0.43 mm min$^{-1}$ vs. 2.11 ± 0.21 mm min$^{-1}$, respectively; $n = 5$) across the cerebral hemisphere. In contrast, transient stimuli produced significantly fewer numbers of CSD events (3.8 ± 0.5 events per animal, $n = 5$) than did sustained stimuli (7.4 ± 0.5 events per animal, mean ± S.E.M., $n = 5$, $P = 0.002$). The peak ADC deflection of their primary CSD events declined by ~30% as they propagated from their initiation site to the interhemispheric boundary. The primary CSD event following a transient stimulus showed a latency of 1.4 ± 0.1 min. It was followed by successive and smaller secondary ADC deflections that were separated by progressively longer time intervals. Conduction velocities of secondary events were similar to those of primary events. Conduction velocities of both primary and secondary events were slower than their counterparts following a sustained stimulus. ADC changes associated with CSD thus persist at times well after stimulus withdrawal and vary markedly with the nature of the initiating stimulus even in brain regions remote from the stimulus site.

(Corresponding author: C. L.-H. Huang: Physiological Laboratory, Downing Street, Cambridge CB2 3EG, UK. Email: clh11@cam.ac.uk)
and resulting in headache (Hardebo, 1991; Moskowitz & MacFarlane, 1993; Lauritzen, 1994). However, few clinical studies have successfully demonstrated CSD in man (Mayevsky et al. 1996), except indirectly through its vascular consequences (Cao et al. 1999; Hadjikhani et al. 2001). Nevertheless, experimental CSD has been induced empirically (Marshall, 1959; Bureš et al. 1974; Somjen, 2001) by a wide range of agents, including single (Piper et al. 1991) and repetitive (Ebersberger et al. 2001) mechanical injury and chemical (James et al. 1999; Kuge et al. 2000) or anoxic stimuli (Fabricius et al. 1993) in both feline and rodent brain. Furthermore, DC recording electrode arrays successfully detected a propagation of spreading depression (SD) events through layers VI–I in neocortical slices from epileptic human brain after introduction of 3 M KCl solution into layer VI. However, the amplitude of the DC deflection appeared to vary with distance from the stimulus (Gorji et al. 2001).

The extent to which brain from normal animal systems might model the clinical phenomenon could vary with gyrencephalic or lissencephalic brain type (Marshall, 1959). For example, CSD propagation decelerates from 6–8 mm min⁻¹ on gyri to 1–2 mm min⁻¹ in sulci of gyrencephalic porcine brain (Bowyer et al. 1999b). In contrast, SD events in the lissencephalic rabbit brain propagated with a constant velocity of 3.5 mm min⁻¹ (Bowyer et al. 1999a). Furthermore, the ratio of glia to neurons is proportional to brain size (Tower & Young, 1973): glial cells are thought to protect against spreading depression (Szerb, 1991; Largo et al. 1996) by buffering increases in [K⁺], [Na⁺] and extracellular glutamate concentrations (Syková, 1992, 1997; Froes & Campos de Cavalcio, 1998; Zahs, 1998). Finally, CSD propagation involves astrocytic gap junctions (Mantz et al. 1993); the uncoupling agent halothane inhibits SD propagation in the gyrencephalic feline brain (Saito et al. 1995; Piper & Lambert, 1996) and reduces the frequency of SD events in a dose-dependent manner in the lissencephalic rodent brain (Kitahara et al. 2001).

Recent experiments have accordingly used the gyrencephalic feline rather than rodent brain to model human CSD (James et al. 1999; Bockhorst et al. 2000). CSD was detected through measurements of water mobility (self-diffusion) in horizontal cortical planes using diffusion-weighted echo planar imaging (DWI). CSD is associated with large transient increases in [K⁺], [Na⁺] and glutamate. These are accompanied by large decreases in extracellular [Ca²⁺], [Na⁺] and [Cl⁻] and significant transmembrane fluxes of water (Nicholson & Kraig, 1981; Fabricius et al. 1993). The apparent diffusion coefficient (ADC) of water can be measured using DWI. The source of the ADC signal deflection resulting from CSD comes from changes in diffusional properties of both the intra- and extracellular compartments (Duong et al. 1998). Thus, recent studies demonstrated a pattern of regions showing transient reductions in ADC radiating from the site of stimulus application across the cerebral hemisphere but ceasing at the inter-hemispheric boundary during CSD (James et al. 1999).

Experimental CSDs may also differ from their possible clinical counterparts owing to the nature of the initiating stimuli. The known anti-migraine compounds dihydroergotamine, acetylsalicylic acid, lignocaine, metoprolol, clonazepam and valproate failed to alter CSD propagation or the accompanying increases in cerebral blood flow when this was triggered by mechanical stimuli in the cat brain (Kaube & Goadsby, 1994). Other experiments achieved reproducible and measurable CSDs following large and sustained KCl stimuli (James et al. 1999; Bockhorst et al. 2000; Kuge et al. 2000). However, there is the possibility that the delayed secondary responses that followed the initial, primary event actually resulted from a persistent stimulus rather than a continued pathological process. Thus, a sustained presence of KCl produces a prolonged depolarisation (Herreras & Somjen, 1993) in the surrounding tissue; the resulting situation might more closely resemble the irreversible changes following ischaemia or head injury (Nilsson et al. 1993) rather than the reversible and temporary changes expected in migraine aura.

The present experiments accordingly compared the characteristics of CSD events detected by DWI produced by sustained or transient (10 min) agar/KCl stimuli applied to the feline brain and the features of their propagation over the gyrencephalic brain surface in two dimensions. We sought to determine whether different types of CSD exist or not and if these events were different at regions remote from the site of stimulus application. The characteristics of CSD have not been investigated in detail in a gyrencephalic brain and may well be greatly affected by the nature of the initiating stimulus. DWI offers the advantage of measuring changes over many voxels across a two-dimensional plane of the brain surface. Additionally, due to good signal and physiological stability, magnetic resonance imaging (MRI) measurements were made over a long duration (>2 h). They demonstrated that the different stimuli although produced by a similar chemical agent resulted in initial (primary) and subsequent (secondary) events that sharply differed with respect to the changes they generated in the values of their ADCs, their conduction velocities and the total number of events. These differences were observed even at regions remote from the stimulus site and could only be demonstrated using the MRI methods that made it possible to visualise the spread of CSD events in two dimensions. They complement recent results of CSD propagating through different layers within a given region of human neocortex (Gorji et al. 2001).
METHODS

Animal preparation

The experiments conformed to all aspects of the Animals (Scientific Procedures) Act (1986) and were ethically approved by the GlaxoSmithKline Procedures Review Panel. Most details of the experimental procedures have been previously published (James et al. 1999; Bockhorst et al. 2000). Briefly, non-recovery anaesthesia was induced in overnight fasted female cats (n = 10, weight 3.4 ± 0.3 kg; mean ± S.E.M.) using 4–5% halothane in oxygen. Cephalic vein cannulation allowed immediate injection of the maintenance anaesthesia using x-chloralose (100 mg kg⁻¹ I.V.) that subsequently replaced the halothane: the latter quenches CSD in feline species (Saito et al. 1995; Piper & Lambert, 1996). Animals were mechanically ventilated at 20–24 ml min⁻¹ and their core temperature monitored using a rectal probe and maintained at 37°C using a homeothermic blanket throughout the experimental procedure. The femoral vein was cannulated to permit the administration of supplementary anaesthesia as necessary throughout the MRI experiments. The femoral artery was cannulated to permit sampling for blood gas analysis and continuous blood pressure monitoring.

Animals were placed in a custom built stereotaxic frame and ear and eye bars fixed their external acoustic meatus and zygomatic bone, respectively, to minimise rotational and translational movements of the head. The surface of the scalp was then retracted to expose the cranium. Craniotomies made in the sphenoid/ frontal sinuses were filled with a sterile mixture of saline in agar to reduce image distortions and signal loss resulting from tissue–air interfaces during the DWI sequences. A craniotomy was performed over the right suprasylvian gyrus (SG), 1.0 cm lateral to the midline and 1.5 cm posterior to the bregma. To minimise dehydration and to avoid dural trauma during surgery, saline-saturated collagen paper was placed over the exposed dura. Our initial experiments confirmed that brain DC surface potential changes correlate closely with the occurrence of the ADC changes associated with SD (see also Gardner-Medwin et al. 1994; James et al. 1999). Such DC recordings were thus discontinued in later experiments to reduce magnetic susceptibility problems resulting from the presence of the silver recording electrode in the imaging field. A plastic-coated disposable receive surface coil (internal diameter ~3 cm) was bonded symmetrically to the skull surface using cyanoacrylate adhesive. Silastic medical adhesive was used as a supplement and built up in layers around the surface coil forming a well that was then filled with mineral oil. The dura was removed to expose the cortical surface and the well immediately filled with mineral oil through a cannula fixed to the Silastic well, to minimise dehydration of the exposed cortex. The animal was then removed from the steel stereotaxic frame and manoeuvred into a non-magnetic Perspex equivalent. This was then placed in a Perspex bed with built in transmit sine-spaced resonator.

The experiments that delivered sustained stimuli (n = 5) placed a 30 mg KCl crystal upon the surface of the SG through the cranial window and left it in place throughout the duration of the MR experiment. The transient stimulus used a KCl concentration of 300 mM in a KCl–agar solution. These experiments (n = 5) involved the extrusion of ~7 µl of the agar onto the cortical surface through a cannula that was attached to a ramp. The agar cannula was connected to a 50 µl Hamilton syringe outside the bore of the magnet. The KCl–agar remained in contact with the surface of the brain for 10 min only, after which it was retracted.

MR measurements

Image acquisition used a whole-body 2-T superconducting magnet (Oxford Instruments, Oxford, UK) interfaced to a MSL 400 console running Tomikon (Karlsruhe, Germany) software. The reduced bore gradient set was 20.5 cm in diameter and of Maxwell-Golay design. Image localisers were followed by a high resolution multi-slice multi-echo horizontal image slice acquired through both the SG and MG to provide reference sections for comparison with the DWI data that were used for the production of ADC maps. An optimised two-point DWI technique for ADC determination (Xing et al. 1997) used an effective repetition time (TR) of 28 s. The TR represents the time between the pairs of low- and high-b images, i.e. the time for each ADC measurement. Additional imaging parameters were: echo-time (TE) 64 ms, field of view (FoV) 50–60 mm, slice thickness (STh) 3 mm, pixel matrix 64 × 64; the latter was zero-padded to a 128 × 128 matrix prior to Fourier transformation. This looped sequence employed two diffusion-weighted image acquisitions along the z-direction. These used low and high b values of 123 mm² s⁻¹ and 897 mm² s⁻¹, respectively, with a diffusion gradient duration 10 ms and interval time between gradients 39 ms in both cases. Horizontal DWI slices were initially acquired across superficial cortical layers of the MG and SG for 30 min.

The resulting high- and low-b images were then transferred to a Linux Pentium III workstation and processed using customised software (CamRes, Herchel Smith Laboratory for Medicinal Chemistry, University of Cambridge, UK). Regions of interest (ROIs) were defined in both the SG and MG of both experimental (ipsilateral) and control (contralateral) cerebral hemispheres. A series of initial quality assurance ADC measurements performed on the experimental preparation confirmed an absence of artefacts prior to commencement of the definitive experimental measurements. The control imaging period then began 20 min before stimulus application. The experimental preparation within the Faraday cage was accessed 10 min before stimulus application in order to minimise external RF noise artefacts that might affect image quality at the time of KCl application.

The experiments that applied a sustained stimulus briefly interrupted the data acquisition when the KCl crystal was deposited on the surface of the SG where it remained throughout the 60–100 min duration of the experiment. The experiments that used the transient stimuli extruded KCl–agar onto the brain surface using the Hamilton syringe without interruption of the imaging sequence. This was left in place for 10 min and mineral oil was then injected onto the brain to lift the KCl–agar away from the brain surface. The KCl–agar was then retracted from the cranial window by withdrawing the same ejected volume. DWI was continued for a further 90 min.

Finally, a high-resolution T1-weighted 3D spoiled gradient (SPGR) image obtained at the end of the experiment confirmed that brain anatomy was normal in all animals in both groups. In addition the condition of the exposed brain surface was visually checked. The animals were killed at the end of each experiment with an overdose of sodium pentobarbital without recovery from anaesthesia (in accordance with schedule 1, UK Home Office Regulations).

Data analysis

Data were transferred from the MSL-400 console to a Pentium III processor GNU/Debian Linux workstation. The DW images were reconstructed using a reference scan for N/2 ghost minimisation.
Low- and high-\(b\) DW images were concatenated into separate time lapse movies to identify motion artefacts or significant contrast changes, particularly those resulting from RF interference whilst access was being made to and from the Faraday cage. Images displaying such signal artefacts were removed. Data analysis involved calculation of ADC maps from the high and low-\(b\) DWIs, statistical mapping of the resulting ADC maps and plotting ADC changes with time in selected ROIs. The high-\(b\) movies were used to assess qualitatively whether CSD induction was successful; CSD produced hyperintense patterns propagating from their sites of initiation.

Firstly, the spatial patterns with which the CSD events spread over the cortical surface were mapped by applying a running \(t\) test \((P < 0.05,\) Bonferroni corrected) to the time series of the ADC changes as represented in each image pixel using programs written in Interactive Data Language (IDL, Research Systems, Boulder, Colorado, USA). Pixel-by-pixel comparisons were made of successive pairs of ADC images against the remaining frames in the time series. Pixels exhibiting significant changes, whether positive or negative with respect to the mean ADC value in any given frame were false coloured and superimposed on an averaged high-\(b\) image map. The resulting \(t\)-maps thus displayed both the geometry of the cortical areas affected by the CSD and its pattern of spread using NIH Image (National Institutes of Health, Bethesda, MD, USA). The statistical analyses (Student’s \(t\) test, ANOVA and the Kruskal-Wallis test) were performed using Microsoft Excel in conjunction with Analyse-it (version 1.61, Analyse It Software, Ltd, Leeds, UK) and graphs produced by SigmaPlot (SigmaPlot for Windows version 4.00, SPSS Inc, Chicago, IL, USA). A \(P\) value of < 0.05 was considered to be statistically significant. All values are presented as means ± standard errors of the mean (mean ± S.E.M.), unless otherwise stated.

Secondly, the following signal analysis was applied to ROIs made up of groups of 2 \(\times\) 2 pixels for the detection and characterisation of the ADC changes associated with CSD. Three and six such ROIs were selected at fixed positions in the SG and MG, respectively. Such a ROI size represented a compromise between the analysis of data from single pixels smoothed over space and time (Bockhorst et al. 2000) or of extracting biological signals from averaged values derived from larger ROIs (James et al. 1999; Kuge et al. 2000). The former approach would result in lower signal to noise ratios (SNR) and would exaggerate the effect of slight motion artefacts; the latter method would attenuate CSD events that extended over a small number of pixels. The latter was frequently the case for the secondary CSD events. The time series derived from such 2 \(\times\) 2 pixel ROIs were subject to a 3rd order, 3 point Savitzky-Golay smoothing algorithm (Gorry, 1990) to reduce high frequency and random noise but preserve higher order moments of the data, such as peak heights or troughs. This was followed by a semi-automated threshold detection of those deflections that exceeded two standard deviations from the mean (IDL, Research Systems, Boulder, CO, USA).

The original time series ADC data were then used to derive: (1) the value of peak ADC deflection produced by each CSD event; (2) the latency and the time to peak deflection of the ADC response; and (3) the time intervals separating both the primary wave and the subsequent CSD waves and successive individual waves. Finally, the propagation velocities of the CSD events were deduced from the timings of the peak ADC deflections and the positions of the ROIs in which they occurred.

### RESULTS

Physiological variables were similar in both experimental groups and remained within normal limits throughout each experiment. The animals in which CSD was initiated by a sustained stimulus showed the following values: mean arterial blood pressure (MABP) 87 ± 11 mmHg, arterial pH 7.34 ± 0.02, \(P_{O_2}\) 81 ± 3.8 mmHg and \(P_{CO_2}\) 31 ± 2.1 mmHg (mean ± S.E.M., \(n = 5\)). For the group in which CSD was triggered with a transient stimulus, MABP was 89 ± 9 mmHg, arterial pH 7.36 ± 0.05, \(P_{O_2}\) 80 ± 2.9 mmHg and \(P_{CO_2}\) 29 ± 3 mmHg \((n = 5)\). The long imaging times necessitated periodic maintenance administrations of intravenous \(\alpha\)-chloralose anaesthetic through the femoral vein cannula. These produced transient increases in MABP that nevertheless consistently returned to pre-dose baseline values within 2.5 min and were not associated with significant alterations in MR signal.

To validate the MR diffusion values the looped DWI sequence was run for 90 min prior to the CSD experiment on a CuSO\(_4\)-doped water phantom (relaxation times: T\(_1\) 300 ms, T\(_2\) 280 ms). ADC values were measured and calculated as 218 \((\pm 23) \times 10^{-2} \text{ mm}^2 \text{ s}^{-1}\) \((n = 10)\). This was in agreement with previous reports (Atkins, 1983). The SNR and baseline stability were measured in the feline brain to confirm the quality of images acquired during each CSD experiment. SNRs were derived from the signal from 2 \(\times\) 2 pixel ROIs that were selected on the marginal gyrus of the contralateral hemisphere \((n = 10)\) and within the image background \((n = 10)\). These gave values of 35 ± 4.2 and 21 ± 3.5 for the low- and high-\(b\) images, respectively. Additionally, the signal drift derived from comparing the ADC values in the first and last 5% of images was 1.3 ± 3.4% \((n = 10)\).

ADC values were also measured in ROIs from selected sites on the MG in the contralateral hemisphere in each experiment. These ADC values remained stable throughout the acquisition period and therefore both provided control values for each imaging session and confirmed that the CSDs had not invaded the contralateral hemisphere. In addition, three ROIs were selected in areas on the ipsilateral MG that did not show repeated CSD event activity (> 1 CSD event) as reflected in the statistical \(t\)-maps created from the ADC images. ADC measurements were obtained from such ROIs before and after the first CSD event and these gave values that were indistinguishable from corresponding values obtained at the contralateral side. From the same ROIs, the coefficient of variation (CoV) was calculated to provide a percentage mean of the standard deviation (S.D.) in the ADC signal during these baseline periods. Table 1 accordingly demonstrates that there were no significant differences in baseline values between the two stimulus groups and ADC values obtained before and after CSD stimulation in either group. Furthermore, there were no differences in CoV after either stimulus or in any region of the brain.
CSD following transient and sustained stimuli affect similar areas of cerebral cortex

The images in Fig. 1 compare typical patterns of propagation of primary CSD waves over the cortical surface after the application of sustained (A) and transient stimuli (B and C). They display $t$ values as false colours derived from statistical testing of ADC values in time series represented by individual pixels from successive images that were acquired following stimulus application. The $t$-maps are superimposed on the high-$b$ DW images so that ADC changes could be localised on cerebral anatomy. Figure 1A and B shows single, illustrative frames obtained at 4 min after the sustained stimulus and 11.35 min after the transient stimulus, respectively. The primary events that followed either stimulus spread concentrically from the stimulus site (arrows, Fig. 1A and B) as extensive and

---

Table 1. Baseline values of the cerebral apparent diffusion coefficient (mean ± S.E.M., $\times 10^{-6}$ mm$^2$ s$^{-1}$) and the coefficients of variation (CoV, (S.D./mean) $\times 100\%$).

<table>
<thead>
<tr>
<th>Regions of interest (ROI)</th>
<th>Sustained stimuli</th>
<th>Transient stimuli</th>
<th>Sustained stimuli</th>
<th>Transient stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control contralateral hemisphere</td>
<td>866 ± 17</td>
<td>881 ± 24</td>
<td>4.0 ± 2.1</td>
<td>3.8 ± 1.9</td>
</tr>
<tr>
<td>Ipsilateral marginal gyrus, pre-CSD</td>
<td>895 ± 25</td>
<td>908 ± 32</td>
<td>2.8 ± 1.1</td>
<td>3.2 ± 2.0</td>
</tr>
<tr>
<td>Ipsilateral marginal gyrus, post-CSD</td>
<td>892 ± 23</td>
<td>900 ± 31</td>
<td>2.9 ± 2.0</td>
<td>4.5 ± 2.6</td>
</tr>
</tbody>
</table>

$n = 15$. Kruskal-Wallis ANOVA detected no significant differences between values.

---

Figure 1. Statistical ($t$ test) mapping analysis of the spread of CSD

A, single frame obtained from an animated mapping sequence following the induction of CSD by a sustained stimulus applied to the suprasylvian gyrus (yellow arrow). The primary event is seen traversing the marginal (MG) and anterior/dorsal boundaries of the suprasylvian gyrus (SG). B, similar frame from a sequence obtained following a transient stimulus. C, sequence of image frames representing the propagation of the primary event across the brain surface following a transient stimulus; a, 5 min after stimulus; b–o, successive images obtained at 28 s intervals with primary events crossing the suprasylvian sulcus at 9.5 min (l) ($t$ test map: red, $t = 15$; black, $t = 0$). Abbreviations: P, posterior; A, anterior; L, left; R, right. Scale bar 10 mm.
coherent wavefronts over the SG and MG. These were followed by secondary events (Bockhorst et al. 2000) that were more restricted in area and were largely confined to the suprasylvian gyrus.

Figure 1C shows a sequence of images, obtained at 28 s intervals, that followed the time course of the spread of a primary CSD event from 5 min following a transient stimulus. The features of CSD events triggered by transient stimuli appeared similar to those reported for sustained stimuli on earlier occasions (James et al. 1999). Thus, the primary CSD event spread in anterior, posterior and medial directions along the SG and reached the MG approximately 9.5 min after stimulus application (Fig. 1C.

**Figure 2. Extraction of the time course of CSD events from regions of interest (ROIs) based on t-map images**

A–C, mapping of primary CSD wave at intervals of 7, 10 and 11.8 min following stimulus application. Scale bar 10 mm. D–F, ADC traces derived from averaging of signals over each region of interest (ROI) shown in A–C, respectively, plotted against time. ROI sizes and geometry based on the trajectory and area affected by the primary event – shown as false coloured overlays. A and D map the geometrical extent of the primary event at an early stage on the SG and plot the corresponding ADC changes against time in that ROI demonstrating a total of three events, respectively. B and E and C and F do the same for regions that were invaded by the primary event at later stages in its propagation. In B, the primary CSD event is seen crossing into the marginal gyrus; the relevant ROI shows only two significant events over the sampling period (E). In C, the event is propagating across the marginal gyrus, yet the ADC trace in that corresponding ROI shows only one CSD event (F). The bar indicates the time over which the KCl–agar stimulus was applied. *Peak values different from the baseline to a significance level of $P < 0.05$. 
The primary CSD waves showed similar latencies whether stimuli were transient (1.1 ± 0.1 min; \( n = 5 \)) or sustained (1.4 ± 0.1 min; \( n = 5 \)). However, CSD wavefronts following transient stimuli often showed advancing and trailing boundaries that were smoother and less fragmented as they passed over the MG.

Figure 2 illustrates results from the analysis that determined the extent of cortical area affected by such CSD events. The ROIs were chosen to match the topographic regions defined by the \( t \)-maps representing primary CSD waves produced by a transient stimulus. These are superimposed on high-\( b \) DW images early (A), in the course of (B) and at the end of a primary CSD event (C), at 7 min, 10 min and 11.8 min after stimulus application, respectively. Figure 2D–F plots the mean ADC values that were obtained by averaging ADC values from the individual pixels within such relatively large ROIs as has been performed on earlier occasions (James et al. 1999; Kuge et al. 2000).

Each trace clearly demonstrates the primary event that followed the application of the transient stimulus. However ADC deflections produced by the subsequent secondary events appeared diminished in maximal amplitude. Such differences nevertheless could reflect the smaller and different cortical areas affected by secondary events and that would therefore result in a distorted representation of the absolute ADC changes (James et al. 1999). Thus, Table 2 compares the extents of cortical area that were affected by such primary or secondary waves following the sustained and the transient stimuli. These are expressed as a mean percentage of the total area of the ipsilateral hemisphere affected in each image frame. The primary events covered > 20% of the ipsilateral cortical surface area in each frame but secondary CSD events covered significantly smaller fractional areas of the cortical hemisphere (~7%) per frame (\( P < 0.05 \), Mann-Whitney \( U \) test). Thus, Fig. 3 shows typical plots of the percentage areas of ipsilateral hemisphere affected by either the initial primary or subsequent secondary CSD events against the frame number associated with the imposition, as indicated by the horizontal lines, of either sustained (Fig. 3A) or transient (Fig. 3B) stimuli. Both the sustained and transient stimulus elicited primary CSD events of similar extent and duration. In both cases, there followed a sequence of secondary events, each of which consistently affected smaller cortical surface areas. However, the sustained stimuli nevertheless produced significantly larger numbers of secondary CSD events (7.4 ± 0.5) than did the transient stimuli (3.8 ± 0.5) (\( P = 0.002 \), Mann-Whitney \( U \) test).

**Regional variations in CSD responses**
In addition to the differences in area of cortex affected by primary and secondary CSD events, the number of secondary events varied between different regions of cortex following either transient or sustained stimuli.

![Figure 3. Percentage surface areas of ipsilateral hemisphere affected by propagating CSD events over time after either a sustained or a transient stimulus](image)

Typical traces of the percentage area of imaged ipsilateral cortex affected by CSD events after application of either a sustained (A) or transient (B) stimulus. The horizontal (time) axis indicates frame number (each frame obtained at 28 s intervals). The traces illustrate a pattern of successive ADC changes following stimulus application beginning with a primary event and followed by secondary events that involve significantly smaller extents of cortical area. Sustained stimuli gave rise to a larger number of events than did transient stimuli (see text for details). The bar represents stimulus duration.
Figure 4. Geometrical patterns of ADC changes reflecting primary and secondary CSD events extracted from different ROIs placed at selected positions along the SG and MG

The T1-weighted image through the suprasylvian gyrus (SG) and marginal gyrus (MG) of the gyrencephalic cortex in horizontal section indicates positions (a–i) of ROIs consisting of 2 x 2 pixel areas chosen for analysis (inset). Scale bar 10 mm. a–i, the bars indicate the stimulus application period that was consistent through all the traces. Traces are of ADC against time from ROIs positioned on the ipsilateral SG (a–c) and MG (d–i); such a use of small ROIs resulted in the successful detection and characterisation of secondary events, which affected only small regions and consequently would not have been detected using the larger ROIs based on areas affected by primary events as illustrated in Fig. 3. The halo artefact surrounding the brain originated from the mineral oil around the surgical field and the paler area close to ROI (a) resulted from the cranial window to which the transient stimulus was applied.
Figure 4 illustrates the results from a typical analysis of ADC changes following a transient stimulus in which ADC values were deduced from small ROIs made up of 2 × 2 clusters of pixels. These were each selected at fixed points (a–i) on the ipsilateral MG (traces a–c) and SG (d–i) as shown on the inset image. The ADC time series obtained were subject to the Savitzky–Golay smoothing to minimise the high frequency noise while preserving major peaks and troughs. These demonstrated significantly larger numbers of ADC events than did the analysis using the large ROIs in Fig. 2. The individual traces also demonstrated that the number and amplitude of secondary CSD events varied between anatomical regions. Asterisks indicate significant ADC deflections exceeding 2 standard deviations from the mean value. These were absent from ROIs on the SG and MG on the contralateral, unstimulated hemisphere, indicative of an absence of CSD activity (data not shown). In contrast, comparisons of Fig. 4a–i demonstrates that different gyri showed different numbers of events. Regions within the MG continued to show the primary event (Fig. 4d–i) but showed fewer (1–4) secondary events than did ROIs in the SG (2–6 events). Furthermore, even though Fig. 4a–c were all obtained from the ipsilateral SG, Fig. 4a shows 6 significant deflections at a point adjacent to the stimulus site on the SG, but Fig. 4b and c shows different (2 and 4, respectively) numbers of events.

Local ADC changes at the site of stimulation
Application of different, transient or sustained, stimuli also produced contrasting alterations in the diffusional properties of brain areas close to the stimulus site. Figure 5A displays a typical horizontal section through the marginal (M) and suprasylvian gyri (S); the arrow marks the stimulation site and the open and filled squares respectively indicate adjacent (0.8–1.4 mm away) and more remote (1.4–1.7 mm away) ROIs from which ADC measurements were made. Figure 5B and C shows transverse sections of the feline brain obtained by high-resolution 3D-spoiled gradient (SPGR) T1-weighted MRI from an experiment in which the CSD was initiated by sustained (B) or transient (C) stimulus, respectively. The latter images were obtained immediately following the experimental DWI sequence, 90 min after stimulus application. The gyral and sulcal convolutions are characteristic of the gyrencephalic feline cortex. Such images consistently demonstrated hyperintense regions (arrowed) below the site of application of sustained (B) but not transient stimuli (C). These intensity changes were absent either prior to stimulus application or on the contralateral side and might therefore be most simply attributed to the local effects of a sustained presence of KCl (Herreras & Somjen, 1993). Thus, within the 2 × 2 pixel ROI adjacent to the site of the sustained stimulus (Fig. 5A, open square) there was a marked increase in ADC followed by a prolonged decay (Fig. 5D) with time and recognisable CSD events were absent. In contrast, the remote ROIs (e.g. Fig. 5E) showed ADC traces made up of stable baselines interrupted by a series of the negative deflections that have previously been associated with CSD activity (James et al. 1999; Bockhorst et al. 2000). Asterisks mark those deflections that exceeded 2 standard deviations (S.D.) from the baseline ADC.

In contrast, following transient stimuli, ROIs both close to (Fig. 5F) and remote from (Fig. 5G) the stimulus showed stable ADC baselines interrupted by negative deflections associated with the CSD phenomenon (James et al. 1999; Bockhorst et al. 2000). However, the successive secondary events declined progressively in magnitude and were separated by progressively increasing time intervals whether the ROIs were adjacent to or remote from the stimulus site. Thus, comparisons of Fig. 5D and F demonstrate ADC changes at regions close to the site of stimulus application and thus establish that different stimuli indeed produced different effects at the stimulus site. Furthermore, the findings in Fig. 5B and D confirm predictions made by Bureš et al. (1974) of the spread of changes following the sustained application of a high concentration of KCl solution to a fixed site on the brain. Yet a comparison of Fig. 5E and G even 1.4–1.7 mm away from the site of stimulus application indicate that the changes in steady state ADC are absent with either stimulus. Thus, local ADC changes in either case had fully decayed even at these distances.

ADC changes produced by primary waves of CSD
Figure 6 summarises typical measurements of the amplitudes of the ADC changes produced by primary CSD waves at different sites along their propagation path across the cortical surface. Figure 6A shows an image of a horizontal section through the SG and MG that indicates the three, typical, ROIs from which such ADC changes were measured. The first recording point (S) was made close (1.3–1.5 mm) to the stimulation site. The middle point (M) on the MG was adjacent to the sulcus separating the SG and MG, 2.6–2.9 mm away from the stimulus site. The end-recording site (E) was adjacent to the central sulcus on the MG, 5.6–5.8 mm away from the stimulus site. Figure 6B and Ci–iii shows traces of the corresponding ADC values, as measured from their respective ROIs, plotted against time. The plots are normalised to the baseline values that were obtained from the sequence of 10–20 frames that were acquired prior to stimulus

### Table 2. Size of cortical area affected during CSD event per frame (area of CSD visible in plane, as a percentage of ipsilateral hemisphere)

<table>
<thead>
<tr>
<th>Event type</th>
<th>Sustained</th>
<th>Transient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary event</td>
<td>22.1 ± 2a</td>
<td>23.6 ± 1.6b</td>
</tr>
<tr>
<td>Secondary event</td>
<td>7.2 ± 1.7a</td>
<td>6.6 ± 1.9b</td>
</tr>
</tbody>
</table>

a,bP < 0.05 (Mann-Whitney U test).

FIG. 5E
application. The primary ADC deflections showed progressively increasing latencies as would be expected from an existence of propagation delays from the site of stimulus application. Nevertheless, the amplitudes of the ADC deflections that followed the sustained stimuli were conserved with the distance from the point of stimulus application (Fig. 6B). In contrast, the amplitudes of such ADC changes that followed transient stimuli declined with distance (Fig. 6C). Figure 6D and E summarises the mean ADC deflections at the three different points along the propagation pathway as a result of the primary CSD event. Sustained stimuli gave statistically indistinguishable

![Image of Figure 5](image)

**Figure 5. Changes in apparent diffusion coefficient at regions of interest (ROIs) close to and remote from the site of application of the sustained and transient stimuli**

A, T1-weighted image displaying marginal (M) and suprasylvian (S) gyri in horizontal section, and the anterior (A) and posterior (P) ends of the cortex. The arrow marks the stimulus application site. The open and filled squares indicate the position of 2 × 2 pixel ROIs along the ipsilateral SG adjacent to and remote from the stimulus site. B, representative coronal T1-weighted brain slice showing marginal (M), suprasylvian (S) and ectosylvian gyri (E) obtained following an experiment in which CSD was induced by sustained application of solid KCl. Following application of the sustained stimulus, a hyperintense region developed below the stimulus site (hatched arrow). C, representative coronal T1-weighted section of brain from an animal in which CSD was initiated by a transient stimulus; such hyperintense regions below the stimulus site then were not observed. Scale bar 10 mm (A–C). D, ADC trace extracted from a ROI adjacent to the site of sustained stimulus (open square in A); ADC values were normalised to those obtained in the 10–20 frames acquired before stimulus application: ADC increased rapidly after stimulus application (frames 0–10) then declined steadily over time. E, ADC values at a region remote from the stimulus site on the SG (filled square in panel A) that show characteristic ADC waves (* denotes significant deflections; \( P < 0.05 \)). F, ADC trace obtained from a ROI adjacent to the site of transient stimulus demonstrating a typical ADC trace with multiple ADC deflections. One large primary event was initiated following stimulus application (bar over frames 35–60). Subsequent secondary events following stimulus withdrawal were much smaller in amplitude than the first event. G, ADC trace taken from a 2 × 2 pixel ROI remote from the stimulus site on the SD revealed a similar pattern of a relatively large deflection resulting from the primary CSD event followed by smaller subsequent events. D–G, large-dashed line indicates mean value; small-dashed lines indicate ± 2 standard deviations.
deflections of $26 \pm 2.1\%$ (S), $27 \pm 2.1\%$ (M), and $25 \pm 3.0\%$ (E) (mean ± S.E.M.; $n = 5$) respectively. Conversely, transient stimuli gave deflections of $36 \pm 3.2\%$ (S), $28 \pm 3.9\%$ (M) $25 \pm 2.5\%$ (E), resulting in a significant difference between values at sites S and E ($P < 0.05$, using a Kruskal-Wallis test for comparison of multiple, rather than paired non-parametric data sets).

ADC changes produced by secondary CSD events

The amplitudes of ADC deflections produced by the secondary events also varied with the nature of the stimulus. Figure 7 displays frequency histograms for the amplitudes of both primary (clear bars) and secondary events (hatched bars) after either sustained (A) or transient (B) stimuli. Sustained stimuli produced primary and secondary CSD events whose mean ADC deflections showed amplitudes of $24.7 \pm 1.5\%$ and $25.8 \pm 1.4\%$ (Fig. 7A) from baseline, respectively, that were not significantly different (Mann-Whitney U test, $P < 0.05$) and accordingly formed a single distribution of ADC amplitudes. In contrast, the amplitude histograms resulting from transient stimuli formed two distributions, in which the secondary events produced the smaller deflections

Figure 6. Investigation of the amplitudes of ADC events at different distances from the stimulus site after the application of a sustained or transient stimulus

A, high resolution image showing SG and MG in horizontal section denoting typical regions of interest (ROIs) chosen for measurements of ADC changes resulting from primary CSD events at the start (S), middle (M), and end (E) of their propagation, in order to detect and follow changes in their amplitude in the course of their propagation. ADC values were normalised to baseline control values obtained prior to stimulus application. B, normalised ADC changes in response to application of a sustained stimulus, obtained at the start (i), middle (ii) and end (iii) of their propagation. The break in the frame axis indicates times over which sampling was interrupted for stimulus application. The deflections in the ADC trace attributed to CSD events in the ROI concerned show a clear temporal shift along the frame axis between successive ROIs reflecting a propagation velocity in this example of $3.63 \text{ mm min}^{-1}$. The maximum deflection in the ADC trace associated with such CSD events remained unchanged at different distances from the stimulus site (deflections of $-31.89\%$ (i), $-31.32\%$ (ii) and $-32.04\%$ (iii), respectively). C, results from regions chosen at the start (i), middle (ii) and end (iii) of the primary wave path of a CSD event that followed a transient stimulus. The bar denotes the stimulus period. Such ADC traces typically showed differences in both timing and amplitude of the ADC deflections with distance (deflections of $-40.55\%$ (i), $-32.84\%$ (ii) and $-23.12\%$ (iii), respectively); the event illustrated propagated with a velocity of $2.21 \pm 0.28 \text{ mm min}^{-1}$. D, histogram of ADC amplitudes normalised to their respective baselines after the sustained stimulus: the amplitude of the ADC deflection remained unchanged at different distances from the stimulus site. E, histogram of the ADC amplitudes after a transient stimulus: the amplitude of the ADC deflection declined with distance from the stimulus site. Error bars represent ± S.E.M. * Significant difference between the amplitude of the event at its end from its amplitude at its start; $P < 0.05$ (Kruskal-Wallis test ANOVA).
Thus, primary events gave a mean deflection of 38.2±3.0% from baseline; this was significantly greater than the deflection produced by sustained events (Mann-Whitney U test P < 0.05, n = 10). Secondary events gave deflections of 16.9±2.5% after transient stimuli. Moreover, the first secondary event that followed each transient stimulus was significantly reduced by 30±7.6% (n = 5; Mann-Whitney U test P < 0.05) in amplitude relative to the preceding primary CSD event.

Propagation velocities of primary and secondary CSD events following transient and sustained stimuli

Table 3 compares the mean velocities of individual primary and secondary events whose propagation pathway over the cortex was followed with the aid of successive t-maps over the duration of the MRI experiments. The velocities of primary CSD events were easily determined, as these covered relatively extensive areas of cortex and normally propagated over the SG and MG as coherent wavefronts of reduced ADC and only fragmented or faded when the waves reached the interhemispheric boundary. In contrast the secondary events affected much smaller cortical areas, with each successive event invariably affecting different areas of cortex, were more fragmented and failed to propagate from the SG into the MG on occasion. Nevertheless, in both cases, the t-maps could be used to (1) locate, track the progress of and determine the centres of the areas successively affected by either type of event as they spread over the ipsilateral hemisphere. It was thus possible to (2) identify successive affected ROIs at different times along the relevant propagation paths, and therefore their distances apart. ADC values obtained from such ROIs would then provide (3) the time course of their ADC deflections produced by the CSD event under investigation. The results of (2) and (3) would then make it possible to determine (4) the latencies to peak deflection. (5) The overall propagation velocity was derived from the distance between the ROIs and latency of the deflections from within each ROI.

Values for such propagation velocities were obtained for the cerebral hemispheres ipsilateral to the stimulus both for the hemisphere as a whole and within each individual gyrus in that hemisphere. Thus, the overall propagation velocity over the entire hemisphere was calculated using ROIs located on the MG close to the midline and an ROI located adjacent to the stimulus site on the SG. For SD events that propagated through the suprasylvian sulcus the increased path length due to the sulcal fold was determined from the anatomical images and included in velocity calculations. For animals used to investigate the effects of transient and sustained stimuli, this distance was 3.8±0.1mm and 3.6±0.1mm, respectively. In contrast, the propagation velocity over the SG alone used one ROI located at a site adjacent to the stimulus and one ROI on the same gyrus but adjacent to the suprasylvian sulcus. Finally, the propagation velocity across the MG was calculated using one ROI located adjacent to the supra-

---

**Figure 7. Frequency distributions of the amplitudes of primary and secondary ADC deflections after either a sustained or transient stimulus**

Frequency histogram plots comparing the percentage ADC deflections of primary (open) and secondary (hatched) CSD events after sustained (A) and transient (B) stimuli. After the sustained stimulus the amplitudes of ADC deflection of primary and secondary CSD events produced a single mean. In contrast, the histogram from the amplitudes of the ADC deflections after a transient stimulus resulted in two distributions. The larger ADC deflections were produced by the primary CSD event, while the smaller deflections resulted from secondary CSD events.
sylvian sulcus on the MG and the other ROI near the midline.

Table 3 summarises the results from the above calculations of propagation velocities. The velocities fall within the range 1–4 mm min\(^{-1}\) and therefore generally agree both with previous experimental data (Bures˘ et al. 1974), and with the classical clinical observations made for propagation over the cortex of the visual disturbance of the classical migraine with aura (Lashley, 1941). However, they showed important differences in detail. Thus, the primary CSDs that followed the sustained stimuli showed higher velocities than the equivalent events elicited by the transient stimulus whether averaged over the entire cerebral hemisphere (Table 3\(^a\)) or measured over the SG adjacent to the application site (Table 3\(^c\)). However, this was not the case for the MG that was more remote from the site of stimulus application. Similarly, propagation velocities of secondary CSD events generated by the sustained stimulus were consistently higher than those of the corresponding events elicited by the transient stimulus over both the SG and MG (Table 3\(^d\) and \(^e\)). Finally, primary events propagated significantly more rapidly than secondary events following sustained (Table 3\(^b\)), but not transient stimuli.

**Intervals between successive CSD events**

Figure 8 demonstrates that the nature of the initiating stimuli also influenced the timings of successive events. It plots time intervals separating successive CSD events (denoted 1–2, 2–3, 3–4 and 4–5) observed from ROIs that were positioned adjacent to either the sustained (A) or transient (B) stimulus site (~1.4–1.7 mm). The latency of the primary CSD events following either sustained (1.1 ± 0.1 min; \(n = 5\)) or transient (1.4 ± 0.1 min; \(n = 5\)) stimuli were significantly shorter than the time intervals between either the primary event and first secondary event, or between successive secondary events. The primary event that followed a sustained stimulus was followed by successive secondary events with progressively longer time intervals between events of 8.1 ± 2.2 min, 9.1 ± 2.9 min, 13.5 ± 1.5 min and 17 ± 3.1 min (\(n = 5\)), respectively. Thus, both stimuli produced recurrent CSD events separated by a return to baseline consistent with a period of refractoriness following each individual event even in the face of a sustained stimulus. The repeated CSD events persisted even after withdrawal of the transient stimulus. Furthermore, CSD events were separated by constant refractory periods in the presence of a sustained stimulus and progressively longer intervals following withdrawal of the transient stimulus.

**DISCUSSION**

Spreading depression (SD) phenomena have been associated with a wide range of clinical situations (Gorji, 2001). Thus, cortical SD (CSD) has been implicated in the aura of classical migraine (Hadjikhani et al. 2001) and peri-infarct depolarisation (PID)-like phenomena have been reported in association with head trauma (Mayevsky et al. 1996). Both experimental CSD and PID are accompanied by similar, large perturbations of ion and amino acid distributions between the intra- and extracellular spaces and characteristic deflections in DC potential as recorded on the brain surface (Fabricius et al. 1993; Nallet et al. 1999). However, the detailed features of SD phenomena vary with their initiating pathology. CSD itself has not been associated with recognisable long-term histological changes under conditions of normal tissue oxygenation (Nedergaard & Hansen, 1988). In contrast the PID phenomena that follow transient or permanent middle cerebral artery occlusion (MCAO) in anaesthetised animals have been implicated in the progression of the ischaemic injury (Hossman, 1996).

Furthermore, the detailed characteristics of even experimental CSD vary with experimental conditions (Martins-Ferreira & Do Carmo, 1987) and brain type (lissencephalic vs. gyrencephalic; Bowyer et al. 1999a,b). For example, repeated, transient micropipette applications
of 3 M KCl each consistently produced a single SD event detectable by DC recording in rat parietal cortical slices (Kruger et al. 1996). Similarly, applications of successive pinpricks in rat cortex in vivo each reproducibly elicited a single SD event (Ebersberger et al. 2001). In contrast, single mechanical stimuli applied to feline cerebral cortex variously produced single (Kaufe & Goadsby, 1994) or multiple (Lambert et al. 1999) CSD events. Multiple CSD events similarly followed a single transient application of solid KCl to the cortical surface of the feline brain (Smith et al. 2000).

In addition to their overall number, propagation velocities of SD events also varied with the anatomical features of the brain regions in which they occurred. Thus, successive CSD events each elicited by repeated applications of KCl in parietal cortical slice preparations from adult rats showed no significant variations in propagation velocity (Kruger et al. 1996). In contrast, a single sustained KCl stimulus elicited multiple CSD events in feline cerebral cortex studied using DWI (Bockhorst et al. 2000). The initial CSD or primary event (James et al. 1999) was followed by secondary events that gave rise to ADC deflections of similar amplitude but lower propagation velocities. Similarly, measurements of laser Doppler flow and DC deflections attributable to successive CSD events following the application of single mechanical stimuli at 30 min intervals in the feline gyrencephalic brain gave deflections of similar amplitude but whose propagation velocities following the initial response were smaller than that of the first CSD event (Kaufe & Goadsby, 1994).

The present experiments adopted more closely defined conditions to explore the extent to which the detailed features of individual CSD events might vary with the nature of the applied stimulus itself and the extent to which such findings might parallel features of SD in different clinical situations. First, they employed feline rather than rodent brains in view of their gyrencephalic rather than lissencephalic topography shared with the human cerebral cortex, whose presence of sulci may reduce the propagation velocities of CSD events. Thus, single CSD events initiated by local electrical stimulation of the MG in porcine gyrencephalic cortex measured using both magnetoencephalography and electrocorticography initially propagated with a velocity of ~8 mm min⁻¹. However, this decreased to ~2 mm min⁻¹ when the wave entered the large coronal sulcus, but recovered to ~6 mm min⁻¹ when the CSD subsequently emerged at the neighbouring gyrus (Bowyer et al. 1999b). Vascular events associated with CSD similarly failed to traverse prominent sulci during human migrainous aura (Hadjikhani et al. 2001). Furthermore, cerebral cellular layers are better differentiated into grey and white matter in feline and human cerebral cortex: SD is more readily produced in superficial grey matter (Bureš et al. 1984). Finally, the ratio of cortical glial cells to neurons increases with brain size giving a higher gliazneuron ratio in feline (and human) brain compared to that of rodents (Tower & Young, 1973). Glial cells are known to be involved in CSD initiation and propagation (Largo et al. 1996; Largo et al. 1997).

Secondly, as adopted in previous protocols, the experiments applied KCl to initiate CSD (Herreras & Somjen, 1993; Lehmkühler & Richter, 1993; Kruger et al. 1996; James et al. 1999; Kuge et al. 2000; Smith et al. 2000). In these earlier studies, there were a similar number of SD events with similar frequencies and similar propagation velocities observed within a similar time period. Furthermore, the concentrations of KCl we used were similar to that used in these earlier reports in order for our results to be comparable to existing CSD data. Other studies have used mechanical stimuli that have been proven to reliably initiate CSD (Kaufe & Goadsby, 1994; Lambert et al. 1999). However, they leave permanent anatomical changes at the site of application (Syková et al. 2000). Other stimuli have included faradic stimulation (Leão, 1944; Bureš et al. 1974) or direct current (Leão & Morrison, 1945; Ochs, 1962), application of sodium cyanide (Bureš et al. 1974) or ouabain (Menna et al. 2000) or exposure to air or local cooling (Bureš et al. 1974). However, these interventions do not reproduce the changes occurring in vivo (Somjen, 2001) and did not induce CSD as reproducibly as did the application of KCl. In contrast, KCl has proven to be a reliable stimulus leading to reproducible events on earlier occasions in both non-imaging (Lehmankühler & Richter, 1993; Read et al. 1999; Smith et al. 2000) and imaging studies (Gardner-Medwin et al. 1994; de Crespiigny et al. 1996, 1998; Bockhorst et al. 2000; Kuge et al. 2000).

Changes in [K⁺]o might well be involved in pathophysiological processes in human brain tissue (Mayevsky et al. 1996; Nicholson & Syková, 1998). Increases in [K⁺]o to around 6–10 mM and to 50–60 mM have been associated with excessive neuronal activity (Paulson & Newman, 1987; Iadecola & Kraig, 1991) and experimental SD phenomena (Nicholson & Kraig, 1981), respectively. In parallel with these correlations, neuronal hyperexcitability in the occipital cortex has been associated with migraine aura (Aurora 1999). Experimental models for migrainous aura therefore might appropriately initiate CSD events using a transient [K⁺]o change (Smith et al. 2000) and models studying more prolonged SD phenomena might employ a larger more sustained [K⁺]o change (Reid et al. 1988; Bockhorst et al. 2000; Kuge et al. 2000). The use of either transient or sustained applications of KCl to the cortical surface provided two different types of stimulus. The transient stimulus used a significantly smaller [K⁺] and modified previous experimental procedures by using a remote applicator to extrude KCl in agar during image acquisition thus minimising disturbance of the preparation. Such a measure permitted ADC values at selected regions
on the cerebral cortex to be compared before and after application of the stimulus.

Thirdly, the resulting SD events were detected and characterised using DWI. This approach has previously successfully detected and mapped the propagation of both PID in rodent brain (Röther et al. 1996) and CSD in both feline (James et al. 1999) and rodent brain (de Crespigny et al. 1998) through the demonstration of propagating areas of reduced ADC. It offers advantages over electrode and probe measurements that record from only one or two selected points on the cortical surface. The present study proceeded to a quantitative spatial analysis of SD activity and its propagation over the cerebral cortex. It thus determined the magnitude and time course of ADC deflections, specified the cerebral areas affected during CSD generation and determined the propagation velocity of events over selected cortical regions.

Finally, the ADC values were derived from original values found in small ROIs made up of 2 x 2 pixels positioned at specific, fixed locations on both the SG and MG of the cerebral cortex. This contrasts with the considerably larger ROIs based on either the areas affected by the primary CSD event (James et al. 1999) or on different brain nuclei (Kuge et al. 2000). However, secondary events affected fewer and often different pixels from their preceding primary event. The latter approaches would therefore have yielded averaged ADC measurements that would have under-represented the ADC deflections actually occurring within individual image pixels produced by such secondary events. The present experiments therefore could provide absolute values of both baseline ADC values and ADC deflections associated with CSD that could be compared quantitatively both within and between experimental animals. They represent a further development of work described by Bockhorst et al. (2000) in comparing the effects of sustained and transient applications of KCl upon the nature of CSD responses for the first time, under comparable conditions.

The present experiments demonstrated that SD events spread with similar overall patterns and affected similar quantities of cortical surface, with secondary events affecting smaller areas than primary events whether in response to sustained or transient stimuli. It also made a number of novel findings concerning the detailed characteristics of such phenomena. Firstly, T1-weighted images demonstrated a localised hyperintensity while DW images produced prolonged ADC changes, previously undetected, in regions around the site of a sustained but not a transient stimulus. These were particularly clear when such images were compared to findings before stimulus application. The high [K+]o may cause vasogenic oedema and possibly osmotically induce blood–brain barrier opening. Such local changes could potentially form a source of prolonged stimulation for repeated CSD activity (Herreras & Somjen, 1993; Lehmkühler & Richter, 1993; Bockhorst et al. 2000); and might even partially account for the observed secondary events. They were not observed at the site of application of transient stimuli that showed a characteristic pattern of ADC deflections resembling the more familiar findings at sites remote from stimulus sites. In any case, such transient stimuli would have been withdrawn (within 10 min) before any observed secondary events had taken place (James et al. 1999; Bockhorst et al. 2000).

Secondly, both the primary and secondary events resulting from the two kinds of stimulus differed in detailed characteristics. Thus, following sustained stimuli, the primary and secondary events produced ADC deflections where amplitudes were similar, and did not alter significantly with propagation. Furthermore their velocities were similarly conserved but secondary events always assumed lower velocities than did primary events. Successive CSD events were separated by similar time intervals and recurred throughout the (~90 min) experiments. In contrast, transient stimuli gave rise to fewer SD events. The amplitude of their ADC deflections declined with distance from stimulus site and their secondary deflections had consistently smaller amplitudes than primary CSD events. Nevertheless, the velocities of primary and all the successive secondary events were similar and were conserved with propagation. Furthermore, successive secondary ADC deflections measured from any given ROI progressively declined in amplitude and were separated by progressively increasing time intervals to eventually leave a flat ADC baseline.

Thus, responses following transient stimuli differed significantly from those produced by sustained stimuli even at regions remote from the site of application of the stimulus, that could be expected to be beyond the diffusional range of the applied KCl. Thus, earlier reports have extensively considered the diffusion of K+ through the brain. Pape & Katzman (1972) calculated that simple K+ diffusion through the extracellular space would require 7 min to achieve 90% of the surface value of an imposed increase in [K+]o even at a distance of 200 μm. However, movement of K+ through the mammalian brain in vivo is additionally affected by changes in KCl concentration at the stimulus site, its removal into the blood stream and the tortuosity and volume of the extracellular space (Nicholson & Syková, 1998; Syková et al. 2000). In addition, cells in the central nervous system themselves can take up K+. This will buffer increases in [K+]o (Walz, 2000). Accordingly, a rise in [K+]o in brain regions over a diameter of 200 μm is reduced by 75% within a period of minutes through this buffering action (Gardner-Medwin, 1983). Finally, Herreras & Somjen (1993) found that over a 90 min interval K+ ions did not diffuse beyond 200 μm from a dialysis fibre perfused with high [K+] solution. In contrast,
tetramethylammonium ion appeared to diffuse freely through the extracellular space.

The present experimental observations also suggest that high levels of K$^+$ had not diffused large distances from the site of application of the sustained stimulus. Thus, local changes in ADC resulting from the application of the sustained stimulus had fully decayed ~1.4 mm away from the site of stimulus. Tissue in such regions showed normal baseline ADC values and the characteristic ADC changes due to recurrent CSDs. Thus, the differences in the magnitude of the ADC events following sustained and transient stimuli were observed at distances from the site of stimulus application far greater than expected for the diffusion of the applied KCl. This is difficult to reconcile with a single regenerative mechanism for initiating spread of SD. However, CSD initiation is known to involve changes in both [K$^+$]$_o$ and extracellular glutamate (Grafstein, 1956; Saito et al. 1995; Basarsky et al. 1999). The relative activation of the two mechanisms might well be a function of the mass of tissue affected (Bureš et al. 1974) by an elevated K$^+$ resulting from the different methods of stimulus application. Local application of K$^+$ (15–60 mmol l$^{-1}$) through a microdialysis probe produces a DC deflection attributable to SD whose amplitude depends on [K$^+$]$_o$ (Obrenovitch & Zilkha, 1995). However, applying 100–250 μmol l$^{-1}$ glutamate in the absence of K$^+$ through the microdialysis probe fails to induce or enhance SD propagation in the rat cerebral cortex (Obrenovitch & Zilkha, 1995). Nevertheless, depolarisations resulting from release of [K$^+$]$_o$ into the extracellular compartment would be expected to decrease [Na$^+$]. (Nicholson & Kraig, 1981). The latter in turn could cause a net release of intracellular glutamate into the extracellular space through inhibition of glutamate uptake or a reversal of glutamate transporters (Szatkowski et al. 1990; Billups et al. 1998). Extracellular glutamate concentration does increase with increasing [K$^+$]$_o$ (Saito et al. 1995), to an extent that may vary with the nature of the initiating stimulus. The released glutamate may then act on N-methyl-D-aspartate (NMDA) receptors and thereby contribute to SD: NMDA blockade reduces the rate of propagation of SD and attenuates its associated DC deflections (Kaube & Goadsby, 1994; Basarsky et al. 1999).

The present experiments demonstrated that different stimuli produced CSD events in which the nature of the stimulus influenced (1) their total number and therefore the period of time over which such events took place, (2) their maximum ADC deflection, primary or secondary events, and how these varied with distance from the site of their initiation, (3) the time intervals between successive events, and (4) the propagation velocities of primary or secondary events and how they altered with distance from the stimulus. CSD thus need not be an ‘all or nothing’ phenomenon as might be inferred from measurements obtained from sustained stimuli (Bockhorst et al. 2000). This may account for the different characteristics of CSD in different clinical circumstances that might be exemplified in the transient nature of migraine with aura (Hadjikhani et al. 2001) and the more prolonged phenomena associated with PFO following head injury (Mayevsky et al. 1996). Finally, the variability of the ADC events observed in the gyrencephalic brain may be responsible for difficulties in detecting CSD in humans by MRI (Cao et al. 1999). Our findings may help in studies exploring modifications of the conditions under which observations are made with a view to enhancing the likelihood of detecting CSD in humans.

REFERENCES


**Acknowledgements**

The research was supported by GlaxoSmithKline (to M.F.J., M.I.S., A.A.P. and K.H.J.B) and the Biotechnology and Biological Research Council (BBSRC; to D.P.B., C.L.-H.H. and N.G.P.), the University of Cambridge (to J.M.S.) and the Herchel Smith Endowment (L.D.H.). C.L.-H.H. would also like to thank the Royal Society, the Wellcome Trust and the Medical Research Council (MRC) for funding support.

**Authors’ present addresses**


J. M. Smith: MRC Biochemical & Clinical Magnetic Resonance Unit, Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK.

N. G. Papadakis: Department of Psychiatry, Longely Centre, Northern General Hospital, Norwood Grange Drive, Sheffield, S5 7JT, UK.