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Magnetic fields associated with spreading depression in anaesthetised rabbits

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Magnetic fields were measured with SQUID magnetometry outside the skull of anaesthetised rabbits during initiation and propagation of spreading depression (SD) in the cortex. Slowly changing fields (up to 1.4 pT) were observed during the propagation phase, from 4–8.5 min after initiation of SD with KCl application, with maxima at about 6 min. The peak amplitude of the equivalent net dipole generators in the brain was ca. 28 μA·mm, substantially less than previously observed with SD in vitro, but large enough that similar signals might be detectable in man.

INTRODUCTION

Spreading depression, first described in the anaesthetised rabbit by Leão[6], is a profound, stereotyped, and usually transient disturbance of neural tissue that, once initiated, can often propagate through otherwise normal tissue. It is associated with a diminution of normal neural activity, profound disturbances of ion homeostasis, large (ca. −20 mV) extracellular voltage shifts and increases of tissue impedance (see e.g. refs. 2, 9 for reviews). Currents flow within the tissue that may be expected to give rise, in some geometrical situations, to external magnetic fields.

The importance of magnetic studies of SD is chiefly that they may lead to non-invasive assessment of SD-like phenomena in the human brain. Such information could be valuable in relation to the migraine aura, since recent research has revealed new similarities between SD and the aura[3,5], which strengthen the long-standing hypothesis that the two phenonena may be related[7,8].

Magnetic fields during SD were first measured by Okada et al.[10], in isolated turtle cerebellum. We have studied the fields during SD in the intact brain in situ in anaesthetised rabbits. Preliminary details have been published[1,4].

MATERIALS AND METHODS

Magnetic fields (DC–50 Hz) were measured with a 7 channel, DC-coupled SQUID magnetometer (BTI Model 607) in a magnetically shielded room. Second order gradiometers, with pickup coils 18 mm in diameter, were in a hexagonal array with 21.5 mm axial spacing. The 6 peripheral coils were tilted at 7° so that their axes converged about 150 mm below the Dewar vessel containing the coils, which itself prevented access closer than about 15 mm below the coils. This arrangement gives approximately radial field measurements around the human skull. With the rabbit, the measured field components were closer to vertical than radial.

Rabbits (New Zealand White, 1.5–2.5 kg) were anaesthetised with i.v. urethane (750 mg/kg) and chloralose (40 mg/kg), with 10–20% maintenance doses given every 1–2 h as required. The animal respired normally through a tracheal cannula. A 5 mm diameter opening was made in the skull and dura, centred 5 mm left of the midline and 5 mm behind the coronal suture. A chamber was formed by cementing a perspex cylinder (4 mm high) to the bone around the hole and covering this with a cap moulded in silicone rubber. The cap was fitted with silicone tubes for inflow (0.4 mm i.d., up to 3 m long) and outflow (0.8 mm i.d., 50 mm long). The inflow from a manually operated syringe either inside or outside the screened room, was angled to stream fluid directly over the brain. The outflow passed to an adjacent beaker for waste, generating excess pressure within the chamber of only a few mm H_2O. Inflow and outflow tubes were taped securely to the head and beaker, since movement and dripping in the outflow were found to cause magnetic artefacts. The chamber was normally flushed at least every 30 min with 1 ml saline (147 mM NaCl + 3 mM KCl). To elicit SD, 2 ml 150 mM KCl was passed through the tubes and chamber during about 30 s. The same volume of normal saline was flushed through 2 min later and also, for control observations, instead of the KCl. SD was verified by voltage recording at a subdural electrode (0.5 mm dia. Ag/AgCl) positioned through a small craniotomy 6 mm anterior from the anterior edge of the chamber. A reference electrode (Ag/AgCl) was inserted in neck muscle.

The animal was arranged with the exposed brain surface in the chamber horizontal. The centre coil of the magnetometer was directly over this, 20–25 mm from the brain, with its axis vertical. Positions of the peripheral coils are identified relative to the anterior

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direction: 0° = anterior, 90° = right, etc. Other geometrical arrangements were occasionally used, in an effort to record field components in other directions. The measurement distance had to be greater under these conditions, and no consistent signals were observed.

Magnetic artefacts were minimised by the use of only plastic, silicone rubber and silver wire in the animal preparation. Degaussing of the preparation with a commercial tape eraser sometimes helped to reduce artefacts from gross movement. Steady field gradients around the head were measured in one experiment by moving the animal back and forth on plastic rollers, and were found[4] to be less than 0.2 pT/mm. Any movements during most recording sessions were monitored by an experimenter in the room, and recorded with an event marker. Twitches of facial muscle and limb movements sometimes caused detectable, transient, magnetic artefacts. These occurred spontaneously, and sometimes immediately on KCl application; they were not observed in association with the consistent slow field changes seen during the 10 min following initiation of SD. Artefacts from sources outside the screened room were sometimes observed. Magnetic signals from distant sources were continuously monitored with three orthogonal reference magnetometers about 300 mm above the preparation. These signals did not in any instance mimic the signals of interest.

RESULTS

Magnetic recordings were made on 5 animals, in which 13 SD episodes were studied using the standard geometrical configuration and conditions. Three of these runs were not analysed: two because of contamination with quantum flux jumps, and one because of a large movement artefact. Ten records from 5 animals remain: 7 with the peripheral coils positioned at 0°, 60°, 120°, etc. and 3 with coils at 30°, 90°, 150°, etc. Fig. 1A shows original records from a run with coils at 0°, 60°, etc. Fig. 1B shows the mean magnetic fields (± S.E.M.) for all 7 recordings made at these positions.

Perfusion of the chamber for 2 min with KCl reliably elicited a single wave of SD recorded at the subdural electrode in front of the chamber. The voltage record (bottom trace) in Fig. 1A shows typical characteristics. There is a sudden onset of negativity 240 s after KCl application, reaching a peak at 257 s (mean 245 s, range 184–305 s). The negativity is followed by a smaller and more prolonged positivity. Irregular fluctuations of the electrocorticogram at 1–30 Hz due to normal neural activity (evident as a broadening of the trace on this timescale) are much reduced at the time of the slow potential changes during SD and recover about 250 s after onset of the negative wave.

Application of KCl was sometimes immediately followed by small limb, neck, or ear movements, lasting a few seconds. These may have been associated with pain reflexes or arousal reactions due to KCl reaching sensory endings in the meninges. Variable magnetic signals occurred at this time, visible at 240° and 300° in Fig. 1A. In the averages (Fig. 1B) these are evident both as

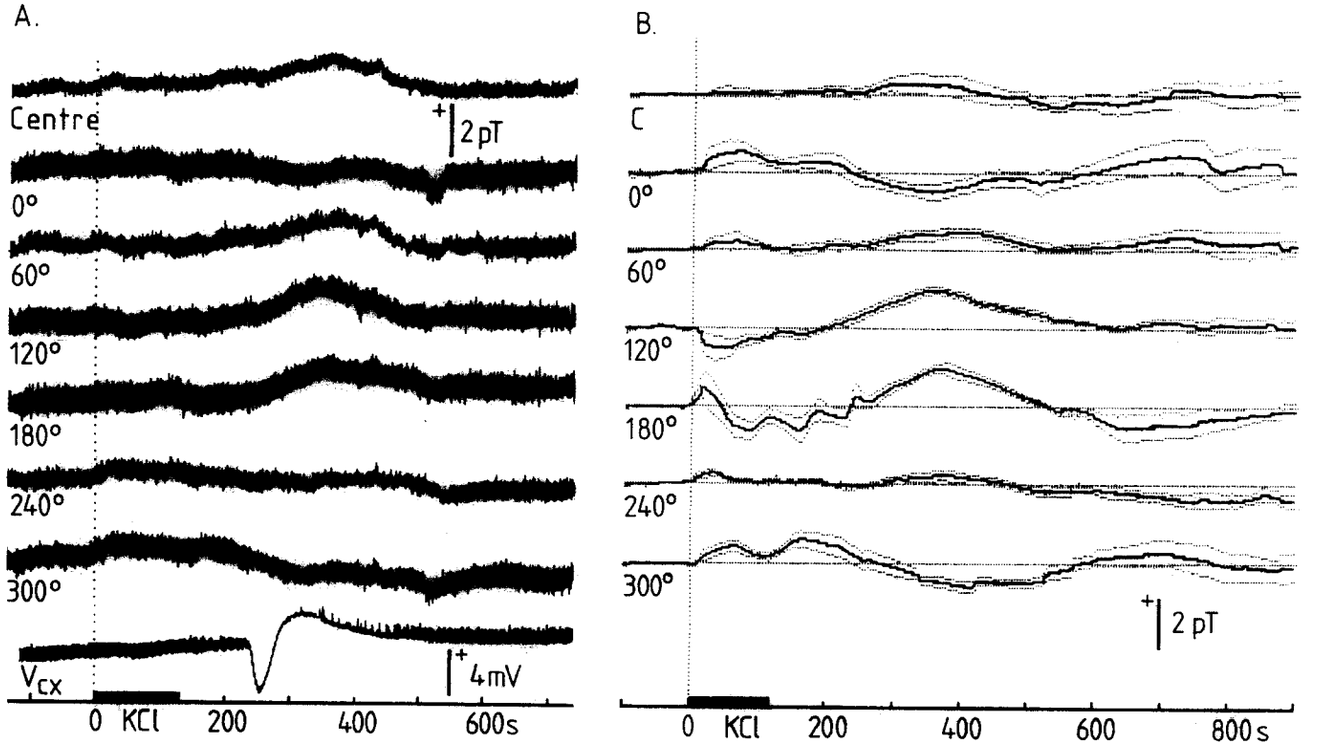

Fig. 1. A: magnetic and electric changes during SD elicited with KCl (bar) applied to the left cortex of an anaesthetised rabbit. Top 7 traces are approximately vertical magnetic fields (+ = upward field), from a coil 25 mm above the initiation site and coils displaced 21.5 mm anterior (0°) and at specified orientations (see plan view, Fig. 2A). V_{cx}: cortical voltage record. B: mean ± S.E.M. (n = 7) for 7 sets of magnetic records as in (A), from 5 animals. Records were shifted relative to the mean baseline before KCl application and aligned in time relative to KCl onset.

increased standard errors of the traces during the period of KCl application and shifts of the mean that were not statistically significant.

Control perfusion of the chamber with standard saline did not produce any visible reactions in the animals. Magnetic shifts were sometimes observed that developed during the 30 s injection period and declined with a time constant of about 30 s afterwards. The explanation for these is unknown. SD was never elicited, and the magnetic shifts that developed with SD some minutes after the injection were not seen.

A consistent magnetic signal was seen at 300–500 s after onset of KCl application (Fig. 1A,B). Field shifts were positive (away from the head) at the coils positioned at 120°–180° in the posterior right quadrant, and negative at 300°–0° (anterior left). Mean amplitudes at 400 s after KCl application are shown on a plan view of the brain at the various recording sites in Fig. 2A.

These data suggest a pattern of current flow equivalent, from the point of view of the magnetic recordings, to leftward and posterior pointing dipole components. Statistical assessment is complicated, however, by the fact that information from the different channels recorded simultaneously in such circumstances is not independent. To deal with this problem, dipole components giving the best (least squares) fit to the fields recorded in each peripheral coil were calculated as a function of time for each run separately, using Eqns. 3, 4 derived in an Appendix. Data from the centre coil were ignored in this analysis, since overall the mean fields from this coil were at no time significantly different from zero (Figs. 1B, 2A).

Fig. 2B shows the mean timecourse of the inferred anterior (0°) and rightward (90°) dipole components. The means are based on all 10 runs, amongst which the numbers of repeats in individual animals were (1, 1, 3, 3, 2). Analysis of variance on the inferred dipole components at 400 s showed that the variability between animals was not significantly different from that between runs repeated on one animal ($F_{4,5}$ = 0.8, 3.6 for the 2 components: both $P > 0.05$). Standard errors for the dipole estimates in Fig. 2B were therefore calculated using n = 10, as for independent measures. This gives Student's t values of 4.8 and 5.8 for the 0° and 90° dipole estimates at 400 s respectively (both significant at $P < 0.001$ in a 2-tailed test). Using just the 5 means on the different animals instead in this analysis (thus throwing away some of the benefit of repeated measures in individual animals), the corresponding t values (n = 5) were 3.3 and 6.1 ($P < 0.05$ and $P < 0.01$ respectively).

The data indicate a slowly developing magnetic signal, commencing at about the time (240 s after KCl onset) when the SD disturbance reached the subdural monitoring site 6 mm from the edge of the cup, and finishing at ca. 520 s. It reached a peak at ca. 360 s. Since the 2 dipole components in Fig. 2B have similar amplitudes and timecourses, the total equivalent dipole required to generate this disturbance is inferred to be approximately oriented at 45° within the posterior left quadrant (225° relative to anterior), with peak amplitude about 28 μA·mm.

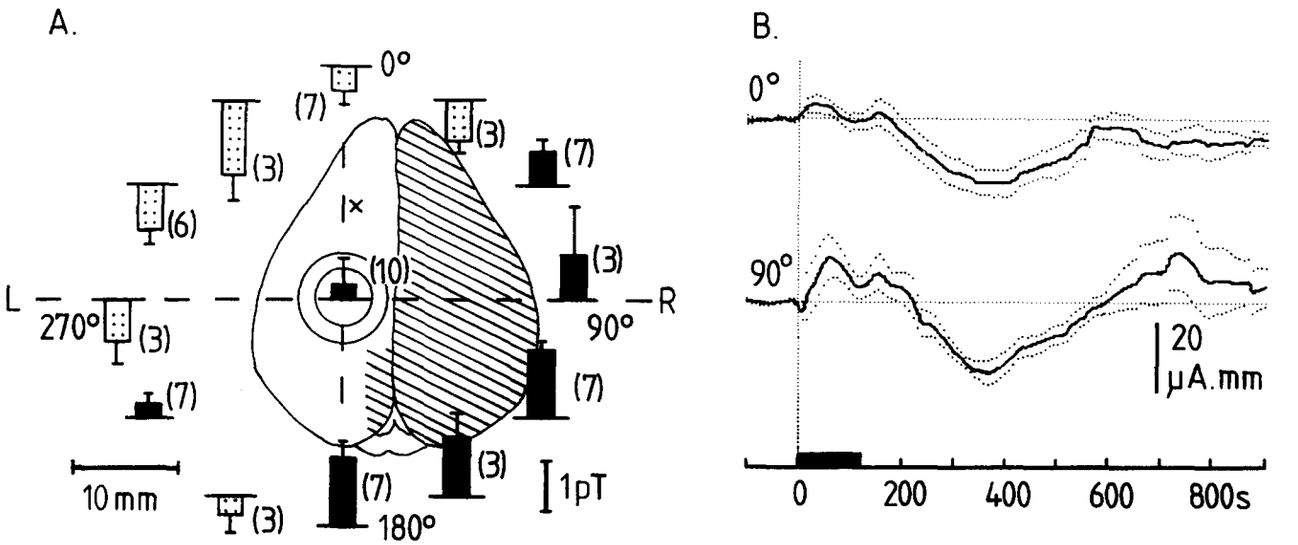

Fig. 2. A: mean and S.E.M. for magnetic field shifts at 400 s after KCl application, shown in plan view. The rabbit cortex is shown with the cup (concentric circles) and site of electric recording (×). Hatching indicates the region of cortex into which SD would not spread [6]. The numbers of observations at the different sites are shown in brackets. B: mean inferred dipole components (± S.E.M., n = 10) in the anterior (0°) and rightward (90°) directions, calculated from fields recorded at the 6 peripheral coils in all 10 runs on 5 animals, according to Eqns. 3 and 4 in the Appendix (α = 21.5 mm, z = 25 mm, ϕ = 7°, giving r = 33 mm, k = 19.6 μA·mm·pT^{-1}).

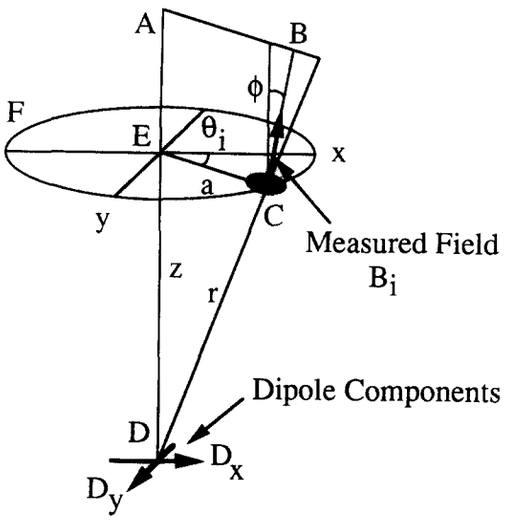

Fig. 3. Geometrical arrangement, showing the basis for estimating dipole components.

DISCUSSION

Elicitation of SD in the anaesthetised rabbit was associated with slow shifts of magnetic field, principally 4–6.5 min after the onset of the stimulus. SD was initiated in these experiments in a flat region of parietal cortex parallel to the skull, where it propagates uniformly in each direction in the ipsilateral hemisphere[2], at a rate of ca. 3 mm/min. The maximum propagation distance is about 15 mm rostrally and caudally and about 20–25 mm over the curved lateral surface. The subdural voltage recordings showed that the SD wave had reached approximately 6 mm from the cup at 240 s after stimulation. This latency includes both initiation time for SD in the cup and a propagation delay for 6–9 mm from the cup. By the end of the magnetic signals (ca. 520 s) there would have been time for propagation over an additional 14 mm, i.e. roughly the maximum propagation distance available within one hemisphere.

So long as SD propagates as a completely symmetrical disturbance in cortex parallel to the skull, no magnetic fields are expected[11]. Symmetry will be broken within a few minutes by two factors: the cortical geometry, and the regions resistant to the invasion of SD (the contralateral hemisphere and the ipsilateral retrosplenial area[6], shown hatched in Fig. 2A). The midline sulcus lies about 3 mm from the cup, where the SD wave reaches the parts of the cingular cortex perpendicular to the skull. The resistant retrosplenial zone parallel to the skull is reached at a similar distance in a more posterior direction (Fig. 2A). Thus the effects of these two breaks in symmetry will have commenced at about 180 s from KCl application.

The equivalent dipoles for SD propagating after these breaks in symmetry can be deduced partly from the arguments and data of Okada et al.[10] showing that much of the net intracellular current during SD flows perpendicular to (and away from) the cortical surface in the affected region. Thus propagation of SD into the cortex adjacent to the midline sulcus would produce an equivalent leftward dipole (270°, Fig. 2A). Where the SD propagates parallel to the skull but in a broken wave due to failure of propagation in the retrosplenial area, the magnetic effects arise from the components of intracellular current parallel to the surface. Because of raised extracellular resistance produced by SD, these currents are predominantly backwards from the centre of the disturbance, producing extracellular positivity in the wake of SD and a loop of current returning via extracellular space mainly in the deeper tissue[3]. The effect is equivalent to a horizontal bar magnet travelling with the SD wave with its south pole on the left of the finite propagating zone and its north pole on the right, or roughly to a current dipole pointing backwards in the propagating zone. The effect of the retrosplenial break should therefore be similar to a dipole pointing in the direction of this zone, i.e. ca. 150° (Fig. 2A).

The combination of these two effects producing dipoles at 270° and 150°may plausibly account for the net dipole at ca. 225° that was observed starting at around 200 s in these experiments. The later parts of the magnetic signals might be expected to be affected by propagation into the extensive frontoparietal and temporal regions of cortex, but the electrical conditions in these regions are too uncertain to speculate on their possible contribution.

Although the rabbit cortex is lissencephalic, the whole hemisphere resembles a single gyrus of the human brain. The fact that signals can be recorded in association with SD in the rabbit brain is encouraging for the prospects of identifying related phenomena in man. The fields are smaller and slower in time course than those previously described in vitro. At a vertical distance of 17 mm and lateral displacement of 12 mm, Okada et al.[10] described fields up to 8 pT, corresponding to a dipole strength ca. 60 μA·mm compared with a peak 28 μA·mm in the present study. The in vitro signals were faster in onset (sometimes reaching a peak in less than 20 s), presumably in part because of synchronous initiation of SD by electrical stimulation in a favourably oriented region of tissue. If SD occurs in man, the conditions at the site and time of initiation are uncertain; it is the slowly changing signals associated with changes of orientation of tissue affected in a propagating wave that can be more confidently predicted, though conditions leading to the faster changes may occur also.

An additional factor in comparison with the in vitro results is that the effective dipole strength may become

larger in vitro because of immersion of the tissue in a highly conductive fluid environment. Physiological saline has a conductivity some 5–10 times that of brain tissue. The resultant 'short circuit' effect tends to reduce the voltage difference between the exposed faces of the tissue, thereby reducing the fraction of the driving current that returns locally within the tissue. This component of the driving current produces little or no magnetic field, since the current paths in the two directions are from the macroscopic point of view almost identical. Thus isolation of tissue and immersion in saline is liable to enhance its magnetic effects.

Since SD is associated with a reduction of neural activity, as seen in electrical records of local activity (Fig. 1A), it is plausible that there might be a reduction of the magnetic fluctuations in the 1–30 Hz bandwidth. We have seen clear reductions in the level of magnetic fluctuations in some recordings[4], but it is not clear whether any component of these reductions was consistently related to the timing of SD. Large sudden drops (up to 70%) were sometimes observed at widely differing times and may have been related to reticular activation or other diffuse influences rather than to SD. The mean drop (ca. 15%) was greatest at about the time of the DC shifts, but was not statistically significant.

APPENDIX

Simultaneously recorded data from two or more coils can be combined in order to estimate the strength and orientation of a dipole at a specific location that best fits the measurements. This involves calculation of the dipole components at that location which minimise the sum of the squares of differences between observed and expected measurements. Since different combinations of dipoles can generate equivalent fields, the calculation does not demonstrate that there *are* dipole components at the specified location, even if the location gives the best possible fit and the fit itself is very good. However, the procedure is valuable if there is reason to think that the dipole sources are within a restricted range of locations (for example, within the head), and it condenses what may be many channels of simultaneous (therefore non-independent) data into a single dipole that can form the basis for statistical comparisons between repeated measurements.

The geometrical arrangement in these experiments is shown in Fig. 3. Coils producing field measurements B_i (i = 1, 2, 3, etc.) are centred at positions such as C, at various points on the horizontal circle CF with radius a. Each centre lies at a different orientation θ_i, relative to the x axis indicated. The coils are tilted outwards at an angle ϕ (7°) relative to the horizontal plane so that they measure the field along a direction CB, close to the vertical. The location D for which dipoles are fitted is at a distance z below the centre of the circle of coils E (a distance r (= $\sqrt{(z^2 + a^2)}$) from each coil centre). The dipole components in the x,y directions are D_x, D_y. The coils are not sensitive to any dipole component in the z direction, which therefore cannot be estimated.

The coil at C is sensitive to the vertical field B_z and (to a small extent due to the tilt angle ϕ) to the field B_{EC} along the direction EC. The fields that would be produced by the dipoles D_x, D_y are:

$$B_z = \frac{\mu}{4\pi r^3}\, a\, (D_y \cos\theta_i - D_x \sin\theta_i)$$

$$B_{EC} = -\frac{\mu}{4\pi r^3}\, z(D_y \cos\theta_i - D_x \sin\theta_i)$$

where μ is the permeability, taken as equivalent to that of free space ($\mu_o = 4\pi\cdot 10^{-7}$ T·m·A^{-1}). Multiplying these components by $\cos\phi$ and $\sin\phi$ respectively gives the contributions to the expected field B_i^* normal to the coil:

$$B_i^* = \frac{\mu}{4\pi r^3}\, (a\cos\phi - z\sin\phi)\, (D_y \cos\theta_i - D_x \sin\theta_i) \quad (1)$$

We wish to choose D_x, D_y to minimise the sum S of the squared discrepancies between the predicted fields B_i^* and the measured fields B_i:

$$S = \Sigma_i\, (B_i^* - B_i)^2 \quad (2)$$

This is achieved by substituting equation (1) in equation (2), setting the partial derivatives with respect to D_x, D_y equal to zero and solving the resultant pair of equations, giving:

$$D_x = k\, \frac{(\Sigma_i B_i \sin\theta_i)(\Sigma_i \cos^2\theta_i) - (\Sigma_i B_i \cos\theta_i)(\Sigma_i \sin\theta_i \cos\theta_i)}{(\Sigma_i \sin\theta_i \cos\theta_i)^2 - (\Sigma_i \sin\theta_i)^2 (\Sigma_i \cos\theta_i)^2} \quad (3)$$

$$D_y = -k\, \frac{(\Sigma_i B_i \cos\theta_i)(\Sigma_i \sin^2\theta_i) - (\Sigma_i B_i \sin\theta_i)(\Sigma_i \sin\theta_i \cos\theta_i)}{(\Sigma_i \sin\theta_i \cos\theta_i)^2 - (\Sigma_i \sin\theta_i)^2 (\Sigma_i \cos\theta_i)^2} \quad (4)$$

where:

$$k = \frac{4\pi(a^2 + z^2)^{1.5}}{\mu(a\cos\phi - z\sin\phi)}$$

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