Problem of Dural Scarring in Recording From Awake, Behaving Monkeys: A Solution Using 5-Fluorouracil


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Problem of dural scarring in recording from awake, behaving monkeys: a solution using 5-fluorouracil. J Neurophysiol 90: 1324–1332, 2003; 10.1152/jn.00169.2003. In studies using single neuron recordings from awake, behaving monkeys, it is necessary to make repeated transdural penetrations using fragile microelectrodes. The tough connective tissue that accumulates after the dura mater is first exposed is often problematic because of electrode breakage and the mechanical stress to the underlying brain tissue caused by excessive dimpling during penetration. We describe the use of an antimitotic compound, 5-fluorouracil (5FU) to control the growth of this connective tissue. 5FU can be safely applied for short periods to the exposed dural tissue on a regular basis provided that it is thoroughly rinsed after application. The advantages of using 5FU are fourfold: first, it depresses fibroblast division and minimizes dural growth and scar tissue formation so that penetrations are easier with less electrode damage or breakage. Second, the frequency of surgical procedures required to remove this tissue are greatly reduced, which benefits both the experiment animal and the experiment. Third, 5FU reduces vascularization of the tissue so that its removal is far easier and without significant blood loss. Finally, 5FU seems to inhibit bacterial infections within the recording chamber. In macaque motor cortex, we performed a quantitative study of electrophysiological data recorded from monkeys with and without 5FU treatment. No significant deleterious side effects produced by 5FU could be detected. Likewise, histological examination of cortical tissue underlying treated dura did not reveal any obvious signs of damage by 5FU. We recommend this approach, with the appropriate safety precautions, to all those neurophysiologists using transdural microelectrode methods in chronically prepared experimental animals. It is also possible that this technique may be useful in other situations where there is dural scarring after surgical intervention or injury.

INTRODUCTION

Multiple microelectrode methods are increasingly being used in awake, behaving monkey studies. In many approaches, this involves the use of relatively fine and fragile electrodes. For example, the Eckhorn multielectrode system (Thomas Recording) (Eckhorn and Thomas 1993) uses ≤16 independently moveable electrodes; they are made of platinum-iridium (Pt-Ir) and insulated with quartz glass. These microelectrodes are very fine, with an external shaft diameter of only 80 μm, and during transdural penetration, dural thickening can cause them to buckle and break.

When the dura mater is first exposed at surgery, in preparation for single-unit recording, it is usually thin, unscarred, and free of any covering tissue and therefore possible to penetrate with fine electrodes. As the inflammatory wound-healing response begins, scar tissue forms and the dura thickens. At first, this is a filamentous growth, which subsequently becomes fibrous and vascularized. Conventional tungsten or Pt-Ir microelectrodes typically have a shank diameter of 150 μm and are still able to penetrate this type of growth successfully (Lemon 1984). In contrast, the thickened dura is almost impenetrable to the more delicate (and expensive) Thomas electrodes within 2 wk. The ratio of broken electrodes to successful penetrations becomes high and costly. Furthermore the quality of recordings is diminished because difficult penetrations cause excessive dimpling of the dura and pressure-induced spreading depression in the underlying cortex. To continue with further recording, the dura must be stripped of this growth. This usually requires a general anesthetic. These surgeries interrupt the routine of chronic recording and adversely affect the performance of the monkey on the behavioral task as well as its general well being.

We describe here a well-tested method to suppress the growth of connective and scar tissue above the exposed dura and to reduce the vascularity of this tissue in the recording chamber. The method involves the topical application of a solution of 5-fluorouracil (5FU) to the exposed dura. 5FU is an antimetabolic, antimitotic agent that reduces tissue growth by interfering with the enzyme reactions essential for nucleic acid synthesis, thus preventing mitosis and therefore the division and proliferation of cells.

The first use of a single 5-min application of 5FU in sponges to prevent scarring after ocular surgery in humans (Smith et al. 1992) was based on the discovery that short, 5-min applications of 5FU caused long-term fibroblast growth arrest in culture with minimal cell death (Khaw et al. 1992a,b). This was subsequently confirmed in an aggressive model of scarring after surgery to create a new outflow channel to drain aqueous fluid out of the eye to reduce intraocular pressure. In vivo the treatment was found to have focal long-term effects on fibro-

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blunt proliferation without overt toxicity (Khaw et al. 1993a,b). These single 5-min applications have also subsequently been shown to prevent cell-mediated collagen contraction in vitro (Occleston et al. 1994) and in models of tendon scarring after injury (Akali et al. 1999; Khan et al. 1997; Moran et al. 2000). An adapted version of this single treatment in combination with heparin has also been shown to significantly reduce retinal scarring in a human trial without any detectable retinal toxicity (Asaria et al. 2001; Kon et al. 1998).

Antimitotic solutions are already used in many clinical situations, for example ophthalmic surgery for conditions such as glaucoma (Khaw and Migdal 1996) and strabismus (Andreo et al. 1997). It is also used in chemotherapy regimes for a range of solid tumors including breast and colorectal cancers (Cunningham and Coleman 2001) and as a topical treatment for nonmelanoma skin cancers such as basal cell and squamous cell carcinomas (Nguyen and Ho 2002).

We therefore believe that the topical use of 5FU may be expected to safely act to retard the growth of dural scar tissue with minimal risk to the individual. We have investigated the actions of 5FU on the dura by assessing its effects on the quality and yield of single neuron recordings in the awake, behaving monkey. By using 5FU to control dural scarring and solve the “tough dura” problem, we have been able to record continuously for periods of ≤3 mo before having to perform a surgery to strip the dura. We have improved the success rate of the penetrations and decreased the rate of breakage of electrodes, thus reducing the cost of each experiment. We have investigated in detail any deleterious effects 5FU might have on the underlying cortex and are confident that it is safe to use when strict guidelines are observed. This method therefore offers significant refinement to and reduction of animal procedures as well as more efficient data capture.

A brief description of these methods has been previously published (Baker et al. 1999).

METHODS

All procedures involving animals were carried out in accordance with the UK Animals (Scientific Procedures) Act 1976.

5FU

5-Fluorouracil (Faulding Pharmaceuticals Plc, see APPENDIX for address) is a potent anti-mitotic agent. It is supplied in aqueous solution in 10 ml vials (250 mg, 25 mg/ml). According to the manufacturers, when given systemically in chemotherapy, it can cause side effects including diarrhea, nausea, vomiting, alopecia, dermatitis, nail pigmentation changes, blood disorders, and electrocardiogram (ECG) changes. If used in pregnancy, it may be teratogenic. It is therefore essential that it is handled carefully for the safety of both experimenter and animal. Vials should be kept in a locked cupboard, and protective clothing (lab coat, disposable mask and gloves) must be worn during use. After use, the solution itself and the fluids used to flush it away are disposed of via the laboratory sink, washed down with copious amounts of tap water. Syringes, needles, cotton wool, gloves, and masks used are disposed of into clinical waste for incineration.

Recording chamber

Our recording methods have been described previously (Baker et al. 1999, 2001). Briefly, during a surgical procedure, a small craniotomy (typically 10 mm in diameter) is made above the area from which recordings are to be taken, and a circular stainless steel chamber is implanted. Chamber position is guided by a previous MRI scan. We use a chamber which has a rather shallow lower section (height only 3.5 mm) that is seated into the surrounding bone so that the upper surface of the chamber (with the lid detached) is ~5 mm from the dural surface. A threaded lid adds additional height to the chamber when it is sealed so that the entire craniotomy can be protected and bathed in sterile saline when the chamber is closed and filled through holes in the lid. The shallow chamber allows easy access to the dura both during the initial surgery and later during the recording period while the monkey is awake and headfixed.

Preparation for recording

At the beginning of each recording session, a small area of the chamber to be recorded from must be prepared for electrode penetration. After opening the chamber, the superficial connective and loose agranular tissue that has accumulated is removed under a binocular microscope using gentle suction applied to a fine glass sucker (tip diameter: ~1.5 mm), together with a pair of fine watchmakers forceps and a fine, 90°, 6 mm corneal hook (we use Lawton, No. 68-1027; U.K. supplier: Caterham Surgical Supplies, Surrey, UK; similar hook available in the U.S.: Fine Science Tools, Foster City, CA, No. 10140-02, see APPENDIX). This is not normally painful to the animal, and monkeys do not usually show any signs of discomfort while clearing this small area. Once it is prepared, the electrodes can be inserted, and the recording session commenced. In our laboratory, the mechanical mounting of the electrode drive is such that we can examine directly the entry of electrodes through the dura. Along with the monitoring of the electrode alignment and electrophysiological activity from the electrode tip, this alerts us to any excessive dimpling of the dura by the electrode.

Removing excess fibrous tissue

This tissue is removed after most recording sessions with the objective of clearing a new area of dura in preparation for the next session. Once the electrodes and drives have been removed, a local anesthetic cream (EMLA, Astra Pharmaceuticals) is applied so as to cover the exposed dura and connective tissue, and this is left in place for 5 min; the cream is then aspirated and the chamber flushed with sterile saline. This application helps to minimize any discomfort felt by the monkey during contact with the dura. The use of local anesthetic is most often necessary when working close to a large dural blood vessel (e.g., middle meningeal artery). Dural nociceptive fibers are known to be positioned predominantly close to such vessels (Wolff 1963).

FIG. 1. Schematic diagram to show the arrangement of the chamber and Thomas Recording multiple electrode drive during recording.
Under microscopic examination, fibrous tissue is removed from the chamber using gentle suction, the corneal hook, straight watchmakers forceps and a pair of miniature surgical scissors [Fine Science Tools (Interfocus in the UK), No. 15002-008]. It is often necessary to peel back layers of fibrous tissue that can accumulate rapidly above the dura. Even when treated with 5FU, this tissue can be very robust and tough enough to prevent electrode entry. However, it is rather avascular, and there is little blood loss during the removal procedure. Once the translucent blue-white appearance of the underlying dura has been revealed by removing the fibrous layers above it, it is unsafe to continue stripping off any further layers, since this may result in tearing of the dura. Once the dura has been stripped of the new growth and the dura can be clearly seen, it is flushed and all fluid aspirated. It is now ready for treatment with 5FU.

Treatment with 5FU

Sufficient 5FU solution to cover the exposed dural area is drawn up using a syringe and hypodermic needle from the container. The exact amount required will vary depending on the depth, size, and angle of the chamber, but a typical quantity will be 0.5 ml in a circular chamber of internal diameter 12 mm, depth 5 mm from the surface to the dura. If the chamber is at an angle, a small piece of sterile cotton wool is placed in the chamber to soak up and distribute the 5FU, as it is important that the solution covers the entire dura. The 5FU is then applied carefully to the dura, ensuring in particular that there is no spillage onto the skin surrounding the chamber. The solution of 5FU is left for 5 min for optimal suppression of tissue growth as is common practice for its use in wound healing after ophthalmic surgery (Merriman et al. 2001; Wilkins et al. 2000). After this time, the 5FU is removed from the chamber using the glass sucker. The chamber is then thoroughly flushed with a large volume of sterile saline which is also aspirated to a safe container; we typically run in ~30 ml direct from a sterile infusion bag. The chamber can now be closed and the monkey returned to its home cage. Note that 5FU is never applied at the beginning of a recording session, but used at the end of the session, immediately after the excess connective tissue has been removed.

Frequency of use

The first application of 5FU is made on the day of the chamber surgery when the dura is first exposed. Subsequently, it is applied around three times per week. When we first used 5FU, it was applied daily, 7 days/wk, with good effect. However, our more recent experience has shown that three times per week is sufficient to maintain successful suppression of growth. This is in accordance with recent literature, which has shown suppression of fibroblast proliferation for ≥3 days after a single dose of 5FU (Merriman et al. 2001).

Safety procedures

In addition to the safety precautions referred to in the preceding text, we take additional measures to ensure that 5FU only comes into minimal contact with dura and connective tissue in the chamber. We restrict the exposure time in the chamber (5 min) and most importantly, a thorough flush of saline is used to completely remove it. We also try to reduce the risk of 5FU crossing the dura during application. For example, if after several months of recording in the same chamber, repeated “picking” or “stripping” of the dura leads to a minor tear or herniation of the dura, we cease to use the 5FU protocol until it has healed. This usually only takes ~1 wk. Although there is no known toxic effect of 5FU on the CNS, these measures seem prudent to avoid the risk of unnecessary uptake, which could lead to unwanted actions or systemic side effects.

RESULTS

We report here our experience with the regular and repeated use of 5FU in the recording chamber. Our observations were gathered over a 6-yr period from 10 monkeys (recordings are still being made in 2 animals) and from a total of 33 chambers implanted on these monkeys. Typically, the period of active recording within a chamber is ~15–30 wk, and 5FU is applied throughout this period.

General observations

We have not observed any behavioral problems resulting from the application of 5FU, or neurological deficits that might have arisen for example from damage to motor cortex caused by its use. None of the known side effects listed by the manufacturer were observed. The topical doses and concentration of the 5FU we have used are, respectively, smaller and lower than those used routinely in trabulectomy surgery, where there is topical application to the eye and follow-up supplementary injections where needed (Mora et al. 1996). The use as described here is therefore well within current safety margins for humans. We have noticed that the treatment is more effective in some animals than in others. Prior to using 5FU, we occasionally had infections within chambers. Since using the 5FU protocol, we have not had a single occurrence of infection in a chamber. This is probably due to 5FU suppressing any potential bacterial proliferation.

Electrophysiology

In our experience, the use of 5FU caused no deterioration in the quality of the either single neuron or local field potential (LFP) data during the recording period in a given area. Initial recording data from a given chamber was qualitatively the same as that obtained toward the end of the recording period in that chamber, which was often ≥3 mo later—after ≥35–40 applications of 5FU.

We attempted to make a quantitative assessment of any effects of 5FU on the electrophysiology of the primary motor cortex (M1) by comparing data recorded in two adult purpose-bred macaques. Both monkeys were trained to perform a precision grip task, and recordings were made from M1. Neurons were identified antidromically as pyramidal tract neurons (PTNs) by stimulation of the medullary pyramidal tract (biphasic current pulse, each phase 0.2-ms duration) (see Baker et al. 2001). At the sites from which PTNs were recorded we also determined the threshold for evoking hand/finger movements by repetitive intracortical microstimulation (rICMS: 13 biphasic pulses at 300-Hz pulse, each phase 0.2-ms duration, delivered at 1 Hz).

In the control monkey (M24), recordings were made with conventional single-electrode techniques, using glass-insulated Pt-Ir electrodes. A total of 68 PTNs were recorded in 48 separate sessions from the right hemisphere (chamber 1); this took 20 wk in all; subsequently a further 60 PTNs were recorded in a further 43 sessions from the left hemisphere (chamber 2) and this took 23 wk. In the second monkey (M36), 5FU was routinely used in the cortical chamber throughout the recording period. In this monkey, a total of 154 PTNs were recorded in 30 sessions from the right hemisphere (chamber 1) and this took 12 wk. A further 196 PTNs were recorded in 55
sessions from the left hemisphere (chamber 2) and this took 22 wk. Thus in M24 the average yield of PTNs was 1.4 per session (128/91), compared with 4.1 in M36 (350/85), and the yield was 3.0 PTNs per week in M24 versus 10.3 in M36. These figures confirm the major increase in the yield of neuronal data using a multiple- versus single-electrode technique. They also demonstrate that PTNs were commonly encountered in cortex immediately beneath 5FU-treated dura.

Four different criteria were investigated: the antidromic latency, threshold, and depth of identified PTNs and rICMS thresholds. We compared data from M24 (no 5FU) with M36 (5FU). For M36, we also compared data collected during the first (early) and second (late) half of the recording period from a given chamber.

**Antidromic Latencies.** The ADL (antidromic latency) of a PTN covaries negatively with the size of neuron (Humphrey and Corrie 1978). If 5FU, for example, selectively damaged large pyramidal neurons, one would expect to see an increase in the mean ADL after treatment with 5FU. As Fig. 2 shows the distribution of ADLs was, in fact, unaffected by use of 5FU. The range of ADLs in PTNs recorded in the two untreated chambers of M24 were 0.6–5.2 and 0.6–4.2 ms, respectively, whereas for the 5FU-treated chambers in M36, the ranges were 0.7–3.9 and 0.4–4.4 ms. The mean antidromic latency was significantly shorter in the 5FU-treated animal than in the control (1.2 ± 0.7 vs. 1.6 ± 1.3 ms, P < 0.01, t-test). The mean values are highly skewed by the higher proportion of slow PTNs (ADL: >2.0 ms) that were targeted in the M24 study (see Bennett et al. 1993). In M24, these comprised 22% but only 8% in M36. When the distribution of fast PTNs (ADL: <2.0 ms) was compared, there was no significant difference (mean ADL: 1.1 ± 0.3 ms in both monkeys). In M36, there was no significant difference between the mean ADL for early versus late recording periods in either of the chambers (mean: 1.2 ± 0.6 ms, n = 83 PTNs vs. 1.4 ± 0.5 ms, n = 71) for chamber 1 and (mean: 1.3 ± 0.9 ms, n = 96 vs. 1.1 ± 0.6 ms, n = 100) for chamber 2 (see Fig. 2).

**Antidromic Thresholds of PTNs.** The antidromic threshold of a given PTN is influenced both by the size of the axon and the precise location of the PT stimulating electrodes relative to the axon (Lemon 1984). If 5FU damaged PTNs in any size-dependent manner, the mean antidromic threshold might be affected. However, in neither animal was there any significant correlation between ADL and antidromic threshold, and it is therefore questionable whether such damage would have been detected by a change in threshold.

**Depths of PTNs.** If 5FU damaged the cortex, it might be expected to affect the more superficial layers first, which would be closest to the epidural sites of 5FU application. This should result in PTNs, located in layer V, being encountered at shallower depths after 5FU treatment. Depths of PTNs were measured relative to the point at which neuronal activity as first detected after transdural penetration. In M24, PTNs were recorded over depths varying from 0.8 to 6.3 mm; the mean depth was 3.4 ± 1.6 mm (see Fig. 3). In the 5FU-treated monkey (M36), PTNs were again recorded between 0.8 and 6.3 mm, but the mean depth (2.3 ± 0.6 mm) was significantly shallower (P<0.001). In addition, in M36, there were some small but significant decreases in the mean depth of recorded PTNs from the first half to the second half of the recording period (Fig. 3): from 2.2 ± 0.6 to 2.0 ± 0.4 mm in chamber 1 (P < 0.01) and from 2.7 ± 0.7 to 2.4 ± 0.7 mm in chamber 2. (P < 0.02). These differences may reflect 5FU-induced changes in the recorded cortical tissue, although other factors must be taken into account, including the location of penetrations (anterior bank of central sulcus vs. precentral convexity of gyrus) and differences in the type of electrode used and amount of dimpling (see Discussion).

**rICMS Thresholds.** Movements evoked by rICMS in M1 depend on complex trans-synaptic activation of PTNs and require the integrity of both superficial and deep cortical layers (Asanuma and Sakata 1967; Jankowska et al. 1975; Lemon et al. 1987; Stoney et al. 1968). Thresholds for these effects might vary depending on the location of the PT stimulating electrodes relative to the point at which neuronal activity as first detected after transdural penetration. In M24, PTNs were recorded using conventional techniques from chambers over M1 without 5-fluorouracil (5FU) treatment. In M36, PTNs were recorded using a multiple electrode drive from chambers treated regularly with 5FU. Data are also shown for the early and late parts of recording in M36 from M1 on the right (R) and left (L) hemispheres. A further comparison is made between the 2 monkeys for "fast" PTNs with antidromic latencies (ADLs) of <2.0 ms. See text for further details.

**FIG. 2.** Mean antidromic latency (±SD) of identified pyramidal tract neurons (PTNs) recorded in 2 macaque monkeys (M24 and M36), numbers given in brackets. In M24, PTNs were recorded using conventional techniques from chambers over M1 without 5-fluorouracil (5FU) treatment. In M36, PTNs were recorded using a multiple electrode drive from chambers treated regularly with 5FU. Data are also shown for the early and late parts of recording in M36 from M1 on the right (R) and left (L) hemispheres. A further comparison is made between the 2 monkeys for "fast" PTNs with antidromic latencies (ADLs) of <2.0 ms. See text for further details.
be expected to be very sensitive to any damage to the cortical neuropil by 5FU. In fact, as Fig. 4 shows, there was no significant difference in the mean rICMS threshold for hand and finger movements in the two monkeys: in M24, the mean threshold was identical in both chambers at 12.9 ± 6.3 and 12.9 ± 6.5 μA (n = 32 and 28 sites, chamber 1 and 2, respectively). In M36, the mean thresholds for each chamber were 15.7 ± 4.9 μA (n = 115 sites) and 13.0 ± 5.1 μA (n = 162 sites). There was no significant difference overall between the threshold in M24 versus that in M36 (t-test, \( P = 0.15 \)). In monkey M36, the average threshold for rICMS effects showed a small but insignificant decrease from the first to the second half of the recording period. For chamber 1, it decreased from 13.3 ± 4.8 μA (n = 64) to 12.9 ± 5.4 μA (n = 98), and for chamber 2, it fell from 16.9 ± 4.6 μA (n = 47) to 14.8 ± 4.9 μA (n = 68). Thus these data suggest that 5FU had no demonstrable effect on the mean rICMS threshold.

**Histology of cortex underlying 5FU-treated dura mater**

To confirm that no abnormalities of the cortex were present, an histological analysis of the cortical tissue underlying the
5FU-treated dura was undertaken. In M36, the analysis was carried out on blocks of primary motor cortex (M1) from the left and right hemispheres. This cortical tissue was accessed through chambers in which the dura had been treated approximately three times per week for 32 and 13 wk, respectively. As can be seen from Fig. 5A, the histological appearance of the M1 cortex was normal. The major distinguishing features of M1 are clear to see, including the thickened cortex and presence of large pyramidal (Betz) cells in the deep part of lamina V. Note that the superficial layers also have a normal appearance. In the left M1, a small (100 nL) injection of the tracer biotinylated dextra amine (BDA, 10% in saline) was made in the anterior bank of the central sulcus three days prior to sacrifice of the monkey. Figure 5B shows a section through M1 taken a few millimetres away from the injection site and showing several retrogradely labeled pyramidal neurons. This established that normal axonal transport was present in these neurons. Cortical tissue from two other 5FU-treated monkeys also appeared normal.

Effects on recorded data yield from monkeys treated with 5FU

The use of 5FU improved the efficiency of our recording sessions and increased the total yield of data from each chamber. Prior to the use of 5FU, recording periods had to be interrupted approximately every 2 wk to perform a surgical strip or removal of excess tough connective tissue in the chamber. This was carried out under general anesthetic or deep sedation. This meant a smaller number of sessions over a given period compared with what we were able to achieve with 5FU treatment. In our experience, we were able to continue recording within a given chamber for much longer periods before such an operation became necessary. For instance, in M36, major dura “strips” under sedation were carried out roughly once every 6 wk. These operations were generally rather straightforward, largely because the suppression by 5FU of tissue growth and vascularization within the chamber substantially reduced the amount of bleeding.

Electrode usage and breakage when using 5FU

In our experience, there are two main causes of electrode breakage—the major one is attempting to penetrate tissue that is too tough or thick and the other is operator error during loading and unloading of electrodes from the drive. It would have been ideal to compare the experience of using the Thomas multiple-electrode recording system in chambers with and without treatment with 5FU. This might have yielded a better comparison than with data obtained using conventional single electrode methods, as reported in the preceding text. However, such a comparison was not possible because in our experience within 2 wk of exposing the dura at the start of new recording period, it becomes virtually impenetrable to the fragile Thomas electrodes. Indeed, we found that unless we stripped the dura every 2 wk or so under anesthetic, recording became impossible and many or most electrodes were damaged; based on a small sample of recordings, we were able to use each electrode on average for less than two recording sessions before it broke or failed. This is in contrast to recording in M36, when each electrode lasted an average of 8.5 ± 4.6 sessions (range: 1–22 sessions).

Discussion

We report here in detail the use of 5FU to treat the dura mater in chambers used for recording single neurons with transdural electrodes in the awake, behaving monkey. The benefits of treating the dura in such chambers appear to be fourfold. The first and major advantage is that because 5FU suppresses fibroblast growth in the chambers, dural penetration with microelectrodes is easier. There is therefore less damage to the cortex (due to excessive downward pressure or dimpling when attempting to penetrate tough dura) and to electrodes (which frequently break during such attempts). Second, with 5FU treatment, it is possible to carry on successful recording sessions for longer periods than for untreated dura, with fewer interruptions for surgical removal of connective tissue build up. The reduction in the number of procedures under anesthesia represents a considerable improvement in animal well-being and leads to improved task performance and also greater recording time. Third, because 5FU affects tissue regrowth and vascularization, these operations to strip or scrape the dura are relatively easy and avascular. Finally, the antimitotic properties of 5FU appear to help in controlling bacterial infections within the recording chamber.

The advantages of 5FU treatment are reflected in the excellent yield of high-quality data recorded simultaneously from single neurons in different cortical motor areas (see Baker and Lemon 2000; Baker et al. 1999, 2001, 2003). Although the major beneficiaries of this approach will be those using relatively fine microelectrodes (such as those from Thomas Recording), we believe the advantages of 5FU usage also apply to those using larger, stronger electrodes. The benefits of 5FU are obtained from relatively short periods of exposure (5 min) repeated up to three times a week. We should like to stress that it may be very important to limit the action of 5FU to these 5-min applications by very thorough irrigation and rinsing of the chamber with sterile saline after treatment. Because 5FU is a powerful anti-mitotic agent, it is important to be sure that it has no deleterious side effects either on the cortex or the monkey’s general health. Our introduction to the use of 5FU came about as a result of its use in suppressing scars following operations on the sclera for glaucoma and other ophthalmological disorders (Asaria et al. 2001; Smith et al. 1992). As far as we are aware, this treatment, which is now very widely used in ophthalmology clinics, has not resulted in any retinal damage or deficit, which might be expected if 5FU had toxic actions on neurons. Moreover, there is a wealth of literature on its use in cancer treatment, which involves the administration of systemic doses without any CNS side effects. We have seen no sign of unwanted side effects in either the cortex or the monkey’s general health and well-being.

We have also been unable to detect any obvious deleterious changes in either the electrophysiology or histology of the cortex underlying dura treated regularly for long periods with 5FU. For example, in our studies of identified PTNs, the antidromic threshold and recorded depths of these neurons was very similar in M1 cortex beneath 5FU-treated dura and in a control experiment in which PTNs were recorded with conventional techniques and without 5FU treatment in the chamber. In addition, we did not see any obvious changes in the properties of the neurons recorded in the early phase of 5FU treatment (in the first weeks after the chamber had been im-
planted) compared with those sampled during later periods of recording toward the end of the chamber. We stress in particular the high yield of properly identified PTNs in 5FU-treated chambers and the close similarity of thresholds for rICMS evoking hand and digit movements in monkeys with and without 5FU treatment. Because the production of such movements depends on the activation of complex local circuits leading to repetitive discharge of corticospinal neurons, it is assumed that any damage caused by 5FU should reflect an increased threshold for rICMS effects. This was not the case (Fig. 4).

There were some small changes in the mean depth at which PTNs were recorded, being significantly deeper in the control cortex (monkey M24) than in the 5FU-treated monkey (M36, see Fig. 3). This might have implied damage of superficial layers in the treated monkey, such that PTNs were encountered at shallower depths than in the control. However, we did not observe any loss of superficial layers after 5FU (see Fig. 5, A and B). The difference in PTN depths could result from other factors; for example, the proportion of tracks made in the convexity of the precentral gyrus, where the deepest PTNs are encountered at depths up to ~3 mm, compared with those made in the anterior bank of the central sulcus, where PTNs can be encountered up ~6 mm. Further, it is possible that the larger conventional microelectrodes used in the control monkey may actually have caused more damage to the relatively superficial layer V PTNs, and deeper penetrations were required to find other PTNs.

Use of 5FU in other laboratories

We have conducted a recent survey of 11 other laboratories in the United Kingdom, Europe, the U.S., and Japan to which we introduced the method of using 5FU to control growth of dural connective tissue. Of these 11, 9 are currently using 5FU and all of them are satisfied with the technique. The other two are not currently involved in studies where the use of 5FU would be relevant.

Conclusion

We believe that the use of 5FU as an anti-mitotic agent to control the growth of tough extradural connective tissue is a
safe and useful method with several advantages. It refines the method of single-neuron recording from awake, behaving monkeys and reduces the number and severity of procedures. It can improve the amount and quality of data collected from a given area and reduce both the time taken and cost of electrodes. We have not found any evidence that treatment along the lines described here damages or otherwise changes the properties of the underlying cortical tissue. We therefore recommend its use to other neurophysiologists using transdural microelectrode methods. We also believe it may be of use in other situations where there is dural scarring after surgical intervention or injury.

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DISCLOSURES

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