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Gelatine-embedded electrodes—a novel biocompatible vehicle allowing implantation of highly flexible microelectrodes

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Short title: Gelatine-embedded electrodes

Abstract

Chronic neural interfaces that are both structurally and functionally stable inside the brain over years or decades hold great promise to become an invaluable clinical tool in the near future. A main flaw in the current electrode interfaces is that their recording capabilities deteriorate over time, possibly due to lack of flexibility, which causes movements in relation to the neural tissue that result in small inflammations and loss of electrode function. We have developed a new neural probe using the stabilizing property of gelatine that allows the implantation of ultra-thin and flexible electrodes into the central nervous system. The microglial and astrocytic reactions evoked by implanted gelatine needles, as well as the wire bundles in combination with gelatine, were investigated using immunohistochemistry and fluorescence microscopy up to 12 weeks after implantation. The results indicate that pure gelatine needles were stiff enough to penetrate the brain tissue on their own, and evoked a significantly smaller chronic scar than stab wounds. Moreover, gelatine embedding appeared to reduce the acute reactions caused by the implants and we found no adverse effects of gelatine or gelatine-embedded electrodes. Successful electrophysiological recordings were made from very thin electrodes implanted in this fashion.

1. Introduction

Chronic Neural Interfaces (CNI), implantable multichannel electrodes for both recording and stimulating neurons, have a great potential for both fundamental neurophysiological research and clinical applications ranging from tetraplegia to affective disorders [1-3]. The ability to use such interfaces for recording from awake and behaving animals have opened doors for completely new kinds of neurophysiological experiments, most famously in the form of Brain-Machine Interfaces (BMI). The development of BMIs have already led to significant advances, such as restoration of motor control and control of a robotic arm in rats and non-human primates [4-7] and even the control of computer cursors by tetraplegic humans [8-10]. At present, the most common types of CNIs are either microfabricated silicon structures, such as the Michigan probe [11] or the Utah array [12], or arrays of microwires, typically 33-50 μm in diameter, made from for instance tungsten or stainless steel [13, 14].

A common problem of the electrodes available today is that the recording quality usually deteriorates over time [14-16]. Possible reasons for this include tissue injury and tissue reactions with the implant [17-19]. Conceivably, micromotions between the electrode and the tissue play a part in sustaining these tissue reactions [20]. It is hypothesized that as the electrodes used are relatively inflexible and anchored in the skull, they do not follow the movements of the brain parenchyma easily. Hence, shear forces between the electrode and the tissue arise at the electrode tips that may cause small injuries and result in microinflammations, including infiltration of glial cells and encapsulation, which result in the isolation of the electrode [15]. If such micromotions are of importance for the durability of the implant, highly flexible CNIs that can follow tissue movements need to be developed. A few different designs of flexible electrodes have been suggested, using a polymer material as backbone of the electrode [21-23]. However, very flexible electrodes are difficult to insert through the pia mater and thus new methods for implantation have been developed. These include incising the pia mater [23], treating it with collagenase [24], attaching the implant to a stiffer guide [25] or coating the electrode in a sugar solution [26] or a dissolvable polymer [27].

The aim of this study was to assess whether gelatine needles are suitable vehicles for implantation of electrodes that are too flexible to penetrate the brain tissue on their own. Gelatine is a natural material that provides the needed stiffness during insertion, independent of electrode type, but then dissolves in a matter of minutes. Previous studies *in vitro* have shown that neurons thrive on gelatine surfaces [28], thus making it a particularly interesting biocompatible candidate. To assess the biocompatibility of gelatine *in vivo*, microglial and astrocytic responses surrounding implanted gelatine needles were compared with those observed for stab wounds. Furthermore, the reaction evoked by wire bundles embedded in gelatine needles was compared with that evoked by bundles coated with a very thin layer of gelatine.

2. Methods

2.1. Animals and anaesthesia

All procedures were approved in advance by the Malmö/Lund Animal Ethics Committee on Animal Experiments. Implantations were performed in female Sprague Dawley rats ($n = 44$) (Taconic, Denmark) weighing 200–250 g. Animal handling and anaesthetic procedures are described elsewhere [29]. In brief, animals were anaesthetized with intraperitoneal (i.p.) injections of fentanyl (0.3 mg/kg body weight) and Domitor vet (medetomidin hydrochloride, 0.3 mg/kg body weight). After surgery, the animals received subcutaneous injections of an antidote to the anaesthesia (Antisedan, atipamezole hydrochloride, 0.5 mg/kg body weight) as well as Temgesic (buprenorphine, 50 $\mu\text{g/kg}$ body weight), which was used as analgesia. All animals survived for the duration of the experiments and exhibited signs of well being, such as weight gain, tidy fur and no signs of infection.

2.2. Surgery and implantation

The animals were attached to a stereotactic frame (KOPF Instruments, USA) under anaesthesia prior to any surgical procedure. Craniotomies (1 mm^2) were made over the target area and the dura mater was incised and deflected. Implants were attached to a hydraulic micromanipulator (KOPF Instruments, USA) with gelatine (Type B gelatine from bovine hides, British Drug Houses Ltd., UK). Implantations were made at a speed of 10 $\mu\text{m/s}$ to a depth of 2 mm. The implantations were made

under visual inspection using a microscope (magnification x50). Once the target depth was reached, the gelatine was rinsed in saline solution until it had dissolved and the implant was no longer in contact with the micromanipulator. This method ensured minimal or no movement of the implants when retracting the micromanipulator. The implants were left “free floating”, i.e., without any attachment to the skull. The skin was closed over the craniotomies using surgical clips. The animals were returned to the animal facility after awakening under supervision.

2.3. Implants for histological evaluation

Tissue reactions to the wire bundles embedded in gelatine, as well as to the gelatine itself, were studied. One group of animals ($n = 24$) was implanted with pure gelatine, which was moulded from a 40% solution into a needle shape, 300 μm in diameter and allowed to dry for at least 24 hours until hard enough to be implanted, on one side, and a stab wound was inflicted on the contralateral side using a stainless-steel needle with the same dimensions as the gelatine needle. A second group ($n = 14$) was implanted with wire bundles consisting of 32 tungsten wires that were 7.5 μm in diameter and were covered by 3 μm of the polyimide HML, for insulation (CalFineWire, USA). One hemisphere was implanted with a wire bundle coated with a very thin film of gelatine, henceforth referred to as gelatine-coated wires. The contralateral hemisphere was implanted with a wire bundle moulded into gelatine needles that were 300 μm in diameter (i.e., the same dimension as the pure gelatine needles); these are henceforth referred to as gelatine-embedded wires. These bundles were cut perpendicularly to minimize spread during insertion, thus aiding the process of quantification of the tissue reaction. The differences among these four groups of implants are explained schematically in figure 1. The animals implanted with gelatine needles and stab wounds were kept for either one, six or 12 weeks and the animals implanted with gelatine-coated and gelatine-embedded wire bundles were kept for either one or six weeks.

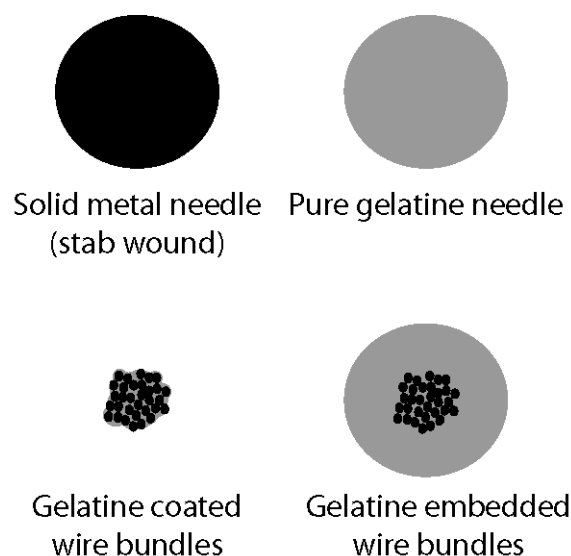


Figure 1. Schematic explanation of the four different groups of implants for histological evaluation; solid-metal needle (used for stab wounds, 300 μm in diameter), gelatine needle (made from pure gelatine, 300 μm in diameter), gelatine-coated wire bundle (bundle of 32 wires coated with a very thin layer of gelatine, approximately 180 μm in diameter) and gelatine-embedded wire bundle (32 wires moulded into a 300 μm needle of gelatine). Grey, gelatine; black, metal.

2.4. Histology

The animals were killed by an i.p. overdose of pentobarbital and were transcardially perfused with cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were dissected and immersed in post-fix solution (same as used for perfusion) overnight. The brains were then cryoprotected in 25% sucrose until they were no longer able to float and were cryosectioned horizontally using a sliding-knife freezing microtome (Microm, Germany) in increments of 30 μm .

After blocking in goat serum to preclude unspecific binding, the sections were incubated with primary rabbit antibodies against glial fibrillary acidic protein (GFAP, which is an astrocytic cytoskeleton protein; 1:5,000, Dako, Denmark) and mouse antibodies against CD68/ED1 (which is expressed in activated microglia; 1:250, AbD Serotec, UK) at room temperature overnight. Thereafter, sections were incubated with 4',6-diamidino-2-phenylindole (DAPI, which stains all cellular nuclei; 1:1,000, Invitrogen, USA), goat anti-rabbit Alexa594 antibodies and goat anti-mouse Alexa488 antibodies (1:500, Invitrogen, USA). Sections were mounted onto chromealum-coated glass slides and coverslipped using PVA/DABCO (Fluka/Sigma-Aldrich, Switzerland).

2.5. Image acquisition

Image capture and analysis were performed using the NIS-Elements 3.0 software (Nikon Instruments). Regions of interest (ROIs) were set at 0–25, 25–50, 50–100 and 100–200 μm distance from the centre of the wound, for stab wounds and gelatine needles, or from the rim of the artefact, for the implanted wires. Because of the variability of the specificity of the different markers for their respective antigens, the thresholds were set at individual levels for each marker, corresponding to differences in the contrast between unspecific background staining and positively stained antigens. The larger the contrast between the background and positively labelled tissues, the higher the threshold was set. Thus, the intensity thresholds for GFAP were set at 3.5 times the background intensity and for ED1 at five times the background intensity. The total area and the area above the intensity thresholds were measured within each ROI. Sections from the middle of the implant tract, i.e., at an approximate depth of 750–1,000 μm , were chosen for imaging. Quantifications were only performed in animals with visible scars. All image analyses were performed in a blinded manner by an experienced histologist who had no information regarding the different groups.

2.6. Visualization of wire spread

To be able to interface a larger region of the cortex than that of the entry zone, we aimed at letting the wires fan out during insertion, as illustrated in figure 2(a). To visualize this feature, gelatine-coated wire bundles consisting of 16 tungsten wires (12.5 μm in diameter) were implanted into rat cerebral cortex, as described above. To aid the process of spreading during insertion, the tip of the bundle was cut obliquely, causing the wires to diverge slightly at the tips, as can be seen in figure 2(a). Implantations were made as described above, until the wire tips had penetrated the pia mater, at which point the implant was rinsed for a few minutes in saline solution before being inserted up to the intended depth. This procedure can be mimicked by advancing the electrodes into the tissue more slowly, to let the gelatine dissolve. After implantation, the rat was killed and was transcardially perfused as described above. The brain was dissected and postfixed overnight, before being sliced manually into a 2 mm thick coronal slice containing the wire bundle. The slice was dehydrated in increasing concentrations of ethanol and was clarified in methyl salicylate (Sigma-Aldrich, Switzerland) for 6 h. Subsequently, the slice was mounted with dibutyl phthalate xylene DPX (Fluka/Sigma-Aldrich, Switzerland) and was examined using dark-field and fluorescence microscopy. This method renders the tissue transparent and thus allows visualization of the electrodes (figure 2(b)).

2.7. Implantation of recording electrodes

Tungsten wires 7.5 or 12.5 μm in diameter that were insulated as above were used as electrode material. The insulation was removed manually with forceps from a 5 mm section at the end of the wire. Thirty-two wires were glued to a connector board (Samtec, USA) with silver conductive paint (Electrolube, UK). The connector was insulated with epoxy (Loctite, Sweden). Impedance measurements were performed on each channel to evaluate the contact between the wires and the connector board. The wires were then glued together with gelatine and moulded into a 300 μm thick needle of gelatine (as was done for the gelatine-embedded bundles described above). To provide a means to identify the position of each channel inside the tissue after implantation, wires were coloured with grains of fluorescent colours (Lefranc & Bourgeois, France) and coated in cyanacrylate (Loctite, Sweden) or epoxy (Loctite, Sweden) (figure 2(c, d)). These layers increased the diameter of each

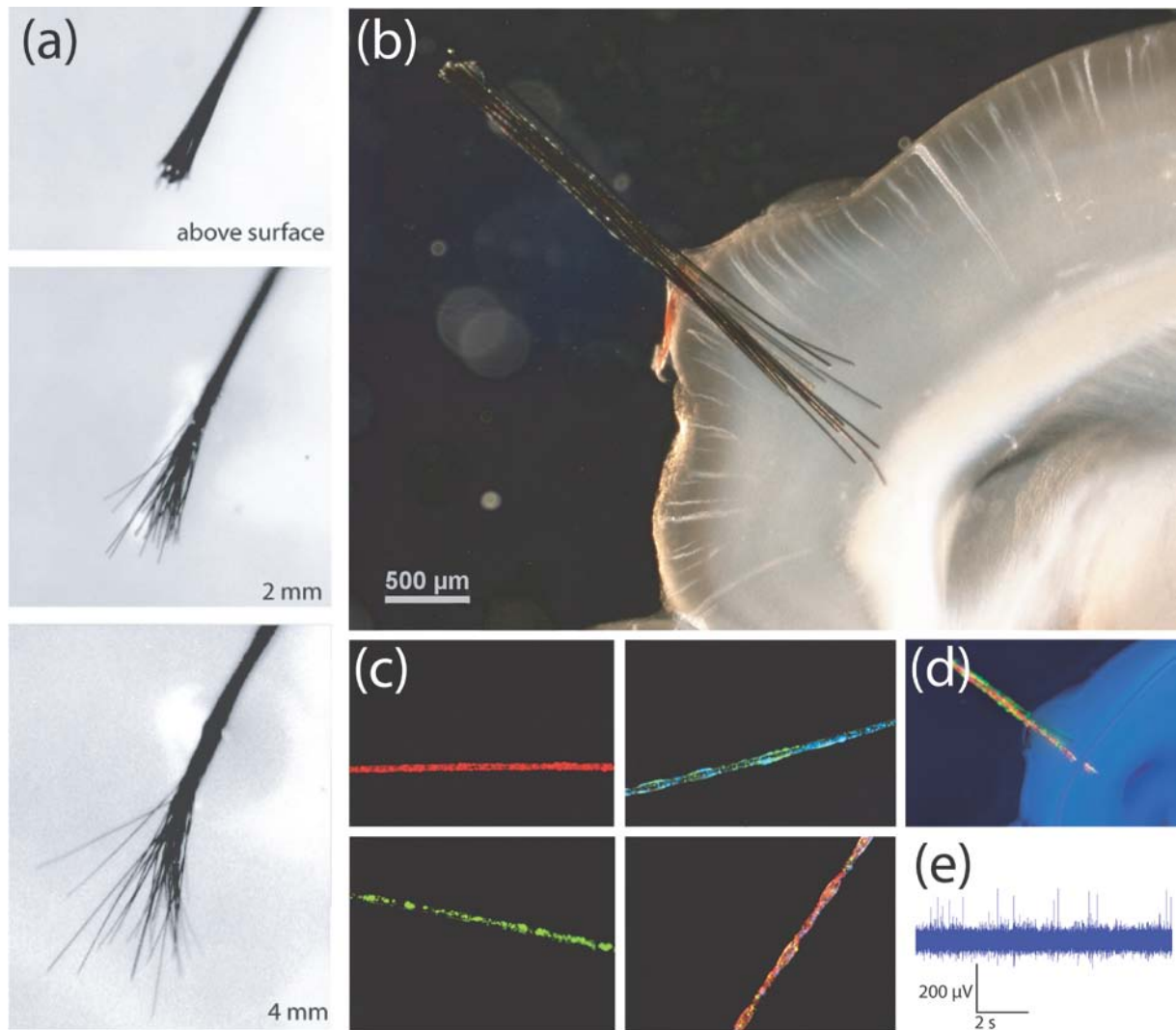


Figure 2. (a) Visualization of the unfolding process of a gelatine-coated wire bundle in an agar gel at indicated depths below the surface. (b) Unfolded wire bundle inside the rat cerebral cortex, clarified in methyl salicylate. (c) Fluorescent colour-coded wires. (d) Fluorescent wire bundle implanted into the rat cerebral cortex, clarified in methyl salicylate. (e) Single-unit recording using a 7.5 µm tungsten wire implanted as part of a gelatine-embedded wire bundle.

individual wire by approximately 5 µm. Implantations were performed as described above. The craniotomy was covered with a small droplet of agar and the connector board was fastened to the skull using three stainless-steel screws and dental acrylic (Kerr Italia, Italy). The skin was closed around the contact with surgical clips. Recording sessions were performed seven days after surgery, at the earliest. The animals were anaesthetized with 2% isoflurane and connected to a 32-channel recording system (Neuralynx Instruments, USA). Recordings were performed under anaesthesia.

2.8. Statistical analyses

Non-parametric statistics, the Kruskal-Wallis test with Dunn's post hoc test, were used in this study. Comparisons among different implants were performed on data recorded from sections from the middle of the respective wounds. The Kruskal-Wallis test was done for each ROI to determine if there were statistical differences between different implant types or over time. The Dunn's post hoc test for selected pairs was then applied on data from the same time point with different implants and on the same implant type between different time points. All p-values in the text are thus corrected for multiple analyses. $P < 0.05$ was considered significant. All values were presented as median values

with indication of the 25 and 75 percentiles (symbols with error bars). All analyses were performed using the GraphPad Prism 5.02 software (GraphPad Software Inc., USA).

3. Results

3.1. General tissue reactions

Generally, the implants tested in this study evoked tissue reactions that were comparable with those seen in other studies conducted in our lab ([30] and unpublished data). All types of implants generally evoked similar reactions after one week, primarily in the form of microglial infiltration, with the exception of two of the gelatine needles, which did not evoke any response at all after one week. All groups exhibited a diffuse distribution of reactive astrocytes after one week, which were not present in normal tissue and were redistributed to a concentrated scar around the implantation site over the following five weeks.

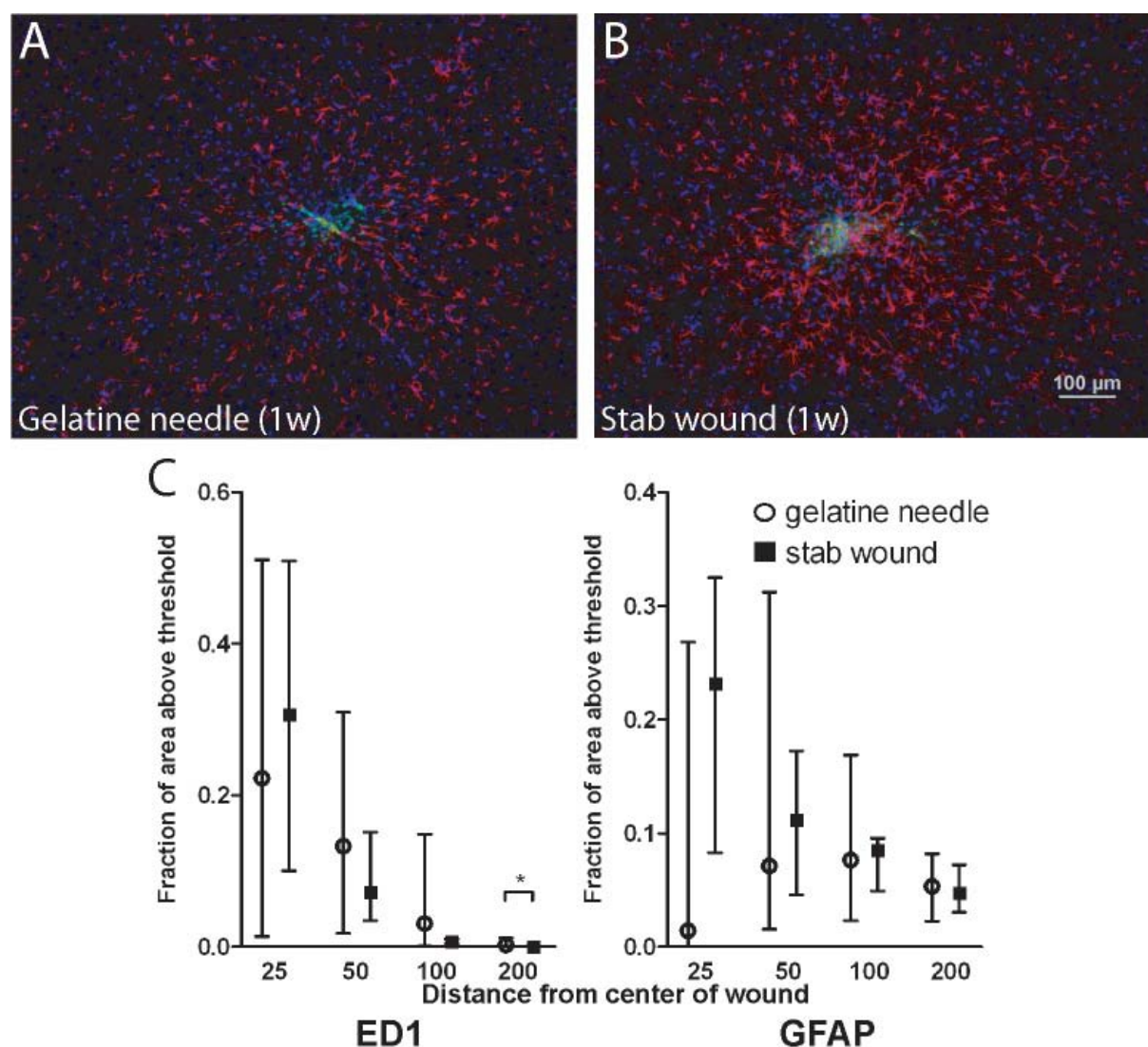


Figure 3. (a, b) Immunohistochemical images of cortical sections stained for GFAP (red), ED1 (green) and DAPI (blue), one week after implantation of a gelatine needle or stab wounding. White asterisk (*) marks the centre of the scar. (c) Quantification of the GFAP and ED1 densities surrounding the implantation sites. Symbols and error bars indicate medians and quartiles. The X axis represents values from the four ROIs: 25 signifies the 0–25 μm ROI, 50 the 25–50 μm ROI, etc. The Y axis indicates the fraction of area within the respective ROI with intensity above the threshold.

3.2. Gelatine needles vs stab wounds

In the first groups of rats, tissue reactions to implanted gelatine needles were compared with stab wounds caused by a metallic needle (diameter, 300 μm). As the gelatine needles dissolved completely in all animals, the only indication that they had been implanted was the glial scar. In the one-week group, two animals did not develop a visible scar from the implanted gelatine needles. While the implantations were made under visual inspection, we cannot be entirely sure that all implants penetrated the tissue to the desired depth since there was no trace of tissue reactions in these animals. However, in the six-week group, only four animals developed a visible scar, and in the 12-week group, none of the eight implanted animals developed a scar from the gelatine needle, while all eight stab-wounded animals exhibited a scar. This may indicate that the long term tissue reactions to gelatine needles are less pronounced than to the stab wounds caused by the metallic needles. More importantly, the presence of scars deep into the tissue one and six weeks after implantation of gelatine needles does show that the gelatine needles are able to penetrate the brain without any support from stiffer electrode materials.

For those animals that did develop scars after the gelatine needle implantation, the tissue reactions appeared similar to those produced by stab wounds. Hence, after one week, a substantial microglial infiltration (which was visualized by ED1 staining) was seen at the implantation site in both groups (figure 3).

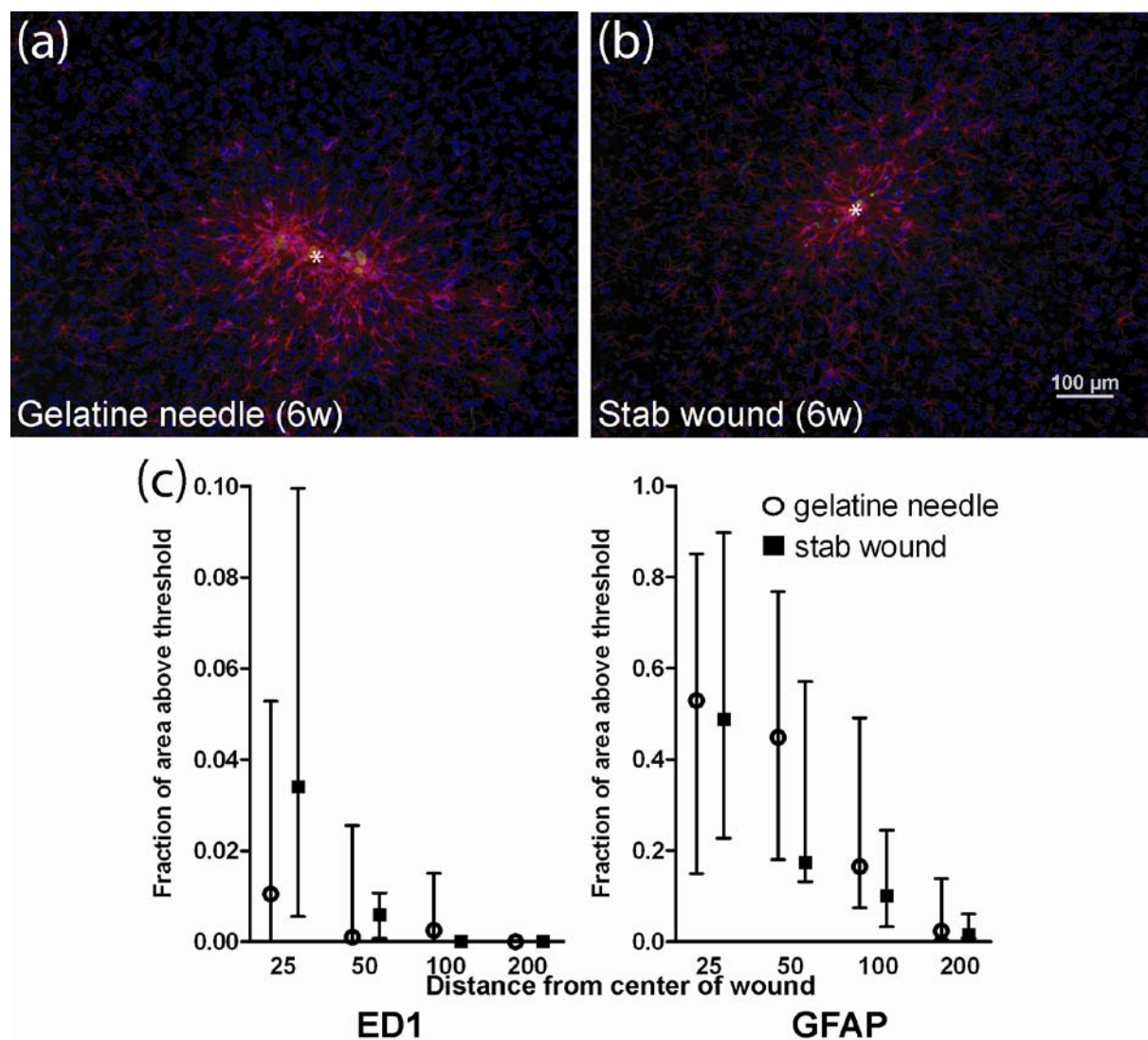


Figure 4. (a, b) Immunohistochemical images of cortical sections stained for GFAP (red), ED1 (green) and DAPI (blue), at six weeks after implantation. The white asterisk (*) marks the scar centre.

(c) Quantification of the GFAP and ED1 densities surrounding the implantation sites. Symbols and error bars indicate medians and quartiles. X and Y axes are as in figure 3(c).

The general impression was that the gelatine needles tended to evoke a more diffuse microglial infiltration, i.e., covering a larger area, than the stab wounds, while they were still only present within the ROIs examined (up to 200 μ m from the centre of the wound). This difference was significant in the outermost ROI, but both groups exhibited very low levels of ED1 cells in this area (figure 3). The astrocytic reactions (which were visualized by GFAP staining) showed a different pattern. Reactive astrocytes aggregated to some extent around the implantation scar after one week. However, the distribution of the astrocytes was much more widespread than that of the microglia, for both stab wounds and gelatine needles, and an increased density of reactive astrocytes was apparent in all parts of the brain examined, i.e., in all horizontal sections with a scar. After six weeks, the microglial reactions had diminished, even though there remained an increased concentration of microglial cells surrounding the implantation sites in both groups (figure 4). The astrocytes exhibited a completely different pattern at this time compared with the one-week time point. A clear astrocytic scar was seen surrounding the site of implantation, while levels in other regions of the brain, i.e., outside the examined ROIs, had diminished (figure 4). Thus, the differences between GFAP response at one and six weeks (figures 3 and 4) might reflect migration of reactive astrocytes towards the scar or, alternatively, an upregulation of reactive astrocytes adjacent to the scar and downregulation of these cells peripherally.

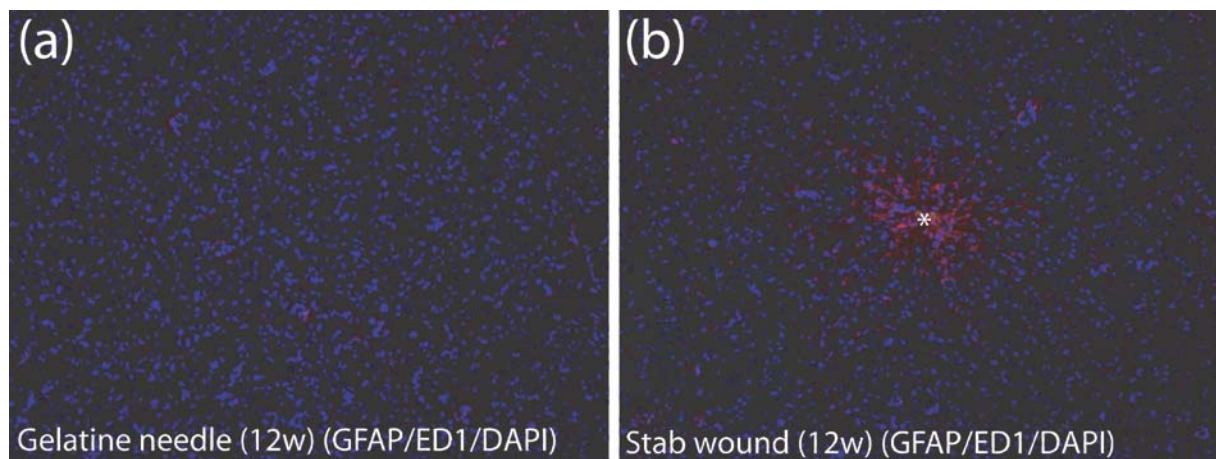


Figure 5. (a, b) Immunohistochemical images of cortical sections stained for GFAP (red), ED1 (green) and DAPI (blue), 12 weeks after implantation of a gelatine needle or stab wounding. Note the lack of visible astrocytic or microglial scar after gelatine implantation, but not after stab wounding. The white asterisk (*) indicates the centre of the scar, as identified by GFAP staining.

No other significant differences between gelatine needles and stab wounds were found in the groups of one or six weeks for ED1 or GFAP. After 12 weeks, ED1-positive microglia cells were found occasionally at the site of the stab wounds. There remained a visible GFAP-positive astrocytic scar, but this appeared less prominent compared with the six-week group (figure 4 and 5). Comparisons over time showed that the GFAP response tended to increase between one and six weeks, whereas ED1 showed the opposite progression (figures 3 and 4). The increase in GFAP was not significant, while the decrease in ED1 was significant in the stab wound group in the 25-50 μ m ROI and in the gelatine needle group in the 100-200 μ m ROI ($P < 0.05$).

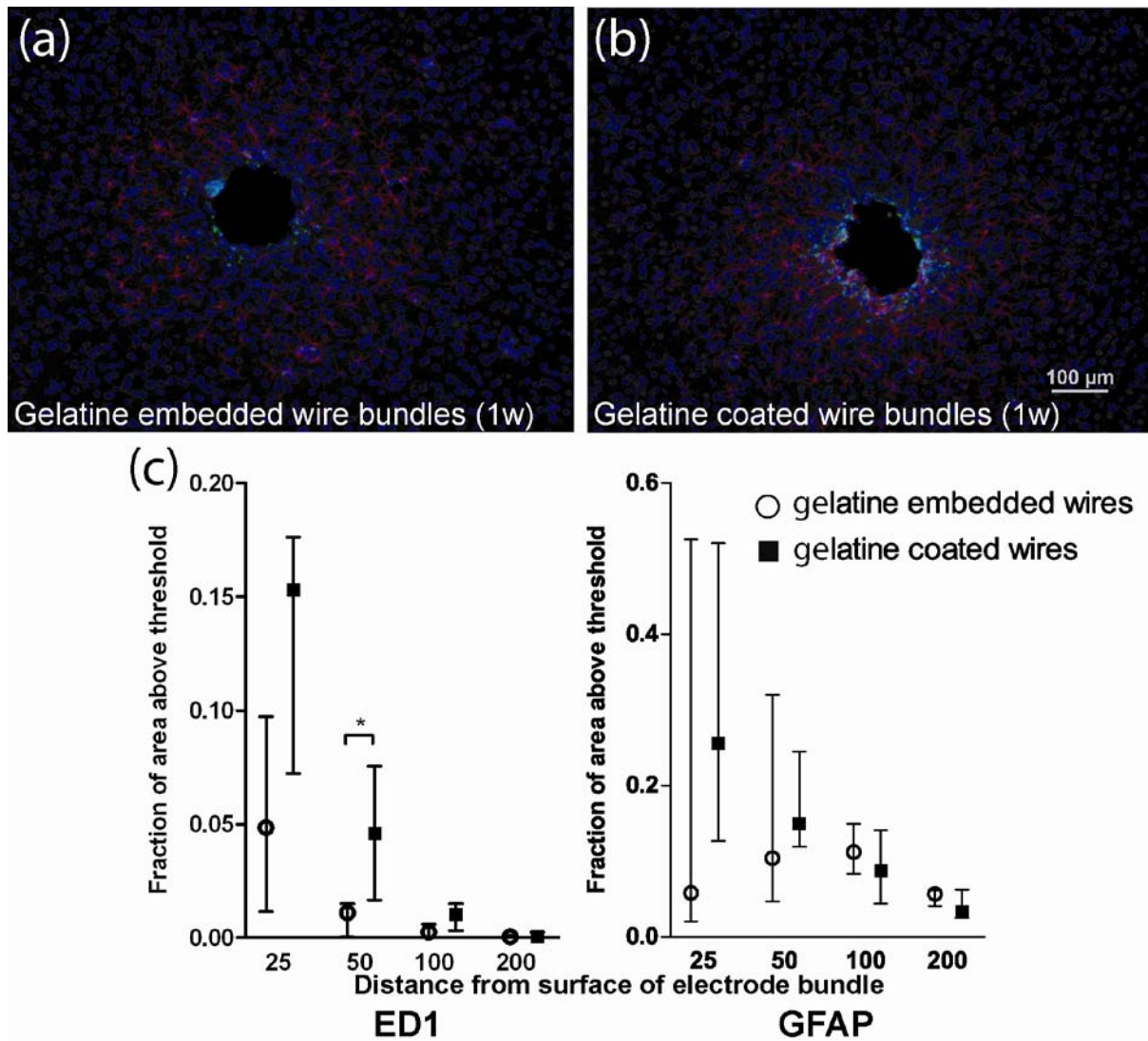


Figure 6. (a, b) Immunohistochemical images of cortical sections stained for GFAP (red), ED1 (green) and DAPI (blue), one week after implantation of gelatine-embedded wire bundles or gelatine-coated wire bundles. (c) Quantification of the GFAP and ED1 densities surrounding the implantation sites. Symbols and error bars indicate medians and quartiles. X and Y axes are as in figure 3(c).

3.3. Gelatine-embedded wire bundles vs gelatine-coated wire bundles

To evaluate reactions to gelatine in combination with implanted electrodes, tissue reactions to gelatine-embedded wire bundles and gelatine-coated wire bundles were examined (figure 1). In almost all of these animals, holes with similar dimensions to the wire bundles ($\sim 180 \mu\text{m}$ in diameter) were clearly visible in the histological sections. The astrocytic and microglial reactions were largely similar to the ones evoked by gelatine needles and stab wounds, in the sense that a rather large microglial infiltration, especially in the innermost ROIs, was seen after one week, which diminished after six weeks, while the astrocytic response was diffusely spread after one week, and was very clearly concentrated around the implant after six weeks (figures 6 and 7). In two animals implanted with wire bundles for one week and one animal implanted for six weeks, a different scar was seen on one side. In these animals, there were no GFAP-positive astrocytes within $100 \mu\text{m}$ of the artefact. Outside this area, however, the astrocytic reaction was similar to that seen in the other animals. Two of these scars were from gelatine-coated wire bundles and one was from a gelatine-embedded wire bundle. The ED1 stain yielded an expected signal in these animals.

The only significant difference between reactions to the gelatine-embedded and the gelatine-coated wire bundles (figures 6 and 7) was found after one week (figure 6), where gelatine-coated wires caused a significantly larger microglial reaction than gelatine-embedded wires. This suggests that the

acute inflammation caused by the implantation was reduced significantly if the implant was embedded in a thicker layer of gelatine.

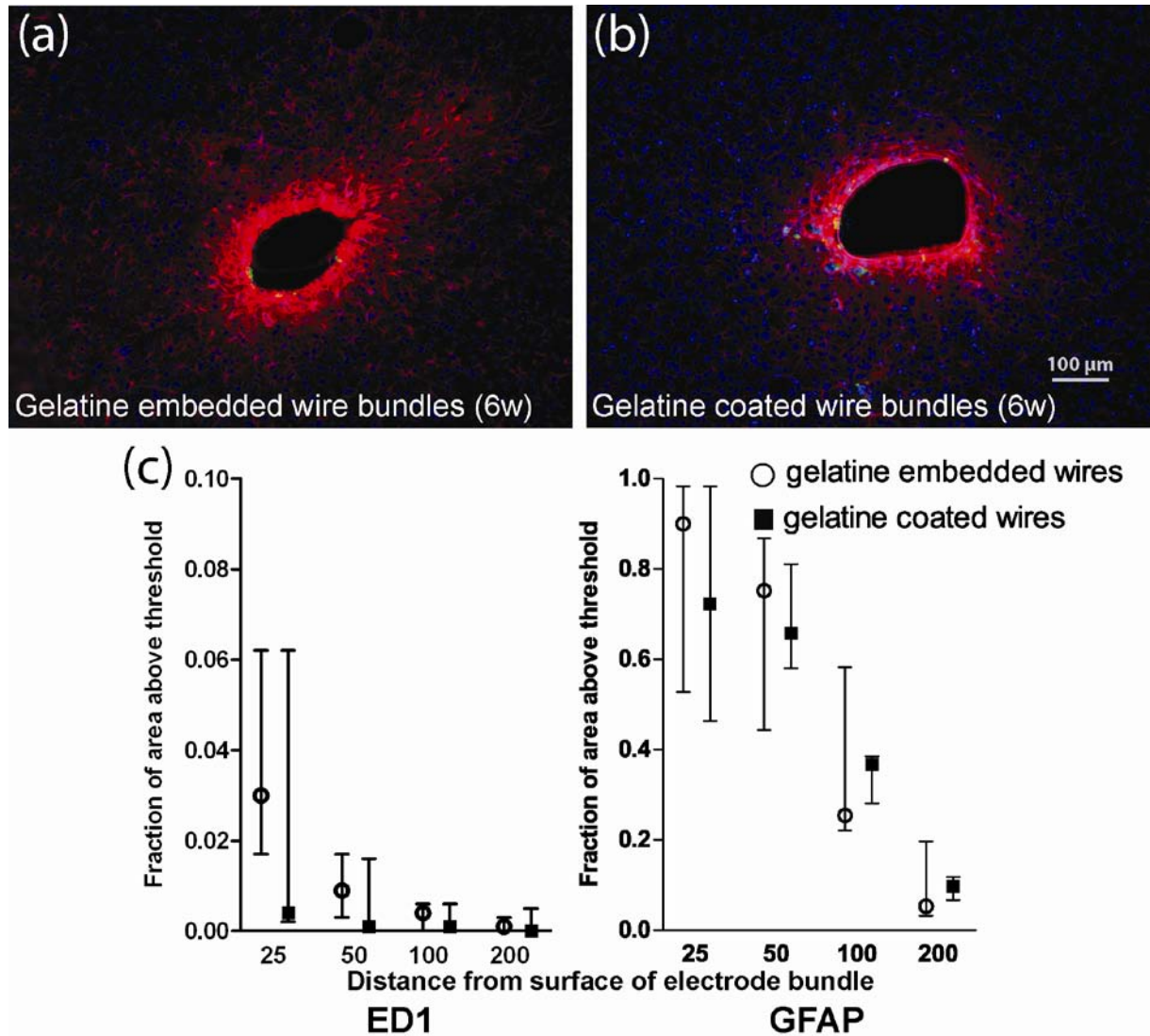


Figure 7. (a, b) Immunohistochemical images of cortical sections stained for GFAP (red), ED1 (green) and DAPI (blue), six weeks after implantation. (c) Quantification of the GFAP and ED1 densities surrounding the implantation sites. Symbols and error bars indicate medians and quartiles. X and Y axes are as in figure 3(c).

Comparisons over time showed patterns similar to those observed for stab wounds and gelatine needles, i.e., an increase in GFAP labelling and a decrease in ED1 labelling. The increase in GFAP between one and six weeks was significant for gelatine-coated bundles in the 50-100 μm ROI and for gelatine-embedded wire bundles in the two innermost ROIs ($P < 0.05$). The decrease in ED1 between one and six weeks was significant for gelatine-coated wire bundles in the innermost ROIs ($P < 0.05$).

3.4. Wire spread and electrical recordings

The method of embedding micro wire bundles in water-soluble gelatine allowed the implantation of ultra-thin wires into the rat cerebral cortex and led them to fan out over a larger area, once inserted. This was demonstrated both *in vitro* (figure 2(a)), by inserting bundles into blocks of agar, and *in vivo* (figure 2(b)). Typically, during implantation to a depth of 2 mm, a bundle of 12.5 μm wires spread out to interface an area with a diameter of approximately 500 μm , as can be seen in figure 2(b). The extent of this spread depended on the configuration of the wire tips, i.e., obliquely cut electrodes deviated more from the track line than transversally cut electrodes. Staining of the electrodes with different

combinations of fluorescent colours (figure 2(c, d)) allowed the visual identification of the position of each channel, which offers the possibility of determining the exact location of each wire while still in the cortex. As shown in figure 2(e), the wire electrodes were found to record single-unit action potentials with satisfactory signal-to-noise ratios. Successful recordings were acquired from both 7.5 and 12.5 μm thick wires (12.5 μm data not shown).

4. Discussion

The present study suggests that gelatine needles are suitable as a stabilizing vehicle for the implantation of very thin electrodes deep into the brain. The fact that the gelatine needles, containing no wires, are sufficiently rigid to penetrate the brain tissue on their own indicates that electrodes with much higher flexibility than the ones used here also can be implanted, since the needed rigidity is provided by the gelatine. Thus, this method can be adopted to implant electrodes that are flexible enough to completely follow the movements of the brain, both the movements between the cortex and the skull and intrinsic movements between different parts of the brain that the electrode penetrates. This could also open up possibilities to chronically interface the spinal cord or peripheral nerves, regions that have been difficult to achieve stable recordings from to date, mainly due to the large movements of these structures in relation to the surrounding tissue. Further studies are needed to assess how flexible an electrode would have to be to completely counteract these motions. A few advantages of thinner electrodes are already apparent; the tissue reaction to wire electrodes is significantly smaller for wires with a diameter of 12.5 μm than to wires of 25 μm [27] and probably even smaller for thinner wires. Furthermore, a much larger number of electrodes can be implanted through an entry wound of a certain area if the electrode diameter is reduced, giving possibilities to increase the recording site resolution of the electrode greatly.

The electrodes used here are considerably thinner and therefore more flexible than other electrodes in use today. In fact, a 7.5 μm wire is about 2000 times more flexible than a 50 μm wire of the same material, which is a very commonly used dimension [5, 13, 14, 31]. This is calculated from the linear elasticity theory for circular cross sections [32], which states that the force needed to bend an elastic wire (these wires are assumed to be elastic on the short distances that are relevant here) is calculated by the equation:

$$F = \frac{3\pi ED^4}{64L^3} \Delta x$$

where F is the force needed to bend a wire with a diameter D and length L the distance Δx and E is the Young's modulus for the material of the wire. Thus the force needed to bend a wire is increased with the diameter to the power of 4. This is only valid for small deviations, but since the motions of the cortex in relation to the skull are below 100 μm [20] it should be valid in the context relevant here. This demonstrates the impact of reducing the diameter of an electrode on the possibilities of allowing it to follow the movements of the brain flexibly and thus removing micromotions between cell and electrode completely. However, whether this difference is sufficient to make a significant impact on the mechanical stability of a cell-electrode interface is not known.

After one week, both gelatine needles and stab wounds elicited a microglial infiltration fully comparable with the one expected from the acute inflammation caused by the implantation procedure [17]. In the acute phase, the gelatine needles seemed to evoke only a slightly larger infiltration than the stab wounds, which possibly reflected the elimination of the implanted gelatine by microglial cells. Importantly, in the more chronic phase, the gelatine needles appeared to evoke lesser reactions than the stab wounds. No reaction to gelatine needles could be found after 12 weeks, while there remained an apparent scar from stab wounds at this point. This suggests that the brain is capable of eliminating the implanted gelatine completely, without forming a permanent scar. The neuronal cell density in the immediate vicinity of the implantation site, irrespective of the method of implantation used, was only slightly reduced, while there still remained viable neurons in all the examined ROIs at all timepoints (see Supplementary material, figures S1 and S2). This was the case for all groups examined, which

suggests that there is a population of neurons that is close enough to the electrode to allow recordings at all time points and for all implant types [15, 33].

The tissue reactions to gelatine-embedded and gelatine-coated wire bundles were largely similar in pattern to those of gelatine needles and stab wounds. A clear microglial infiltration into the tissue adjacent to the wire bundles took place during the first week and diminished over the following five weeks. Similarly, a diffuse astrocytic activation arose during the first week that was limited to a concentrated scar during the following five weeks, which is consistent with findings from other studies on both stab wounds and implanted electrodes [17-19, 27, 34-36]. Further direct comparisons with these studies are however hard to make since the quantification of the histological preparations differ significantly. Notably, the astrocytic scar after six weeks was more prominent from the wire bundles than from the gelatine needles and stab wounds, while it was fully comparable with the reactions to other kinds of chronically implanted electrodes. This reflects the formation of a glial encapsulation, which is an important component of the chronic reaction to any object implanted in the brain [15]. Importantly, the microglial reaction after one week was significantly smaller for the bundles embedded in a thick layer of gelatine than for the ones coated with a thin layer of gelatine, which implies a smaller acute inflammation caused by the implantation. This may indicate that the gelatine reduced the injury caused by the implantation or had other beneficial properties. This might be due to the slow dissolution of the gelatine during insertion, which provides a smooth and slippery surface that causes minimal amounts of shear forces on the tissue. Another contributory effect might be that collagen, which is the main component of gelatine, is a strong activator of haemostasis [37]. Thus, gelatine might work as a haemostatic agent that reduces the small haemorrhages caused by the implantation. The reduced damage also indicates that the neural circuits that are to be recorded from are more physiologically intact, increasing the validity of the recordings. If these notions prove to be true, it is conceivable that many types of implantations could be aided by embedding in gelatine-like materials.

In summary, the present study suggests that the gelatine vehicle developed here is suitable for long-term implantation of extremely flexible electrodes into the central nervous system. The gelatine used as a support matrix does not induce any substantial tissue reactions. It appears that gelatine-embedded wire bundles even induce lesser acute reactions than gelatine-coated wire bundles, indicating that the gelatine alleviates the damage caused during implantation. Since the gelatine needles are stiff enough to penetrate the pia mater on their own, this method should allow the implantation of electrodes of any thickness or flexibility. Further studies are called upon to determine how these electrodes should be designed to eliminate the micromotions between electrode and tissue completely. Furthermore, the extremely thin wires used in this study were fully capable of recording single-unit activity with a satisfactory signal-to-noise ratio. Further studies are required to assess the long-term stability of these electrodes, or even more flexible electrodes.

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