Ice coating – A new method of brain device insertion to mitigate acute injuries

Mohsin Mohammeda,*, Jonas Thelinb, Lina Gällentofta, Palmi Thor Thorbergssona, Lucas S. Kumosa, Jens Schouenborga,b, Lina M.E. Petterssona,b,*

a Neuronano Research Center, Department of Experimental Medicine, Lund University, Lund, Sweden
b NanoLund, Lund University, Professorsgatan 1, SE-223 63, Lund, Sweden

ARTICLE INFO

Keywords:
Neurosurgical method
Brain machine interface
Biocompatibility
Implantation method
Deep brain stimulation

ABSTRACT

Background: Reduction of insertion injury is likely important to approach physiological conditions in the vicinity of implanted devices intended to interface with the surrounding brain.

New methods: We have developed a novel, low-friction coating around frozen, gelatin embedded needles. By introducing a layer of thawing ice onto the gelatin, decreasing surface friction, we mitigate damage caused by the implantation.

Results and comparison with existing methods: The acute effects of a transient stab on neuronal density and glial reactions were assessed 1 and 7 days post stab in rat cortex and striatum both within and outside the insertion track using immunohistochemical staining. The addition of a coat of melting ice to the frozen gelatin embedded needles reduced the insertion force with around 50 %, substantially reduced the loss neurons (i.e. reduced neuronal void), and yielded near normal levels of astrocytes within the insertion track 1 day after insertion, as compared to gelatin coated probes of the same temperature without ice coating. There were negligible effects on glial reactions and neuronal density immediately outside the insertion track of both ice coated and cold gelatin embedded needles. This new method of implantation presents a considerable improvement compared to existing modes of device insertion.

Conclusions: Acute brain injuries following insertion of e.g. ultra-flexible electrodes, can be reduced by providing an outer coat of ultra-slippery thawing ice. No adverse effect of lowered implant temperature was found, opening the possibility of locking fragile electrode construct configurations in frozen gelatin, prior to implantation into the brain.

https://doi.org/10.1016/j.jneumeth.2020.108842

Received 15 May 2020; Received in revised form 22 June 2020; Accepted 2 July 2020
Available online 03 July 2020

* Corresponding authors.
E-mail addresses: mohsin.mohammed@med.lu.se (M. Mohammed), lina.pettersson@med.lu.se (L.M.E. Pettersson).
1. Introduction

Biocompatible neural interfaces that can monitor and interact with neuronal networks in the brain over long periods of time are of great interest for neuroscience. However, current implantable devices usually induce neuronal loss and glial reactions (Biran et al., 2005; Koziol et al., 2012; Stence et al., 2001), despite being made of non-toxic materials. From systematic studies, it has become clear that the microforces between implanted electrodes and surrounding tissue play a key role in triggering glial responses and loss of nearby neurons (Biran et al., 2007; Gilletti and Muthuswamy, 2006; Lind et al., 2013). Such microforces occur if implanted electrodes are unable to smoothly follow tissue movements (Köhler et al., 2015). As a consequence, major efforts towards creating implantable ultra-flexible electrodes have been made (Agorelius et al., 2015; Lind et al., 2010a; Zhou et al., 2017). To aid implantation of such delicate structures, we developed a technique of embedding ultra-thin electrodes in stiff needle-shaped gelatin (Etemadi et al., 2016; Lind et al., 2010a).

While most research in the field of neural interfaces is currently focused on optimizing electrodes, less attention has been given to the surgical methods of implantation (Krüger et al., 2010; Prodanov and Delbeke, 2016). It is known that damage to nervous tissue caused by a transient insertion causes acute tissue reactions similar to those generated around an implanted probe (Potter et al., 2012; Wellman et al., 2019). Moreover, brain tissue reactions caused by briefly inserting a syringe and injecting buffered saline solution can be quite long lasting (> 1 year in rats) (Gällentoft et al., 2015). A possible contributing factor to mechanical injuries resulting from insertion is the commonly observed dimpling of the brain surface and ensuing forces when traversing the nano/micro fiber mesh of the arachnoidea and pia mater (Andrei et al., 2012, 2011). Measurements performed during transient insertions demonstrate that considerable force remains following insertion, known as “resting” force, likely a consequence of unresolved dimpling and friction of the probe surface (Casanova et al., 2014a; Welkenhuyzen et al., 2011). In pilot experiments we observed that dimpling could be markedly reduced by cutting the arachnoidea and pia layers with an extremely sharp diamond knife prior to insertion.

Biocompatible gelatin, derived from collagen, has beneficial effects on reducing acute injury sequelae (Köhler et al., 2015; Kumosa et al., 2018; Lind et al., 2010b). In a recent study we examined the tissue reactions caused by controlled insertion and retraction (“stab wounds”) of either a stainless steel needle or a needle embedded in gelatin. It was found that coating a probe with gelatin not only reduced the microglial reactions but also significantly reduced leakage of the blood brain barrier (Kumosa et al., 2018). The combination of gelatin embedding and an ultra-flexible probe mitigated for the first time the ubiquitous loss of neurons in the immediately adjacent tissues (often referred to as the “kill zone”) six weeks after insertion (Köhler et al., 2015). This beneficial effect of gelatin coating may partly be due to reduced friction during insertion as the gelatin hydrates. Motivated by this notion, we in this study explore the effects of adding a coat of thawing ice to the gelatin embedded needles, as melting ice surfaces are well known to exhibit very low friction (Blau, 1995). Given that hypothermia is also known to protect brain tissue (Darwazeh and Yan, 2013; FAY, 1959; Polderman and Herold, 2009), an added beneficial effect of the lowered temperature is conceivable.

The main aim of the present study was to develop a novel technique utilizing a thawing ice coating of gelatin embedded probes to reduce trauma during device insertion. To this end we evaluated whether such a coating, compared to just lowering the temperature to around 0 °C, would be able to mitigate acute “stab wound” reactions caused by probe insertion into the brain. We first examined any potential effects of lowering the temperature of the probe, and then analysed the effect of adding a low friction, melting ice coat. A second aim was to assess whether the acute tissue reactions could be further reduced by cutting the pia mater with a diamond knife prior to insertion, potentially reducing dimpling of the cortical surface. The effects on cortical and underlying striatal tissue were examined 1, and 7 days post stab (PS). We find significantly reduced acute tissue reactions after adding an ice coat to the probe, but no added beneficial effects of cutting the pia mater. The results highlight the importance of reducing the mechanical trauma during implantation into the brain and may likely be of relevance for many neurosurgical interventions.

2. Material and methods

2.1. Manufacturing of needle implants and force measurement equipment

2.1.1. Needle implants

Stainless steel needles with a diameter of 100 μm (Austerlitz Minutien 0.10 Pin. Agnethos AB, Sweden) were used. Individual needles were positioned and centered in a custom-made plexiglass mould (Prototech AB, Helsingborg, Sweden) for gelatin embedding. The moulds consisted of two halves, with a central canal (diameter of 350 μm), a conical tip and a channel for gelatin injection. The two halves of the moulds were held together with 4 screws to achieve a precise fit. A 30 % W/V gelatin solution was prepared by dissolving 3 g of 101 Bloom strength gelatin (Gelita, Medella Pro 1500, USA) in 7 mL of deionized water at 70 °C. The 30 % gelatin solution was sonicated (Emmi-12HC, Emag technologies, Germany) at 55 °C to remove gas bubbles. The 55 °C solution was injected into the mould using a temperature-controlled syringe (Model TC-124A, Warner Instruments, 1125 Dixwell Avenue Hamden, USA), via the injection channel. After 4 h in room temperature (RT; 22 °C ± 1 °C) the gelatinized needles were dry enough to be removed from the moulds and were subsequently vacuum dried for a minimum of 6 h. As a result of the drying process, the diameter of the gelatinized needles shrunk to around 300 μm. The dried, gelatinized needles were then placed in polystyrene petri dishes (PS606 ~ 400EA, Sigma-Aldrich, Sweden) and stored in a freezer at ~18 °C until surgery. The gelatinized needles were split into three 3 groups i.e., 1) gelatinized needles at room temperature (RT needles), 2) frozen gelatinized needles (cold needles), and 3) frozen gelatinized needles with an icecoat (ice coat needles) (Fig. 1). The ice coat layer was produced on the day of surgery, by freezing the gelatinized needles

Fig. 1. Schematic illustration of the three kinds of probes used in the study. All stainless steel needles were embedded in gelatin, after which two groups of needles were frozen and one of these further coated with a layer of ice. The three groups of needles inserted thus were: i) gelatinized needles at room temperature (RT), ii) cold gelatinized needles, and iii) cold gelatinized needles with a melting layer of ice, i.e. ice coated needles.
on dry ice (CO₂-ice) for 10 min, subsequently dipping them in 4 °C Milli-Q water (Merck Millipore, Solna, Sweden) twice with a 5 min freezing step between, after which they were kept on dry ice along with the cold gelatinized needles, until implantation. The needles are illustrated in Figs. 1 and 2.

2.1.2. Force measurement equipment

To measure insertion forces during insertion of needles into rat brain tissue, a Micro Load Cell (0−100 g, CZL639HD) in conjunction with a PhidgetBridge 4-Input analog-to-digital USB interface (both from Phidgets, Inc., Canada) were used. An adapter was 3D-printed using an acrylic-like substrate to attach the load sensor to the Kopf Micromanipulator and stereotax on one end, and a syringe at the other. Gelatinized insect needles (cold or ice coat needles) affixed to dispos-able Luer-lock hypodermic needles, were quickly attached/detached to the syringe/probe holder. A Matlab interface was developed to record, plot and export force measurement data using manufacturer provided software drivers for the Microsoft Windows environment. All force recordings were performed with a 25 Hz sampling frequency and smoothed using a moving-average algorithm with a 0.5 s window. Calibration of the sensor was performed before each gelatin-embedded needle insertion (see below) using a sacrificial gelatinized needle, verified and adjusted using a 60 s recording immediately following each measurement procedure once the needle was retracted from the brain. Two outlier signals requiring adjustment more than 20 % of maximum recorded force were excluded. Recordings could not be performed before insertion due to the rapid interval needed between removal of each probe from the dry ice container to insertion into brain.

Maximum insertion values were defined as the maximum smoothed insertion force during the insertion procedure.

2.2. Animals and surgery

2.2.1. General surgery

All animal experiments were approved by Malmo /Lund Animal Ethics Committee on Animal Experiments (Permit number M76−16). Twenty-seven, adult, female/male Sprague-Dawley rats (Taconic, Denmark) were used. Allsurgical procedures were performed under deep anaesthesia. Induction of anaesthesia was done with a mixture of 2.5−1.8% Isoflurane (Isoba vet, Apoteksbolget, Sweden) in 30−40 % oxygen and 60−70 % nitrous oxide and then kept at 1.2−2 % Isoflurane, delivered through a rat nose mask (Model 906, Rat anaesthesia mask, David Kopf Instruments, California, USA). After shaving the head, the rats received a local anaesthetic subcutaneous injection of 0.4−0.6 ml xylocaine (2 mg/mL) + adrenalin (1.25 μgram/mL) (Dentsply Ltd, Surrey, United Kingdom) in the surgery area. During the whole surgical procedure, rats were kept on a heating pad, set at 37 °C, with temperature feedback from a rectal probe. Rats were mounted in a ste-reotactic frame, and a midline incision was made to expose the area between bregma and lambda. After removing the overlying connective tissue, the skullbone was removed over the insertion sites, two in each hemisphere; i) ± 2.4 mm lateral from the midline and 1 mm rostral to bregma, and ii) ± 3.4 mm lateral from the midline and 1 mm caudal to bregma Previous studies have shown that there are no significant inter-actions between multiple implants positioned at least 1 mm apart (Lind et al., 2012).

2.2.2. Implantation of probes for 1 and 7 days

At 3 out of the 4 insertion sites the dura was removed, using forceps and Iris scissors, just before implantation. In the 4th, the dura, ar-achnoidea and pia mater were cut using a diamond knife (. All of the gelatinized needles were hydraulically inserted using a KOPF Instruments 2650 micro-positioner (David Kopf instruments, Los Angeles, USA) to a depth of 5 mm from the brain surface. The insertion order was alternated (RT needles, cold needles, ice coat needles and ice coat needles with pia cut) between animals. The gelatinized needles were inserted (speed = 1000 μm/sec) and held in the brain for 10 min, after which they were slowly retracted (speed = 100 μm/sec). On the day of surgery the gelatinized needles were taken from storage (at −18 °C), one batch was kept in a dust free container at RT, and the rest were kept on dry ice. The ice coated needles were constantly monitored through the microscope after removal from the dry ice environment, and were implanted at the time when their outer surface shifted from an opaque ice coat to a transparent state (Fig. 2), i.e. when the transition from frozen to liquid water occurred (defined as 0 °C for water). The cold needles were implanted at the same duration after removal from the dry ice container. Measurements using a temperature probe (Testo 108, Testo SE & Co, KGaA, Germany) confirmed that the surface held a temperature of close to 0 °C at the time of implantation. After surgery the rats received a subcutaneous injection of Temgesic (buprenorphine, 0.05 mg/kg, Schering-Plough, Belgium) for postoperative analgesia, and the skin was closed using surgical clips.

2.2.3. Acute, insertion force measurements

After having prepared the surgery area (as per above), the following procedures were performed to measure the insertion force. The probe holder connected to the force meter was moved to the correct location, calibration was performed (see above), the dura was cut using fine forceps and a pair of iris scissors. Finally, the probe to be tested was swiftly mounted in the probe holder and inserted at a speed of 1000 μm/sec to a depth of 5 mm. The implants were constantly monitored through the microscope after removal from the dry ice environment and were implanted as described above. Needles were held for 10 min inside the brain, after which they were explanted at a speed of 100 μm/sec. The force subjected to the load cell was measured continuously throughout the experiment until 60 s after retraction above the brain surface (see above). After completion of the surgery, the animals were injected with an overdose of pentobarbital (150−200 mg/kg, i.p.) and when deeply anaesthetized killed by heart puncture.

![Fig. 2. Light field images of gelatine embedded needles without and with an ice coating. The structure of the RT gelatin embedded needle is shown (A), compared to the structure of the cold gelatin embedded needle with a melting ice coat (B). The transition from a frozen layer of ice to a melting ice layer is captured (b). 1 = stainless steel rod, 2 = gelatin layer, 3 = ice coat in a frozen state, and 4 = ice coat at melting state.](image-url)
2.3. Perfusion and tissue preparation

One, or seven days PS rats were deeply anaesthetized with an overdose of pentobarbital (150−200 mg/kg, i.p.). When animals showed no response to painful stimulation, they were transcardially perfused with 0.9 % RT saline until the tissue was cleared from blood (80−100 mL), followed by ~320 mL of ice cold 4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and finally ~80 mL of RT 0.9 % saline. The brains were dissected and cryoprotected in a 20 % sucrose solution at 4 °C for 22−30 hrs, snap frozen in isopentane on dry ice and stored at -80 °C until sectioning. The brains were sectioned (16 µm horizontal sections) in a cryostat (Microm HM 560, Microm GmbH, Walldorf, Germany), mounted onto glass slides (Super Frost plus, Menzel-Gläser, Germany), and stored at -20 °C.

2.4. Immunohistochemistry

Immunohistological staining of brain sections was performed to enable evaluation of neuronal density and microglia and astrocyte response. The frozen tissue sections were kept at RT for 30 min, and after rehydration in phosphate buffered saline (PBS; 3 × 10 min), the sections were blocked with 5 % goat serum in 0.25 % Triton X-100 in PBS to prevent nonspecific binding. The slides were incubated with primary antibodies overnight at RT. The following primary antibodies were used; i) mouse anti-CD68/ED1 (a marker for activated microglial cells, 1:250, Cat. Nr. MCA341R, AbD Serotec, UK), ii) rabbit anti-glial fibrillary acid protein (GFAP, an astrocytic cytoskeletal protein; 1:5000, Cat. Nr. Z0334, Dako, Denmark), or iii) rabbit anti-neuronal nuclei (NeuN, expressed n neuronal nuclei 1:500, Cat. Nr. ab 104225, Abcam, USA). Following another rinse in PBS (3 × 10 min), the slides were incubated in light sealed chambers with DAPI (4´, 6-diamidino-2-phenylindole, 1:1000, Invitrogen, USA), and the following secondary antibodies; i) goat anti-rabbit Alexa 594 (1:500, Invitrogen, USA) and ii) goat anti-mouse Alexa 488 (1:500, Invitrogen, USA), for 2 h at RT. After the final rinse in PBS (3 × 10 min), the slides were coverslipped with polyvinyl alcohol mounting medium with 1,4-diazabicyclo-(2.2.2) octane (PVA-DABCO, Fluka/Sigma Aldrich, Switzerland), and stored at 4 °C.

2.5. Image acquisition and analysis

Sections stained for NeuN, ED1, GFAP and DAPI from cortex (900−1300 µm i.e. within lamina 5) and striatum (3500−4200 µm), were screened and captured using a Nikon DS-Ri1 camera mounted on a Nikon Eclipse 80i microscope. Stab wound sites were photographed using a 10x objective, and the same gain, contrast and exposure times for the respective markers were used. For image acquisition and analysis NIS-Elements BR software 3.05 (NIS-Elements, Nikon Instruments, Japan) was utilized.

For evaluation of neuronal density, defined as NeuN density in stained tissue, regions of interest (ROIs), with the following radiiuses (r) originating from the center of the stab injury, were analysed; i) ROI 1: r = 0−75 µm, ii) ROI 2: r = 75−150 µm, iii) ROI 3: r = 150−250µmand iv) ROI 4: r = 350−450 µm. The ROI 4 was positioned far away from the injury and used as a reference for normalization of neuronal density data. The detection threshold for NeuN staining was set at a fixed ratio, 3.3 times above the mean background intensity, to ensure that only specific staining was analysed. The neuronal density is expressed as the fraction of the area above threshold, related to the total ROI area. For striatal sections containing part of a ventricle inside the ROI, the ventricular area was subtracted from the quantified ROI area, and sections where the stab was inside a ventricle were excluded. For comparison between the needle groups, the neuronal density was normalized to the density in the outer ROI (ROI 4), in the same section.

For analysis of the extent of the area deprived of neurons, the area around the injury was investigated with respect to presence of NeuN staining. The area devoid of detectable neuronal cell bodies, i.e. the “neuronal void” area, was encircled and measured around each of the insertion sites using image analysis, NIS-Elements.

Pixel-intensity profiles, for staining of ED1 and GFAP, were calculated using in-house developed software in Matlab (MATLAB ® and Statistics Toolbox Release 2019b, The MathWorks, Inc., Natick, Massachusetts, United States). For each captured section, the pixel-intensity profiles along 36 lines, 450 µm long and symmetrically radiating from the mid-point of the stab-wound, were calculated and averaged. Lines intersected by a ventricle were invalidated and excluded from the average. The distances from the mid-point were binned into four ROIs in the same way as for the neuronal density measurements, with the modification of ROI 4 being defined as 400−450 µm to ensure that it is outside the glial reaction area. The mean intensity value in each ROI was used for statistical analysis. For comparison between needle groups, the intensity profiles were normalized with respect to ROI 4 by dividing with the mean intensity in ROI 4. When comparing ROI 1−3 with ROI 4, the non-normalized data were used.

2.6. Statistical analysis

All the analyses were performed using GraphPad Prism 8.1.2 software (GraphPad Software Inc., USA). For each animal the mean value of two sections was used for cortex and striatum respectively, with the exception of three animals where only one section either in cortex or striatum of sufficient quality was available. Data is expressed as the mean value and the standard error of the mean. For comparing ROI-1, 2 and 3 with ROI-4 non-parametric Friedman test with Dunn’s multiple comparison test was used. For comparisons between the experimental groups i.e., RT vs cold or ice coat vs cold, either non-parametric Friedman (where matching data points were available; cortex 1 day PS) or Kruskal-Wallis test (for remaining groups, where matching values were missing), with Dunn’s multiple comparison test was used. For the force insertion measurements, the Student’s t-test was used. P-value of <0.05 was considered statistically significant. In case of statistical significance, the Q, H or T values are provided in the Results section.

3. Results

3.1. Technique used for implantation of frozen probes into the brain

The present study builds on our previous findings that a gelatin coating around implants has beneficial effects on the brain tissue response (Köhler et al., 2015; Kumosa et al., 2018; Lind et al., 2010a). To improve this technique further, we here introduced cold probes, with or without a low friction surface. The intention was to provide an outer layer of thawing ice (frozen probes dip coated in water and re-frozen), to yield minimal friction against the tissue during insertion (Fig. 2). We made use of the property that the opacity of the ice coated needles changes when thawing, from non-transparent to translucent, to initiate the insertion at around 0 °C. Below, the tissue reactions to implantation of cold and RT needles are compared, as well as reactions to cold and ice coated needles, to evaluate the effects of hypothermia and low friction surface coating, respectively. In addition, results from the insertion force measurements are shown for comparison of the differential forces subjected to the brain tissue in response to insertion of cold or ice coated needles.

3.2. Effects of hypothermia and ice coating

3.2.1. Neuronal density (NeuN) and neuronal void

To determine the effects on neurons of lowering the temperature of an implant, from room temperature (RT) to around 0 °C, or adding a slippery coat on the probes, the neuronal density in cortex and striatum was compared (1 and 7 days PS), between RT gelatinized needles and cold gelatinized needles, and between ice coated gelatinized needles.
and cold gelatinized needles, respectively. In addition, the neuronal density within and peripheral to the area of the stab (i.e. ROI 1–2, and ROI 3, respectively) was compared with the normal neuronal density located far from the injury site (ROI 4) (Table 1). In general, very small differences were found in neuronal density between RT gelatinized needles and cold gelatinized needles. However, when examining the stab wounds after ice coated needles compared to cold needles, it was evident that effects on neuronal density were less pronounced after addition of the ice coat.

Comparison of RT vs cold implants 1 day PS: No significant difference in neuronal density was found in cortex or striatum in any of the ROIs when comparing the RT with cold needles 1 day PS (Fig. 3). Compared to respective ROI 4, both groups showed a significant decrease in neuronal density in cortex in ROI 1 (95 % in cold, 89 % in RT; P < 0.0001) and ROI 2 (69 % in cold P < 0.01; 55 % in RT, P < 0.05), but not in ROI 3. In striatum, there was a significant reduction in neuronal density in ROI 1 for both groups (82 % in cold and 90 % in RT; P < 0.001), but not in the other ROIs.

Comparison of ice coated vs cold implants 1 day PS: The neuronal density in cortex was found to be significantly higher in ROI 1 and 2 in response to stab with ice coated needles (394 %, P < 0.05; 116 %, P < 0.001, respectively) compared to cold needles 1 day PS (Fig. 3). Compared to ROI 4, there was a significant decrease in neuronal density in cortex, in ROI 1 (95 %; P < 0.0001) and ROI 2 (69 %; P < 0.01) in response to cold needle stab, but only in ROI 1 (79 %; P < 0.01) in the ice coated group. In striatum, a significantly decreased neuronal density was only observed in the cold needle group, where it was limited to ROI 1 (82 %; P < 0.001), i.e. for the ice coated needles there was no significant reduction in neuronal density in striatum in any of the ROIs compared to ROI 4. These findings indicate a significant mitigation of neuronal loss by the ice coating.

Comparison of RT vs cold implants 7 days PS: There was no significant difference in the neuronal density in the tissue in or surrounding the stab injury when comparing RT and cold needles, in cortex or striatum 7 days PS (Fig. 4). Compared to respective outer ROI 4, a significant reduction in neuronal density in ROI 1 (87 % in cold and 74 % in RT; P < 0.05), but not in ROI 2 and ROI 3, was found in cortex in both groups. In striatum, there was a significant decrease in neuronal density in both ROI 1 and ROI 2 (52 % and 40 % respectively; P < 0.01), but not in ROI 3 after insertion of cold needles. The results were similar for RT needles but did not reach statistical significance in any of the ROIs.

Comparison of ice coated vs cold implants 7 days PS: No significant difference in neuronal density was found between ice coated and cold needles in cortex or striatum 7 days PS (Fig. 4). Compared to ROI 4, a significant reduction in neuronal density was found in cortex in ROI 1 in both groups (67 % for ice coat, 87 % for cold; P < 0.05) and in striatum in ROI 1 and 2 (52 % and 40 % respectively; P < 0.01) for cold needles.

RT vs cold implants 1 and 7 days PS: Notably, for both types of needles, a centrally located area devoid of NeuN staining referred to as the neuronal void, was found at 1 day PS, but less markedly at 7 days PS (Fig. 5). No significant difference in the extent of the neuronal void was found when comparing the RT and cold needles, in cortex or striatum, at either of the time points examined.

Ice coat vs cold implants 1 and 7 days PS: The neuronal void at one day PS was significantly smaller in cortex (P < 0.01) for the stab wounds induced by ice coated needles as compared to cold needles (Fig. 5). However, at 7 days PS, the neuronal voids had decreased and there was no longer any statistically significant difference between the two needle groups in cortex or striatum.

3.2.2. Microglia activation (ED1)

Comparison of RT vs cold implants 1 day PS: There was no significant change in the microglia activation after stab with RT needles compared to cold needles in cortex or striatum, in any of the ROIs at 1 day PS (Fig. 6). Compared to respective ROI 4 (Table 1), there was a significant increase in the microglial activation in cortex in ROI 1 (77 %, P < 0.001 for cold; 67 % for RT, P < 0.0001) and ROI 2 (70 %, P < 0.0001 for cold, 36 %, P < 0.01 for RT,) for both RT and cold needles. In striatum, there was significant increase in microglial activation in ROI 1 (79 % for cold; 65 % for RT; P < 0.01), and ROI 2 (36 % for cold and 45 % for RT; P < 0.05).

Comparison of ice coated vs cold implants 1 day PS: There was no significant difference in the microglial activation to stabs by ice coated compared to cold needles in cortex or striatum 1 day PS (Fig. 6). However, compared to ROI 4 (Table 1), both types of stabs did produce a significant increase in the microglial activation in ROI 1 in both cortex (122 %, P < 0.0001 for ice coat, 77 %, P < 0.001 for cold) and striatum (97 % P < 0.0001 for ice coat, 79 %, P < 0.01 for cold), as well as in ROI 2 (35 %, P < 0.0001 for cold, 44 %, P < 0.01 for ice coat) in cortex and striatum.

Comparison of RT vs cold implants 7 days PS: There was no significant difference in the microglia activation to stab with a RT needle as compared to a cold needle in cortex or striatum at 7 days PS (Fig. 7). Compared to ROI 4 (Table 1), there was, however, still a significantly increased activation of the microglia in both cortex and striatum in ROI 1 (Cortex: 210 % for cold, 235 % for RT, P < 0.001; Striatum: 309 % for cold, 307 % RT, P < 0.0001) and ROI 2 (Cortex: 63 % for cold, 53 % RT; Striatum: 59 % for cold, 75 % RT, P < 0.01) for both groups at this time point.

Comparison of ice coated vs cold implants 7 days PS: There was still no significant difference in microglia activation between the two needle groups in cortex or striatum at 7 days PS (Fig. 7). Compared to ROI 4 (Table 1), there was a significantly increased microglia activation for both needle groups in cortex ROI 1 (225 % for ice coat, 210 % for cold, P < 0.001), ROI 2 (40 % for ice coat, 63 % for cold, P < 0.01), and in

| Time    | Tissue   | Marker | Experimental group | Cold       | RT       | Ice coat  |
|---------|----------|--------|-------------------|------------|----------|-----------|
| 1 day PS | Cortex   | Neun   | Q = 27.4          | Q = 27.1   | Q = 22.6 |
|         |          | ED1    | Q = 22.6          | Q = 19.4   | Q = 30   |
|         |          | GFAP   | Q = 13.3          | Q = 22.3   | Q = 13.3 |
|         | Striatum | Neun   | Q = 16.3          | Q = 21.6   | Q = 16.6 |
|         |          | ED1    | Q = 15.0          | Q = 13.5   | Q = 28   |
|         |          | GFAP   | Q = 15.3          | Q = 15.2   | Q = 15.2 |
| 7 days PS| Cortex   | Neun   | Q = 16.3          | Q = 11.9   | Q = 15.8 |
|         |          | ED1    | Q = 18.4          | Q = 26.7   | Q = 27   |
|         |          | GFAP   | Q = 8.3           | Q = 16.2   | Q = 25.9 |
|         | Striatum | Neun   | Q = 15.3          | NS         | NS       |
|         |          | ED1    | Q = 30.0          | Q = 27.0   | Q = 25.9 |
|         |          | GFAP   | Q = 25.6          | Q = 27.0   | Q = 20.6 |

Summary of Q values after statistical analysis comparing tissue reactions in ROI 1, 2 and 3 with ROI 4, at 1 and 7 days PS in cortex and striatum using Friedman test (Degrees of freedom; Df = 3 for all groups). NS indicates that no significant difference was found in the comparison and further testing was not performed.

### Table 1

| Time    | Tissue   | Marker | Experimental group | Cold       | RT       | Ice coat  |
|---------|----------|--------|-------------------|------------|----------|-----------|
| 1 day PS | Cortex   | Neun   | Q = 27.4          | Q = 27.1   | Q = 22.6 |
|         |          | ED1    | Q = 22.6          | Q = 19.4   | Q = 30   |
|         |          | GFAP   | Q = 13.3          | Q = 22.3   | Q = 13.3 |
|         | Striatum | Neun   | Q = 16.3          | Q = 21.6   | Q = 16.6 |
|         |          | ED1    | Q = 15.0          | Q = 13.5   | Q = 28   |
|         |          | GFAP   | Q = 15.3          | Q = 15.2   | Q = 15.2 |
| 7 days PS| Cortex   | Neun   | Q = 16.3          | Q = 11.9   | Q = 15.8 |
|         |          | ED1    | Q = 18.4          | Q = 26.7   | Q = 27   |
|         |          | GFAP   | Q = 8.3           | Q = 16.2   | Q = 25.9 |
|         | Striatum | Neun   | Q = 15.3          | NS         | NS       |
|         |          | ED1    | Q = 30.0          | Q = 27.0   | Q = 25.9 |
|         |          | GFAP   | Q = 25.6          | Q = 27.0   | Q = 20.6 |

P < 0.01
striatum in ROI 1 (248 % for ice coat, 308 % for cold, P < 0.0001), and ROI 2 (57 % for ice coat, 59 % for cold, P < 0.01).

3.2.3. Astrocytic response (GFAP)

Comparison of RT vs cold implants 1 day PS: There was no significant difference in the astrocytic response when comparing RT and cold needles in cortex or striatum 1 day PS (Fig. 8). Compared to ROI 4 (Table 1), a significantly increased astrocytic response was only found in cortex in ROI 3 for RT needles (11 %; P < 0.05), and in striatum in ROI 2 for cold needles (16 %; P < 0.05).

Comparison of ice coated vs cold implants 1 day PS: The astrocytic response in the cold group was significantly lower than in the ice coated
needlegroupincortexinROI1(28%,P<0.001),andROI3(11%,P<0.05),whereasnodifferencewasfoundinstriatum1dayPS(Fig.8).

Compared to respective ROI 4 (Table 1), the astrocytic response in cortex was not significantly different in the cold group, whereas it was increased (16%, P < 0.01) in the ice coated group in ROI 3 only. In striatum, the astrocytic response was increased in ROI 2 (18%, P < 0.001) and ROI 3 (21%, P < 0.01) for ice coated needles, and in ROI 2 (16%, P < 0.05) for cold needles.

Comparison of RT vs cold implants 7 days PS: There was no significant difference in astrocytic response when comparing RT with cold needles in cortex and striatum 7 days PS (Fig. 9). Compared to ROI 4 (Table 1), an increased astrocytic response was found in cortex in ROI 1 (71%; P < 0.001) and ROI 2 (50%; P < 0.01) for RT needles and in ROI 2 (53%; P < 0.05) (but not reaching statistical significance in ROI

**Fig. 4.** Effect of stab wound injury on neuronal density after 7 days. (A) Representative histological images of NeuN stained sections of cortex and striatum 7 days after stab injury inflicted by cold gelatinized rods, gelatinized rods at RT and gelatinized rods with a melting ice coat. (B) Quantification of neuronal density, expressed as the ratio between the NeuN-stained area above threshold and total area of the ROI. (In cortex; n = 8 for cold, n = 9 for RT and ice coated needles, Kruskal-Wallis test, Df = 2, NS. In striatum; n = 9 for cold and ice coat, n = 8 for RT needles Kruskal-Wallis test, Df = 2, NS).
1) for cold needles, but in neither case in ROI 3 (i.e. outside the stab). Similarly, in striatum, there was a significant increase in astrocytic response in ROI 1 (91 % for cold, 89 % for RT; P < 0.0001) and ROI 2 (41 %, P < 0.01 for RT; 35 %, P < 0.05 for cold), but not in ROI 3, for both RT and cold needles.

Comparison of ice coated vs cold implants 7 days PS: The astrocytic reaction was less spread/ diffuse in the tissue and more concentrated to the insertion track 7 days PS. There was no significant difference in the astrocytic response when comparing the effects of ice coated and cold needles in cortex or striatum (Fig. 9). Compared to respective ROI 4 (Table 1), there was an increased astrocytic response for ice coated needles in cortex in ROI 1 (108 %, P < 0.0001) and ROI 2 (64 %, P < 0.01) but not ROI 3, and cold needles in ROI 2 only (53 %, P < 0.05). In striatum, the astrocytic response was significantly increased in ROI 1 (59 % for ice coat, 91 % for cold, P < 0.0001) and ROI 2 (46 %, P < 0.01 for ice coat, 35 %, P < 0.05 for cold), but not in ROI 3, for both groups.

3.2.4. Effects of cutting the pia mater

Finally, we analysed whether a sharp cut of the pia mater with a diamond knife, prior to insertion of ice coated needles, has any further effect on the tissue response. However, we found no evidence of any such effects at 1 or 7 days PS in cortex or striatum (data not shown).

3.3. Effects of an ice coat on insertion force

Having found differences in tissue responses between cold gelatinized and ice coated gelatinized needles, we evaluated the insertion forces exerted by these two groups of implants.

The effect of a melting ice coat layer around the gelatinized needles in comparison to gelatinized needles without this layer, was evaluated with regards to changes in insertion force during implantation into the brain. There was a pronounced lowering of the insertion force when the needles were coated with melting ice. The mean penetration force (0–5 seconds) of ice coated gelatinized needles (6.1 ± 2.3 mN) was 45 % lower than (P < 0.01) the mean insertion force observed in response to cold gelatinized needles (11.1 ± 3.9 mN) (Fig. 10). Mean total force experienced by the tissue during the first 5 min of the insertion for the ice coated gelatinized needles (3.2 ± 1.4 mN) was 56 % less (P < 0.01) than for the cold gelatinized needles (7.2 ± 3.0 mN). Mean resting forces between 8–10 min post insertion were found to be similarly low for both ice coated and cold gelatinized needles (~0.4 mN, less than 5% of maximum).

4. Discussion

Both for brain science and clinical therapies/diagnosis, it is of considerable importance to minimize acute damage and tissue reactions following invasive neurosurgery, e.g. brain biopsies or implantation of electronic devices for deep brain stimulation (DBS)/recording (Campbell and Wu, 2018; Gulino et al., 2019; Zhang et al., 2013). In the present study, we built on our gelatin embedding method for implantation which previously has proved to successfully mitigate tissue responses and loss of neurons around implanted electrodes in the brain (Köhler et al., 2015; Lind et al., 2010b). For the first time, the effects of a low-friction, thawing ice coat was analyzed. Addition of an ultra-slippery surface around the dry gelatin indeed further mitigates the acute loss of neurons in the brain, one day following insertion, as compared to cold gelatin embedded needles. A week after stab injury, there is still a trend towards higher neuronal density along the track for the ice coated compared to the cold needles. Neuronal density in cortex and striatum was not different from reference levels with the exception of the innermost region of the track in cortex. Importantly, for ice coated probes, near normal levels of the astrocytic response was found along the cortical part of the track at day one PS. Together the present data indicate that the initial injury can be significantly reduced by the addition of an ice coat. Lastly, the finding that no significant adverse effects are caused by lowering the temperature of the probe from room temperature to around 0 °C presents novel opportunities for utilizing frozen constructs.

4.1. Effects on neurons and glial cells

As already mentioned, there is strong evidence that the addition of a gelatin layer around needles has beneficial effects on the nervous tissue injury response. Initial studies implanting pure gelatin needles in rat cortex demonstrated that tissues healed by 12 weeks after implantation as determined by no observable glial reaction (Lind et al., 2010a). More recent studies have shown preservation of neurons around implanted gelatin embedded ultra-flexible probes (Köhler et al., 2015). The present study confirms the usefulness of gelatin embedding and, in addition, shows that an added ice coat further reduces the loss of neurons and severity of a neuronal void in the innermost ROIs 1 and 2 (i.e. within the insertion track) one day after stab injury. It is also worth noting that no significant reduction in neuronal density was found outside the insertion track (i.e. in ROI 3) in any of the regions and time points studied.

The reduction of the neuronal void after seven days, for all of the needle types, clearly suggests that the tissue readjusts over time, bringing along neurons from the periphery into the central ROIs. It is thus conceivable that neurons from tissue adjacent to the track may partly “replace” neurons lost early on, thus partially masking a loss of neurons in the inner ROIs at this later time point.

In general, negligible differences were found with regard to microgli activation and astrocytic response between the probe types in the present study. However, whereas the astrocytic response was close to control levels in animals in the ice coated group 1 day after injury, in the other groups a tendency towards a reduced response was found in the innermost ROI at this time point. Given the reduced loss of neurons and also basically preserved astrocytic reactivity in the innermost ROIs after ice coating, the lack of a mitigating effect on microglia activation was somewhat surprising and may suggest that the degree of the
The microglial response is not directly related to loss of neurons or to astrocytic reactivity in the early phase after injury. However, these interactions are highly complex (Cherry et al., 2014; Liddelow et al., 2017; Mattugini et al., 2019) and outside the scope of this study.

### 4.2. Mechanism related aspects

The present finding, that cold and RT needle insertions did not result in any significant differences with respect to glial responses or neuronal loss, indicate that lowering the temperature of an implant to close to 0 °C do not, in itself, reduce damage. Thus, the mechanisms
underlying the beneficial effects of ice coating do not appear to be due to a lowered temperature. It is, however, known that cooling the brain by a few degrees after an infarct or period of anoxia is beneficial (Darwazeh and Yan, 2013; FAY, 1959; Polderman and Herold, 2009). The lack of beneficial effects of cold probes as compared to RT probes could be due to that the tissue temperature is already reduced a few degrees in both cases and that there is no further effect of reducing the temperature below RT. Alternatively, given the high degree of vascularization of the brain (Andreone et al., 2015), the slender probes are likely to warm up rather quickly and thus significant differences in temperature may not persist long enough to yield a clear effect on neuronal survival. The mitigation of neuronal loss and the reduced central void after insertion of ice coated needles may instead be a consequence of reduced insertion forces as the implant surface becomes
very slippery when the ice coat is melting. Dry gelatin, on the other hand, usually has a somewhat rugged micro/nano structure, which, before being fully wetted, may cause some friction and tearing and possibly rupture nearby cells and vessels. This “mechanical” hypothesis is consistent with the notion that neurons are extremely vulnerable to microforces (Lind et al., 2013).

We found no further beneficial effects of cutting the pia mater with an ultra-sharp knife prior to insertion of an ice coated probe. One possible reason could be that ice coating, by providing a very slippery surface reduces the insertion force to a point where removal of the pia has no added beneficial effect on relief of these forces. Since we used a very small opening in the skull and dura mater, as part of our efforts to reduce surgical damage and prevent post-surgery extrusion of the cortex through the dura opening, we were unable to document the
degree of surface dimpling in the present study. It is assumed that dimpling, by resisting penetration will increase the insertion forces that, in turn, has been linked to tissue damage (Han et al., 2012; Mahvash and Dupont, 2010; Sharp et al., 2009). Resting forces are thus likely a consequence of unresolved dimpling and friction of the probe surface, and usually considerable following insertions (Casanova et al., 2014a; Welkenhuysen et al., 2011). However, in the present study, insertion of gelatin embedded needles, both cold and ice-coated, results in negligible resting forces. This likely contributes favorably to the general neuronal presence for both of these experimental groups at 7 days PS.

While gelatin can provide a very useful structural support for delicate and ultra-flexible probes (Agorelius et al., 2015; Lind et al., 2010a), it rapidly transforms to a soft gel when in contact with fluids.

Fig. 9. Astrocytic response to stab wound 7 days post injury. (A) Representative images of GFAP stained cortex and striatum comparing the astrocytic response to stab wounds generated using cold gelatinized rods, gelatinized rods at RT and gelatinized rods with a melting ice coat. (B) Quantification of the astrocytic response, expressed as the computed pixel intensity above threshold in the respective ROIs. (In cortex; n = 8 for cold and n = 9 for RT and ice coated needles Kruskal-Wallis test, $Df = 2$, NS. In striatum; n = 9 for cold and ice coated and n = 8 for RT needles, Kruskal-Wallis test, $Df = 2$, NS).
Hence the structural support disappears within minutes after introduction into tissue. To avoid deviations from the intended track line in deep implantations, one solution would be to apply a coating that retards water penetration into the gelatin (Etemadi et al., 2016) and/or to increase the insertion speed.

Studies on impact of speed suggest that insertions should be made at either relatively slow speed (2–20 μm/s) (Casanova et al., 2014b; Fiáth et al., 2019) or high speed (1–2 mm/s) (Björnsson et al., 2006; Campbell et al., 1991; Edell et al., 1992; Maynard et al., 2000; Turner et al., 1999) to reduce tissue damage. Given that only high speed is feasible when using a thawing ice coat, we chose to use 1 mm/s throughout the present study, which is also similar to the insertion speeds used during clinical implantations of DBS electrodes. The present finding of rather small tissue reactions to a stab with an ice coated needle suggests that brain injuries in response even to large probes, (as used here) which normally cause substantial injury (Thelin et al., 2011), can be significantly mitigated.

4.3. Novel opportunities from the gel track formed in the brain by a gelatin coated probe

The main aim of the present study was to further reduce tissue injury during insertion of electrodes into the brain, thus increasing safety and improving physiological conditions in the targeted area of the brain. However, the developed technique may also offer additional and novel fields of application. As became evident during surgery, a temporary gelatin space was left in the tissue after extraction of the central pin (stainless steel needle inside the gelatin). This space, consisting of the moist gelatin coat may offer a novel route for subsequent implantation. A conceivable benefit would be to use this “gel track” as a guide, thus a second implantation may be performed with higher spatial precision, as the track will be visible in MRI. For instance, this may be a useful way to increase precision during DBS-electrode implantation, where precision is currently around +/-1 −2 mm in human patients (Bjartmarz and Rehncrona, 2007; D’haese et al., 2010; Von Langsdorf et al., 2015).

4.4. Concluding remarks

Improved surgical methods which mitigate brain injury upon insertion of electrodes, or other objects, are of considerable interest in both neuroscience research and clinical therapy since procedures such as biopsies, implantation of catheters, injection of solutes, etc. all include insertion of a device into the brain. The present study introduces a novel method, utilizing an ice coated gelatin embedded construct suitable for e.g. implanting probes into the brain, which show promising neuroprotective effects in the acute phase after implantation.

Author contribution

MM: Conceiving the study, contributing to experimental work, data analysis, and writing the manuscript
JT: contributing to the experimental work and manuscript writing
LG: contributing to the histology.
PTT: contributing to the data analysis, and manuscript writing
LSK: developing force-sensing adapter and Matlab interface, contributing to force measurements, and manuscript writing.
JS: Conceiving the study and writing the manuscript
LMEP: Conceiving the study, contributing to experimental work and data analysis, and writing the manuscript

Declaration of Competing Interest

Jens Schouenborg is the inventor of a pending patent on low temperature probes (WO2018217147) and of issued patents on flexible electrodes embedded in dissolvable matrix material and is a cofounder of Neuronom AB, Sweden which owns the patents. The other authors have no competing financial interests. There has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

This project was sponsored by grant from Swedish Research council (Grant number: 2016-06195), strategic funding from Lund University (Grant number: 03074) Lund, Sweden and grant from Skåne County Council (Grant number: F2018/1490). We are thankful to Redoxis for immunohistochemical work performed, to Dr. Michael Aagaard Andersen for his helpful comments on statistics, to Dr. Nedjeljka Ivica for help in preparing gelatinized needles, and to Axel Tojo for providing 3D-printing services.

References

Agorelius, J., Tsanakalis, F., Friberg, A., Thorbergsson, P.T., Petterson, L.M.E., Schouenborg, J., 2015. An array of highly flexible electrodes with a tailored configuration locked by gelatin during implantation-initial evaluation in cortex cerebri of awake rats. Front. Neurosci. 9, 331. https://doi.org/10.3389/fnins.2015.00331.
Andrei, A., Welkenhuysen, M., Ameyo, L., Nuttin, B., Eberle, W., 2011. Chronic behavior evaluation of a micro-machined neural implant with optimized design based on an experimentally derived model. In: Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society. EMBS. pp. 2292–2295. https://doi.org/10.1109/EMBS.2011.6090577.
Andrei, A., Welkenhuysen, M., Nuttin, B., Eberle, W., 2012. A response surface model predicting the in vivo insertion behavior of micromachined neural implants. J. Neural Eng. https://doi.org/10.1088/1741-2550/9/1/016005.
Anderounge, B.J., Lacoste, B., Gu, C., 2015. Neuronal and Vascular Interactions. Annu. Rev. Neurosci. 38, 25–46. https://doi.org/10.1146/annurev-neuro-071714-033835.
Biran, R., Martin, D.C., Tesco, P.A., 2005. Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays. Exp. Neurol. https://doi.org/10.1016/j.expneurol.2005.04.020.
Biran, R., Martin, D.C., Tesco, P.A., 2007. The brain tissue response to implanted silicon microelectrode arrays is increased when the device is tethered to the skull. J. Biomater. Mater. Res. – Part A. https://doi.org/10.1002/jbm.a.31138.
Bjartmarz, H.I., Rehncrona, S., 2007. Comparison of accuracy and precision between frame-based and frameless stereotactic navigation for deep brain stimulation electrode implantation. Stereotact. Funct. Neurosurg. 85, 235–242. https://doi.org/10.1159/000103262.
Björnsson, C.S., Oh, S.J., Al-Kofahi, Y.A., Lim, Y.J., Smith, K.L., Turner, J.N., De, S., Roysam, B., Shain, W., Kim, S.J., 2006. Effects of insertion conditions on tissue strain and vascular damage during neuroprosthetic device insertion. J. Neural Eng. 3, 196–207. https://doi.org/10.1088/1741-2550/3/3/002.
Blau, P.J., 1995. Friction Science and Technology. CRC press.
Casanova, F., Carney, P.R., Sarntinoranont, M., 2014b. Effect of needle insertion speed on tissue injury, stress, and backflow distribution for convection-enhanced delivery in the rat brain. PLoS One 237, 79–89. https://doi.org/10.1371/journal.pone.0094919.

Cherry, J.D., Olschowka, J.A., O'Banion, M.K., 2014. Neuroinflammation and M2 microglia: The good, the bad, and the inflamed. J. Neuroinflammation 11, 98. https://doi.org/10.1186/1742-2094-11-98.

D’haese, P.F., Pallavaram, S., Konrad, P.E., Neimat, J., Fitzpatrick, J.M., Dawant, B.M., 2010. Clinical accuracy of a customized stereotactic platform for deep brain stimulation after accounting for brain shift. Stereotact. Funct. Neurosurg. 88, 81–87. https://doi.org/10.1057/000271825.

Darwazeh, R., Yan, Y., 2013. Mild hypothermia as a treatment for central nervous system injuries: positive or negative effects? Neural Regen. Res. 8, 2677–2686. https://doi.org/10.3969/j.issn.1673-5374.2013.28.010.

Edell, D.J., Van Toi, V., McNeill, V.M., Clark, L.D., 1992. Factors Influencing the Biocompatibility of Insertable Silicon Microshafts in Cerebral Cortex. IEEE Trans. Biomed. Eng. 39, 635–643. https://doi.org/10.1109/10.141202.

Etemadi, L., Mohammed, M., Mohammed, et al. Journal of Neuroscience Methods 343 (2020) 108842

Fay, T., 1959. Early experiences with local and generalized refrigeration of the human brain. PLoS One 237, 79–89. https://doi.org/10.1371/journal.pone.0094919.

Maynard, E.M., Fernandez, E., Normann, R.A., 2000. A technique to prevent dural adhesions to chronically implanted microelectrode arrays. J. Neurosci. Methods. https://doi.org/10.1016/j.jneumeth.2014.08.012.

M. Mohammed, et al. Journal of Neuroscience Methods 343 (2020) 108842

V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes are immune response in the brain. Proc. Natl. Acad. Sci. U. S. A. 114, 5894–5899. https://doi.org/10.1073/pnas.1705091114.

Lind, G., Limsmeier, C.E., Thelin, J., Schouenborg, J., 2010a. Gelatine-embedded electrodes - A novel biocompatible vehicle allowing implantation of highly flexible microelectrodes. J. Neural Eng. 7, 046005. https://doi.org/10.1088/1741-2560/7/4/046005.

Lind, G., Limsmeier, C.E., Thelin, J., Schouenborg, J., 2010b. Gelatine-embedded electrodes—a novel biocompatible vehicle allowing implantation of highly flexible microelectrodes. J. Neural Eng. 7, 046005. https://doi.org/10.1088/1741-2560/7/4/046005.

Lind, G., Gallentoft, L., Danielsén, N., Schouenborg, J., Pettersson, L.M.E., 2012. Multiple implants do not aggravate the tissue reaction in rat brain. PLoS One 7, e47509. https://doi.org/10.1371/journal.pone.0047509.

Sharp, A.A., Ortega, A.M., Restrepo, D., Curran-Everett, D., Gall, K., 2009. In vivo penetration mechanics and mechanical properties of mouse brain tissue at micrometer scales. IEEE Trans. Biomed. Eng. https://doi.org/10.1109/TBME.2008.2003261.

Stence, N., Waite, M., 2001. Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices. Glia. https://doi.org/10.1002/1098-1136(200103)33:3<256::AID-GLIA1024>3.0.CO;2-J.

Welkenhuysen, M., Andrei, A., Ameye, L., Eberle, W., Nuttin, B., 2011. Effect of insertion speed on tissue response and insertion mechanics of a chronically implanted silicon-based application accuracy of the Neuromat neurosurgical robot. J. Neurosurg. 122, 191–194. https://doi.org/10.3171/2014.9.JNS14256.

Wellman, S.M., Li, L., Yaxaier, Y., McMamara, I., Kozi, T.D.Y., 2019. Revealing spatial and temporal patterns of cell death, glial proliferation, and blood-brain barrier dysfunction around implanted intracortical neural interfaces. Front. Neurosci. https://doi.org/10.3389/fnins.2019.00493.

Zhang, H., Patel, P.R., Xie, Z., Swanson, S.D., Wang, X., Kotov, N.A., 2013. Tissue-compliant neural implants from microfabricated carbon nanotube multilayer composite. ACS Nano 7, 7619–7629. https://doi.org/10.1021/nn402074y.

Zhou, T., Hong, G., Fu, T.M., Yang, X., Schuhmann, T.G., Viveros, R.D., Lieber, C.M., 2017. Syringe-injectable mesh electronics integrate seamlessly with minimal chronic immune response in the brain. Proc. Natl. Acad. Sci. U. S. A. 114, 5894–5899. https://doi.org/10.1073/pnas.1705091114.