Development of a miniature microdrive recording system for multisite multichannel recording from rodent brains

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Abstract

Introduction
Assessing the electrical performance of cell populations can reveal their functions only partially. To discover the underlying mechanisms it is important to examine how they communicate with other brain regions, which can be achieved by implanting multiple recording devices to all possible cooperating areas. Multisite implantations are limited by available space in small animals, thus the need for a miniaturised yet reliable microdrive that allows for increased implantation counts in a single animal is of utmost importance. Here we describe a miniature microdrive unit that can be used in various experimental settings.

Methodology
Based on a previously used design, but by substituting parts with miniature modelling components, we managed to build a reduced size microdrive which is reliable, biocompatible and customisable. The new device is fully compatible with existing recording solutions but it can be implanted in increased amounts into a single animal, where connector size becomes the only technical limitation.

Discussion
The theoretical number of implantable microdrives is 10 in mice (up to 320 recording channels), 24 in rats (up to 768 recording channels), and is now primarily limited by the connector size. The low profile also allows the use of wireless telemetry to replace the cable and provide unrestricted exploration for the animal as well as interacting animals concurrently, opening the floor to social–behavioural studies.

Conclusion
We successfully modified the concept of the original microdrive and constructed a miniature device that allows the mechanical manipulation of electrode depth in the brain in chronic experiments without the need to anaesthetise the animal. By using the new microdrive, cortical and deep brain structures can be targeted. Although due to the reduced size handling and implantation can be more challenging, the increased number of implantable units makes up for the difficulties and allows the simultaneous examination of multiple brain regions with high accuracy and reliability.

Introduction
An approach to assessing the real-time performance of an area or cell population is the registration of local bioelectrical activity generated by interacting neurons. Although the generating mechanism of the electrophysiological signals still has not yet been revealed entirely, it is considered to reflect the summed electrical activity of several thousand neurons, situated in close vicinity. Due to signal attenuation the activity of deeper brain structures cannot be recorded from the head surface, hence the need for invasive recording techniques enabling to reach deeper cortical layers or subcortical areas. With respect to gradual alterations in brain bioelectrical signals it is believed that subthreshold postsynaptic potential changes on the dendrites of pyramidal cells are responsible for the signal generation, as well as fast, all-or-nothing-like action potentials (units), which are also integral part of the cerebral bioelectrical activity, though their magnitude in the total mass is significantly smaller. Therefore, the development of sophisticated recording equipment capable of registering and separating brain activity is of utmost importance. Deeper brain structures often with nuclear nature require the use of stereotrode or tetrode electrodes, the advantages of which have been widely acknowledged in the separation of unit activity. These techniques allow for the assessment of electrical activity in subcortical structures, but they do not provide information about the network structure of concurrent or cooperating regions in the brain when used on their own. Simultaneous observation of multiple, anatomically related structures is essential for understanding how cerebral networks function under various conditions, as synchronisation and desynchronisation of spatially distant areas play vital roles in learning and memory processes, including behaviour, and numerous other mechanisms of the nervous system. Our aim was to provide a novel tool for accessing multiple brain structures at the same time and further increase the number of implantable devices by miniaturising microdrives that enable the fine tuning of electrode positioning in the brain. Our new microdrive design reduces size without compromising reliability and usability, while supporting the simultaneous examination of electrical activity in spatially distant or close regions.

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There are fundamentally two types of microdrives used today: single electrode microdrives\(^4\) (nDrive, Neuronexus, Michigan, USA) that allow the vertical adjustment of one electrode, and multi-electrode microdrives\(^5-7\) that can be loaded with multiple electrodes and each one’s cerebral depth can be fine-tuned, but these constructions are relatively large in size and weight. Another disadvantage is that all electrodes need to be implanted in a spatially close region preventing recording from spatially distant brain areas.

**Methodology**

**Electrode fabrication**

Multisite, tetrode recording electrodes were constructed manually similar to already existing descriptions in the literature\(^1,8\). Teflon-coated tungsten wires with a diameter of 12 µm (California Fine Wire Company, Grover Beach, USA) were cut to 30 cm segments, then looped twice, fixed to a holder bar and spun on a magnetic spinner with a weight keeping the wires straight until the top of the plate approached the holder bar to about 15 mm. Wires were then allowed to twist backwards for a few turns to release tension. Using a heat gun the insulation was slightly melted to fix the posture and to strengthen the structure of the tetrode. The magnetic weight was then cut down and the electrode was released from the holder bar. Non-twisted wires were cut to have four freely moving wires at the end of the tetrode. The coating was removed about 1 mm from wire tips using a flame using a precision tool and each one’s cerebral depth was cut to desired size with sharp surgical scissors. Impedance values were measured and lowered to ~300 kΩ (measured at 1 kHz), using electroplating with gold solution and NanoZ impedance measuring device (Neuralyx Inc., Montana, USA).

**Microdrive fabrication using the original design**

Commercially available ‘C’-profile brass shaft with a height of 6 mm and a depth of 3 mm was used to produce the frame of the traditional microdrive. Two millimetres wide sections were cut with a professional cutter (Proxxon-KS230, Föhren, Germany), and then a hole with a diameter of 1 mm was drilled down to 0.6 mm vertically to the recognition of the drive. A brass screw (245 µm vertical movement/turn, Precision Technology Supplies, East Grinstead, UK) was placed through the holes and in the middle of a small plastic bit (moving part) that pressed against the inner side of the C-frame and allowed a 5 mm vertical repositioning of the electrode. A nut was wound around the screw from the bottom of the frame to prevent vertical movement of the screw itself. The end of the screw was then cut and sanded to size just to fill the nut, and was glued to the nut using superglue. A spacer shaft was attached to the back side of the microdrive towards the ‘imaginary’ skull to provide an initial fixing to the bone. Prior to implantation the protective cannula (34G, Cooper’s Needle Works, Birmingham, UK) of the tetrode was glued to the moving plastic part and the connector was attached to the back side of the microdrive (Figure 1(b)).

**New microdrive design**

We fabricated the ultra microdrive by using a brass C-profile shaft with a height of 1.5 mm and a depth of 1 mm (Scale Hardware, Ft Lauderdale, Florida, USA). One millimetre wide sections of the C-profile shaft were cut with a professional cutter (Proxxon-KS230, Föhren, Germany), and then a hole with a diameter of 0.6 mm was drilled through the base and top. A brass screw (125 µm vertical movement/turn, THRB-05-B, Scale Hardware, Ft Lauderdale, FL, USA) with a diameter of 0.5 mm was placed through the holes and in the middle of a small (0.3 mm thick) plastic bit (moving part) pressing against the inner side of the C-frame, and enabling the attached cannula with the electrode inside to move vertically 0.6 mm. A nut was fixed on the screw outside the frame to prevent vertical movement, cut into size and then a spacer shaft was attached to the back side of the microdrive as described above. As an alternative solution, the drive was placed and glued into a pipette tip that served as protection. Prior to surgery the protective cannula of the tetrode was glued to the moving plastic part and the connector was attached to the back side of the microdrive (Figure 1(a), (c) and (d)).

**Implantation and recordings**

Thirteen male Sprague-Dawley rats (Charles-River, Margate, UK) weighing between 300 and 350 g were used for implanting chronic recording devices. All procedures conformed to the UK Animals (Scientific Procedures) Act 1986. Implantation surgeries were performed in designated surgical theatres of the Biomedical Services Unit, University of Birmingham, Birmingham, UK, with sterile tools and equipment. The anaesthesia of the animals was induced in an anaesthetic chamber with isoflurane vaporised in oxygen (3% isoflurane and 100% oxygen). The heads of the rats were shaved and were then fixed.
In a stereotactic frame where a low (1.5%) isoflurane anaesthesia was provided via a nose mask. The pinch reflex was checked regularly to assess the depth of anaesthesia. Heart rate, breathing pattern and body temperature were monitored constantly. Body temperature was controlled by a heating pad and maintained at 37°C. Eyes were covered with moisturising agent, ear canals were lubricated with EMLA cream (25 mg lidocaine and 25 mg prilocaine/g) to reduce possible inconvenience caused by the ear bars. After cutting the skin, cleaning and leaving the skull surface to dry, four holes were drilled for anchoring screws to achieve better fixation of dental cement. Small craniotomies were drilled above the targeted regions (coordinates relative to the Bregma were obtained from the Rat Brain Atlas9). The dura was carefully removed and then four tetrodes attached to microdrives were inserted into the craniotomies: two electrodes were lowered to the prefrontal cortex (PFC) on both sides (anterior–posterior (AP): ±3.2 mm, medial–lateral (ML): ±0.8 mm, dorsal–ventral (DV): 3 mm), and two electrodes to the amygdalae on both sides (AP: −2.5 mm, ML: ±4 mm, DV: 7.8 mm). Reference and ground electrodes were connected and placed under one of the posterior anchoring screws. Moving parts (screw, nut and cannula) were embedded in wax to allow the vertical adjustment of the electrodes, whereas microdrive frames, all wires and the connector in the middle were embedded in dental cement. The skin was then stitched with sterile sutures, and following analgesia (Temgesic, 1 mL/kg was administered intraperitoneally) the animal was removed from the stereotactic frame and transported to the recovery room and placed in a container above a heating pad, where sucrose fluid was injected subcutaneously to compensate possible dehydration. Animals recovered and were fed *ad libitum* for 1 week before recordings commenced. A Tucker–Davis Technologies (TDT, Alachua, FL, USA) PZ2 preamplifier was used to collect signals from the brain and the amplified signals were then fed through optical fibre to a TDT RZ2 Z-Series base station. Data were transported through optical fibre to a Windows PC for storage and processing. The original TDT software was used to control the recording and to store the data with the following parameters: 16 channels, 24 kHz sampling rate with a 0.5 Hz–12 kHz band-pass filter and 16-bit signal resolution. At the end of the experiment animals were perfused to perform histology. Recorded files were converted to Matlab (Mathworks, Natick, USA) format after the experiment to feed all data into our custom-made software toolbox.

### Results

All microdrives with attached tetrodes were implanted successfully. Although the difficulty as well as the time required for the implantation procedure increased noticeably due to the challenge in handling the much smaller sized microdrives, the signal quality did not change compared with the signal with the original design. Thus, reduction in the...
drive size had no negative effect on signal reliability, the factor which is absolutely essential. Importantly, the noise level showed the same characteristics both with original and new designs and was satisfactory in the new miniaturised device even without any electrical shielding or other protection. Signal quality was maintained for weeks, although we had recurring problems with keeping the Omnetics connectors clean as the freely moving rats managed to get rid of protecting covers and so allowing dust and dirt into the connector holes, which was prevented by using dummy connectors in the sockets. The screw in the microdrive that is responsible for moving the electrode vertically was marked with a marker pen, so that it was easier to follow its accurate location while turning to move the electrode. The working distance of the microdrive (around 600 µm) was satisfactory to position the electrodes so that multiple neurons’ unit activity was detected, but it might be challenging in larger animals or brain areas if the initial position is not determined adequately. Beyond the size reduction, the weight of the microdrive also radically decreased from 0.192 g (original design) to 0.026 g (new design).

The endurance of the microdrive was verified by physical tests and thanks to the rigid construction, none of the drives was disassembled by a reasonable amount a force. Similarly, no unwanted movements were detected in the implanted devices even when turning the screws.

Although mostly tetrodes were used for acquiring local field potentials and unit recordings, multishank silicon probes were also attached to the drive for testing purposes (Figure 1(e)). Physical tests showed no differences in endurance or reliability when compared with tetrodes. Thus, this mechanical device can also be used for the fine positioning of silicon probes.

In most cases we were able to obtain clean, noiseless and artefact-free signals from which we could register field potential signal as well as single unit activity. To examine action potentials first we filtered the wide band signal with a zero phase-shift band-pass filter between 500 Hz and 5 kHz. Then, after threshold detection we applied principal component analysis for feature extraction, and expectation maximisation algorithm for sorting spiking activity. Results show reliable local field potential signals as well as detectable and separable single and multiunit activity in the range of 50–150 microvolt range (Figure 2). Recordings typically yielded two to six separable cells per tetrode in one single position of the electrode. Local field potential during sleep showed characteristic features of the slow sleep oscillation – up and down states – in the PFC.

**Discussion**

The development of a microdrive that serves the same mechanical functions but at about 1/20th of the size of the original microdrive design is important for reaching deeper structures and recording single cell, multiple unit activity and field potential signals, and also has prominent importance in the neurophysiological examination of behaviour. Rat experiments showed that the new, radically miniaturised design proved to be reliable in terms of the integrity of the components and the achievable signal quality. However, new challenges appeared during implantation. In the absence of a holder equipment that allowed the secure yet gentle grip of the miniature microdrive, we fabricated a screw-controlled precision holder, using pointed scalpels, a spring and epoxy to hold parts together. We also realised that physics seem to have different attributes when such small-sized devices are to be handled: even the smallest drops of wax and dental cement were hard to control as they started spreading immediately after touching the surface, which was quite disadvantageous because of the miniature size of the microdrive. Therefore, extra care and caution were needed.

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**Methodology**

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had to be applied throughout surgical implantations. Due to the size, the implantation procedure is relatively more complicated and needs more concentration and time, but the increased number of implantable devices enables the simultaneous registration of the activity from more brain regions, which is not feasible with the original design. The theoretical limit in the number of implantable microdrives is around 10 in a mouse (Figure 1(f)), 20 in a rat (Figure 1(g)), and is now primarily limited by the size of the connector that also has to be positioned on the skull. A solution to this problem could be the use of connectors with vertical contacts instead of a horizontal socket, in a tall but narrow construction, or alternatively, placing the connector on the neck. It is also worth mentioning that the tight connection of the Omnetics connector requires the experimenter to undergo a training process with the animal as connecting the cable needs an increased amount of force which can be uncomfortable for the animal. Discomfort can also be avoided by slightly anaesthetising the animal for the duration of securely connecting the recording cable, but this procedure is only viable using inhalational anaesthetics. Due to the size of the drive we could use very short cables without any copper shielding of the drive is close to the pivot point (skull) are likely less fragile and more stable. Estimations on the number of implantable devices suggest up to 10 microdrives in mice and 24 in rats based on the surface area required. These calculations suggest that altogether 10 tetrodes (40 channels) or silicon probes (up to 320 channels) can be implanted in mice, whereas 24 tetrodes (96 channels) or silicon probes (up to 768 channels) in rats, which could be a huge advance over behaviour studies.

Conclusion
Besides the fact that our new microdrives have extremely reduced size and weight, based on our experiments they support the reliable recording of both field potential and single unit signals. Implantation tests also revealed they are remarkably biocompatible as no infection or inflammation appeared even months after the implantation, thus they can be used in various experimental settings. Our primary aim was miniaturisation and recording unit activity for several hours that we have achieved. Experiments can require long-term stable recordings (weeks, months) that we have not tested; however, we see no reason why tall and weighty drives could be more stable than our miniature construction. On the contrary, low profile drives where the top of the drive is close to the pivot point (skull) are likely less fragile and more stable. Estimations on the number of implantable devices suggest up to 10 microdrives in mice and 24 in rats based on the surface area required. These calculations suggest that altogether 10 tetrodes (40 channels) or silicon probes (up to 320 channels) can be implanted in mice, whereas 24 tetrodes (96 channels) or silicon probes (up to 768 channels) in rats, which could be a huge advance over currently existing solutions, mostly if the number of simultaneously examined brain regions is taken into account.

References

Abbreviations list
AP, anterior–posterior; DV, dorsal–ventral; ML, medial–lateral; PFC, prefrontal cortex.

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