DESIGN OF A MULTI-SITE, MULTI-REGION MICRODRIVE NEURAL RECORDING SYSTEM

A Thesis in
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by
Myles W. Billard

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The thesis of Myles W. Billard was reviewed and approved* by the following:

Bruce J. Gluckman
Professor of Engineering Science and Mechanics
Thesis Advisor, Chair of Committee

Kevin D. Alloway
Professor of Neural and Behavioral Science
Faculty Reader

Mark W. Horn
Professor of Engineering Science and Mechanics
Faculty Reader

Huanyu Cheng
Dorothy Quiggle Professor in Engineering, and Assistant Professor of Engineering Science and Mechanics
Faculty Reader

Judith A. Todd
P.B. Breneman Chair and Professor of Engineering Science and Mechanics
Department Head

*Signatures are on file in the Graduate School.
Abstract

Current neurophysiology tools for chronic recording experiments in small freely-behaving animals are not capable of driving electrodes to multi-site, multi-regional targets at different angles and with greater than two implant axes. Here, we present a novel microdrive system designed to address these limitations for studying distributed behavioral neural circuits. Our design decouples the surgical implantation of a guide cannula with placement of a flexible tube structure to create multiple, independent drive axes with set drive trajectories and electrode depths. The system leverages tight tube-cannula tolerances and geometric constraints on the flexible drive axis to ensure concentric alignment of electrode bundles within guide cannulas as they move through tissue. Acute experiments included targeting of three separated brain targets at radically different trajectory angles to demonstrate the accuracy of the drive axis placement method. Chronic recording experiments involved targeting and recording from three non co-localized and non-collinear cell groups in brainstem of rats. Data from these experiments included identification of multiple neuron discharge waveforms over multiple spatial points as electrodes were driven through tissue. Additionally, sleep-wake behavior correlates were identified using external sensing modalities supported by the microdrive design. This is the first reported instance of a microdrive capable of monitoring neural activity from simultaneous multiple targets along independently-angled trajectories from a single body structure.
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\[ R \] The length of the placement cannula on a single drive axis implant, p. 28
\[ \alpha \] The length from the top of the guide cannula to the tip of a stereotactic stylus, p. 28
\[ \Delta \] The user-defined to-target driving distance for electrodes, p. 28
\[ \zeta \] The absolute distance of the stereotactic stylus tip to the target, p. 28
I would like to thank Fatemeh Bahari for her efforts in performing surgeries, helping with data analysis, and talking through elements of the microdrive design with me.

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There is a growing interest in neuroscience to study behavior in a systems and circuits context. As more information is gathered, however, it is clear that the systems and circuits of fundamental behaviors are largely non-local and distributed in many regions of brain. One major component of learning, and potentially predicting, the dynamics of such neural network is to observe interactions among relevant cellular nodes within the network. A pragmatic method for observing such dynamics would be to record the activity of neurons, on a single-cell level, and to characterize which details of the activity are correlates of behavioral outputs. If all the interaction rules between cell populations in a circuit can be observed simultaneously and correlated to distinct behavioral outputs, then accurate physiologically-based models of the system can be constructed.

An accurate model of a neuronal system is a powerful tool. From sparse observations of the network, which feed into the model framework, precise decisions can be made about the ongoing dynamics of the system. Predictions of the dynamics can also be projected to future time points. This type of observation and control framework is shown in Figure 1.1. This framework is the core machinery of complex technologies, such as neural prosthetics and active neuro-therapeutics, which require accurate and timely predictions about a patient’s intentions or physiological state using as minimal a number of sensors as possible. In order to develop an accurate model of a neuronal system, however, the system must be well characterized.

Ongoing in the Gluckman lab are efforts to investigate, experimentally and through modeling, the bidirectional interaction between sleep regulation and epilepsy. The development of the microdrive system presented in this thesis was motivated by the need to better characterize
Figure 1.1: **Observation and control framework for neural systems.** Neural network observations from sensors feed information into a closed-loop model-decision-control framework. Decisions about the ongoing network dynamics and future predictions allow for network control through stimulation and intervention. Control structure flow diagram adapted from Bahari et al., 2016 [1].

the sleep-wake (SW) regulatory system. Extensive work has been done to measure and map individual cellular subcomponents that make up the SW circuitry, and existing mathematical models have been developed used those disjointed results. Yet, there are no studies that have shown simultaneous in-vivo interactions of SW cell groups, especially in freely-behaving animals. Thus, current mathematical models are not supported by a complete physiological picture of the network dynamics.

One potential reason for the absence of such studies is that there has not been a tool that can support this kind of neurophysiology experiment on small animals, such as rats or mice. The SW circuit has cellular nodes that are mostly non co-localized and that are, in part, spread out across multiple brain regions. Furthermore, these populations reside within very small and ventral structures, which are typically 200–300 mm in diameter and approximately 7–10 mm deep.
in brain. Simultaneously accessing these cell groups for single-unit observation would require a device that could: target multiple cell groups over multiple brain regions; specifically angle implants to avoid sensitive structures, such as ventricles and sinuses; and, ideally, drive electrodes to targets to maximize recordings from many spatial locations within tissue undamaged by the trauma of initial implantation.

The following chapters of this thesis will present the design, development, and experimental implementation of a microdrive system capable of meeting those outlined criteria. Chapter Two is an introduction to various microdrives and their design subcomponents, as well as the role each has new microdrive has had in progressing the field and neuroscience in general. This provides a good reference for where our microdrive system stands within the literature. In Chapter Three, the design criteria of a multi-site, multi-region microdrive capable of recording from multi-node distributed neural circuits are discussed. Following this, included in Chapter Four are both a general approach to solving those criteria demands, as well as a specific solution embodiment that is our microdrive system.

Chapter Five is a building instructions guide that takes the reader through all the steps needed to construct our microdrive. Chapters Six and Seven are, respectively, the experimental designs, surgical procedures, and results of acute placement and chronic microdrive experiments. Demonstrated in the first of these chapters is confirmation of the accuracy of our placement method for individual drive axes at arbitrary trajectory angling. In the second chapter, we show that our microdrive can be used to record from distributed neural circuits in freely-behaving rats. Together, these chapters validate the first multi-site, multi-region microdrive capable of achieving recordings from three separate, arbitrarily-angle drive axes.

Finally, the thesis ends with a closing remarks chapter. Summarized in this chapter is the work that was done in the thesis. Also included is a discussion about challenges that remain in the current microdrive design, potential applications of this microdrive work within the general neuroscience field, and future experimental goals in the context of our lab’s ongoing sleep-seizure project.
Chapter 2
The Role of Microdrives in Neuroscience

Microdrives are an essential tool in neurophysiology, especially in freely-behaving animal studies. A microdrive, in its simplest description, is a device that can move one or more electrodes through tissue in a controlled fashion. Microdrives can enable researchers to carefully position into and adjust electrodes within brain structures in order to isolate and record from extracellular potential variations of single-cells, which is often referred to as “single-unit activity,” or ensembles of neurons. This maneuvering is done outside the context of a full surgical procedure. Other primary advantages of microdrives include the ability to slowly approach a target from a distance, over the course of many days, and to monitor the activity at many locations in the same brain region over long time periods. Slowly approaching the target from a distance reduces exposure of active electrodes to dead zones created by traumatic implant injury during surgery. Overall, microdrives provide extra flexibility and specificity when studying unit activity of neurons that static electrodes simply cannot.

The idea of using movable bundles of electrodes to record unit activity is not new. Some of the first drives date back to the late 1960s and early 1970s. Ranck introduced his screw-based microdrive design in 1973 [2], which used screws to move shuttles rectilinearly and peri-axially to the motion of a screw driving through a threaded block upon turning. O’Keefe famously used a microdrive in 1971 to discover the phenomenon of place cells in the hippocampus [3]. O’Keefe later shared a Nobel prize for his work on place cells, much of which relied on microdrives for
studying isolated hippocampal neurons. Indeed, much of the research examining place cells, grid
cells, and cell-specific memory function in general, have invented or used microdrives of one design
or another in various studies [4] [5] [6] [7].

The history of microdrives is rich and the details are quite extensive. Over the time-course of
decades, microdrive setups, driving mechanisms, and overall approaches have been altered and
improved upon in order to solve the singular challenge of recording from one or more neuronal
populations with high recording stability, specificity, individual unit isolation, experimental
reliability, and reproducibility. Examined in this chapter are key pieces of the literature with
particular regards to innovations made in driving mechanisms, channel and unit scalability,
multi-site recordings, and long-term recording stability.

This examination will be used as a springboard for arguments that are made in Chapter Three,
where the design criteria for a microdrive suitable for experiments in large-circuit neurophysiological
studies are outlined. Many microdrive achievements and designs will be touched upon, but only
a subset will be treated with a more involved critical discussion. Those particular papers hold
a closer relevance to the microdrive system introduced in this thesis work. For a more general
perspective of the field and its progression, see Keating et al. who provide a synopsis of the core
pieces of the history of microdrives in the introduction of their own microdrive paper [8].

2.1 Driving mechanisms

Microdrives are complex devices that serve to meet the needs of challenging neurophysiological
experiments. The essential element that defines a microdrive is its driving mechanism. The
objective for any driving mechanism is to move electrodes from a starting point to a target
somewhere within brain. The differences in how this task is achieved can dictate the entire design
of a microdrive system and structure. There are only a select few flavors of established driving
elements, but many variations on these mechanisms have been made since their invention.

Ranck’s first microdrive system employed a manual screw-and-shuttle based keyway or key-lock
mechanism. Many iterations and improvements have been made of Ranck’s design [9] [10] [11] [12],
most notably eliminating electrode rotation [11] and increasing driving spatial resolution [12]. A
significant variation on the keyway shuttle design comes from Voigts et al. with the FlexDrive [13].
Their design uses specially patterned thin spring steel to provide rectilinear motion of electrodes
upon the turning of a drive screw. This is achieved by constraining the bottom of the drive spring to the rigid microdrive body. As the screw turns the drive spring, which is angularly constrained between the screw and structure, bows outward, away from the body, and the spring head moves level with the screw head. A major advantage to this setup is a weight reduction of the overall microdrive. An additional advantage is that the springs are externally attached to the microdrive body, instead of being part of the body design. This feature allows for customization of microdrive structures. A minor challenge with the design, however, is maintaining a level spring head throughout the stroke length of the screw; if the angle becomes too steep downward the electrodes may kink within the keyway. Generally, most cost-effective and easy-to-fabricate microdrive designs employ screws and shuttles for their driving mechanisms.

Other major microdrive configurations use stepper motors, hydraulics, or microelectromechanical systems (MEMS) as elements of their driving mechanisms. Stepper motor designs have been around for many decades. Barmack and Hayes described a stepper motor controlled configuration with 1.07 um drive resolution in 1970 [14]. David Hubel used a hydraulic microdrive, along with his initial version of the modern tungsten electrode, to acutely record from neurons in the lateral geniculate nucleus and striate cortex of cats [15] [16]. MEMS microdrives offer high driving resolution and are beneficial for limiting handling of animals during chronic experiments [17] [18]. However, these devices are typically expensive to fabricate, sensitive to liquid damage from blood or cerebral spinal fluid, and are limited to driving rigid silicon probes.

A more recent and notable design for motorized microdrives comes from Fee et al. in 2001 [19] and Long et al. in 2010 [20]. In the 2001 study, Fee et al. describe a motorized microdrive system which was able to independently position three electrodes chronically in brains of freely behaving songbirds. Such a system is useful for moving electrodes toward relevant neurons while an animal is expressing behaviors of interest, such as singing in birds. Isolated units can immediately be qualitatively correlated to relevant behaviors without having to restrain or anesthetize the animal, which could limit or stop expression of the behavior altogether. Long et al. further improved the scheme by achieving intracellular recordings of single vocalization-related units in nucleus HVC with a single-electrode motorize microdrive.
2.2 Channel and Unit Scalability

Alongside changes of the driving mechanisms, another major avenue of microdrive improvements comes from increasing the number of units that can be isolated and the number of channels from which a microdrive system is able to record. In some regards, the true limitations for channel count stems from the channel scalability of preamplifiers, especially ones small enough to be carried on top of a small, freely-behaving animal’s head. This latter point is in concert with good practice of physiological recordings, where digitization and amplification of electrical signals are best done close to their time of acquisition to reduce external electrical interference and signal degradation.

Independent of preamplifier placement, careful attention must be paid to harmonize the structure of a microdrive body with the recording systems that it implements. A popular approach for making large channel connections is through an electronic interface board (EIB). These are custom-designed or commercially-purchased printed circuit boards (PCB) that are used to connect leads of electrodes to channels of a preamplifier. The two main methods for making connections to electrode leads are with gold pins, which were first developed by Bruce McNaughton and Neuralynx in 1998 for place cell experiments on rats in space [6], or by soldering microwire leads [21] [22]. Almost every microdrive system needs an adjunct plug or EIB in order to route electrical signals from their electrodes to amplifiers.

One of the primary ways of increasing unit-yield in microdrives is to use electrode configurations that improve unit isolation and separability of clustered units. One of the first marked improvements in isolation and separation of simultaneous unit activity within single spike trains came from McNaughton et al. in 1983 with the introduction of the stereotrode [23]. The original stereotrode was comprised of two 25 µm platinum-iridium wires tightly twisted together. The stereo configuration aided in discriminating multiple units detected by the wires based on time small latencies between spike peaks. The stereotrode could not fully discriminate the spatial locations of neurons in three dimensions. McNaughton postulated that this could be done with electrodes twisted in a tetrahedral configuration.

The tetrode was first introduced by Okeefe and Reece in 1993 [24]. Similar to the stereotrode, the configuration used a twisted bundle of four 25 µm platinum-iridium wires. The tetrode provided an additional spatial dimension in discriminating between nearby units. Combined
with spike classification techniques, tetrodes can dramatically increase the yield of separable single-units [25] [26]. The use of tetrodes is commonplace in many modern microdrive systems.

Increasing the density of channels on a microdrive system will also invariably increase the yield of detectable single and multi-unit cellular activity. This can be done using either single electrodes or electrode bundles, such as tetrodes. One of the very first microdrives to specifically focus its design around the implementation of a large number of electrode channels is the Hyperdrive. The Hyperdrive appeared in Wilson’s and McNaughton’s 1993 work mapping the spatial mapping dynamics of hippocampal place cells [27], and was later described in full detail by Gothard et al., in 1996 [28]. The specific microdrive described by Gothard, which was made for the purpose of recording pyramidal cells in CA1 of hippocampus, had 14 individually-movable tetrode bundles. The bundles were positioned on keyway shuttles around an outward-sloped conical body and tetrodes were routed through guide cannulas toward a single craniotomy. Neurons were able to be identified and isolated on individual tetrodes and their firing rates could be tracked as animals moved through a circular environment. This progression of microdrive technology enabled the advancement of memory and place-cell studies.

Another example of a microdrive with a high-density channel count comes from Miyakawa et al. That microdrive used a 7-by-7 array of parallel-axis, individually drivable electrodes to map out visual cortical columns in response to visual stimuli. Electrodes had a 250 µm step-size and were spaced 360 µm apart in a hexagonal grid. Electrodes used in this system had diameter of 75 µm. Typically, microdrives use fine microwires that have diameters ranging from 12.5 µm to 25 µm. A 75% - 100% electrode yield was seen in the system for upwards of 12 weeks of implantation [29].

A final example of channel high-density microdrive designs is the FlexDrive. Similar to the Hyperdrive, the FlexDrive employed 16 independently-positioned tetrodes that could be driven in a highly-parallel axis configuration [13]. The FlexDrive was designed for mice, so it is smaller and lighter than the Hyperdrive. Results showed upwards of 20 or more identifiable single units over the duration of chronic experiments. One important feature of their design, similar to the approach in Miyakawa’s work, was the FlexDrive’s ability to pattern tetrodes and optical fibers in specific spatial layouts using arrangements of polyimide tubes. This was useful because the positions and spacing of each tetrode were rigidly defined for all driving configurations. The ability to add drivable optic fibers into their driving arrangement also makes the device useful for
optogenetic experiments.

### 2.3 Multi-Site, Multi-Region Drives

There is an increasing emphasis in neuroscience to understand systems and behaviors by observing network-node dynamics. However, many neural circuits have distributed networks of nodes that are not localized to a single region or that are non-collinear. Furthermore, there are not many microdrives that aim to accommodate distributed systems experiments. It is in this challenge that the microdrive field has the largest opportunity for innovation and improvement. This section examines groups who have developed microdrive systems that focus on recording from multiple targets across multiple brain region.

Lansink et al. developed a microdrive set up for small animals that allowed for movement of 14 independent electrode positions within two split guide cannulas [30]. The guide cannulas were separated 5.8 mm center-to-center for simultaneous targeting of hippocampus and ventral striatum. While the design allowed for simultaneous targeting of structures in separated brain areas, the cannulas were constrained to being parallel by the microdrive structure. Additionally, the microdrive only had two drive axes. This work is also notable for the chronic movement protocol for electrodes which it outlines.

Another multi-region device comes from Santos et al. in 2012 [31]. This was a multi-tetrode construct that utilized large metal guide cannulas, which were approximately 300 μm in diameter, to implant and record from independent-but-parallel axes in primate brain regions at various depths. Details pertaining to surgically setting the implant trajectories and guide cannulas depths were scare or vague, and a protocol for electrode driving was not made clear. While this microdrive was unique relative to other drive systems in its ability to record from multiple, distinctly separate brain regions, the targeting was still limited by rigid geometry of the structure and the orientation of the targets. Additionally, the microdrive was designed for primates and would be difficult to scale down for small rodents.

Marton et al. recently developed a silicon microdrive that was designed to record from brain stem nuclei involved in sleep-wake behavior [32]. The probe had three groups of 12 mm x 12 mm platinum electrode recording sites spaced unevenly along its length, The spacing was such that upon completion of driving each recording group would be in a target structure. While the
authors were able to achieve some success in simultaneously recording from their targets, the method for achieving the recordings had major drawbacks. The most significant problem was that the target nuclei had to be collinear. Thus, their device was no different than commercial laminar electrodes. Additionally, their silicon probe created large amounts of damage to many different brain structures because of its size. Along this same point, recording sites that were lower on the probe shank, as opposed to near the tip, had to record from areas that were already greatly damaged during previous electrode movements. Another problem is that the probe would have to be redesigned for each specific set of targets. While this was the first paper to show simultaneous, chronic recordings in more than two sleep-wake brainstem nuclei in freely-behaving rats, there were very significant problems with the methodology and overall experimental design.

Microdrives with very small footprints can also be used for multi-site or multi-region recordings. The NanoDrive [33], developed by Cambridge NeuroTech, has a 2 mm x 4 mm footprint and weighs 0.5 g. The device uses a standard screw drive mechanism to move laminar silicon probes at a 205 µm per turn resolution. In theory, the microdrive footprint is small enough and the design light enough to place multiple drives on a single animal’s head. The pricing, however, is expensive and the NanoDrive structure does not support EIB or amplifier placement directly on top of its structure. Extra headmount structures would have to address these issues for chronic, freely-behaving recordings that use the NanoDrive.

Headley et al. recently reported a methodology that used 3D-printed technology for the design of small-footprint microdrives that can be placed inside a larger head-cap to achieve multi-site, multi-region recordings [34]. The system incorporated an EIB onto the protective head-cap, thus it overcame one of the major challenges with small-footprint microdrive structures. Additionally, the head-cap was large enough to support a wide distribution of recording sites. This addressed the limitations of the Lansink drive and other similar designs. The authors of the design do not, however, provide guidance for angling of microdrives for non-vertical target trajectories. There are areas in brain, such as along the midline, that have sensitive structures that must be avoided and where vertical approaches cannot be used. A microdrive that could implement multiple, independently-angled drive axes while maintaining the diverse targeting capabilities Headley and others provide would close the loop on innovations and efforts that have been made over the past few decades with respect to multi-site, multi-region microdrive designs.
2.4 Long-Term Recording Stability

A final important aspect of microdrive design to consider is the ability to record single and multi-unit cellular activity, and do so for an extended period of time. Long-term recordings, on the timescales of weeks to months, are not simple to achieve. This is mainly due to the foreign body response (FBR) that biological tissue can have to invasive implants. As part of the FBR, glia encapsulate implants in a sheath that can degrade electrode signal strength and create a dead zone of tissue in the near field of the implants [35]. In part, this is due large tissue displacements caused by the implants. Mechanical mismatch of material strengths between implants and brain also plays a role. Periodic micromotions occur in neural tissue due to physiological processes such as breathing and heart beats [36]. Stiff probes can strain local tissue because they do not comply to the small micromotions; this can promote cellular encapsulation and glial scarring radially up to 100 µm away from the probe [37]. In addition, foreign structures interfere with the dense micro-vasculature of brain. The average distance between any point in cortex to a microvessel is 13 µm [38]. Large implants destroy this microvascular architecture, resulting in a deficit of metabolic nutrients to nearby cells.

Some of these issues can be mitigated by using smaller and more flexible implant materials. Slow insertion speed of implants have been shown to reduce mechanical trauma and cell damage chronically [35]. This is important for microdrives, which drive implants through tissue over many weeks. An additional advantage for microdrives is that they can move electrodes forward from degraded or dead tissue and subsequently recover recording signals from new cells [13].

The following examples to be discussed are referenced for their ability to overcome the challenges of long-term recordings through careful design choices.

Porada et al. used high-density, but non-drivable, 32 to 64 electrode microwire bundles to record visual cortex neurons for over one year in rabbits and marmosets [22]. The group used a micromanipulator to perform a step-wise implantation of a curved guide cannula. The cannula geometry allowed the group to target visual structures near the surface of cortex from below and very far away from the craniotomy. Recording stability was maintained over several months post-implantation with the high density electrode set up. Neuronal spikes were discernible over the same time course, though it is unclear whether many of the identifiable waveforms originated from single or multi-cell activity. One claim the authors make is that the special approach taken
by the microwire bundles shielded the target sites from damage associated with local craniotomies. They also claim that the large number of electrodes used, as well as the electrode lengths, created large enough frictions to prevent tissue from moving relative to the microwires. This latter point is counter to Sridharan’s assertion that stiff probes increase glial scarring by preventing tissue micromotions [36].

McMahon et al. used a similar setup to Porada’s experiment to record visual responses in cortex of single, isolated neurons across months [21]. However, the implant technique varied in a slight, but significant way. Their goal was to use a chronic microdrive system to carefully approach and isolate single units and maintain that connection over the time course of the experiment. Electrode adjustment was performed on awake animals in somewhat low resolution spatial steps of 350 - 700 µm with no electrode adjustment more frequent than every three days. One major problem with the microdrive design is that the high density microwire bundle moved as a complete structure; individual microwires, or smaller bundles, were not able to be driven. This decreased the yield of individual cells from which they could record.

Pieces of the group’s implant technique are also relevant to point out, as other microdrive designs use or purposefully choose not to use them. The depth at which their electrodes would advance to reach the target was set using an implanted guide tube. The electrodes themselves, however, still started dorsally at the top of the guide tube. A pre-surgical MRI scan was used to identify the appropriate depth of implantation of the guide tube. The depth of the electrodes was known by simply traversing the known length of the guide tube. Further, exposed electrode bundles were guided into the guide tube using an xyz-micromanipulator. This is risky because the tips are not protected upon entry and motion through the guide tube itself.

2.5 Conclusions

For many decades, microdrives have had an important role in progressing neuroscience from a single-unit perspective. The different body designs, electrode configurations, and driving mechanisms that have been implemented have all served to improve recording density, electrode stability, and driving spatial resolution. Yet, there are still some limitations on current microdrive designs, and microdrives in general.

Most microdrives are solely designed to accommodate single-unit recordings. From a systems
and circuits perspective, especially if one is studying single-unit activity in the context of behavioral expressions, other electrophysiological signals are needed to better discriminate behavioral states. These signals include: electroencephalogram (EEG), electrocorticogram (ECoG), and local field potentials (LFP). In the case of sleep-wake behavior, EEG and ECoG are useful for determining the state of vigilance an animal is in. Deep slow-wave sleep has characteristic 3 – 4 Hz large amplitude oscillations in cortex, which can be seen in ECoG using cortical screws. Rapid eye-movement (REM) sleep is distinguishable by characteristic 6 – 8 Hz theta rhythms that can be seen on EEG of the hippocampus and ECoG. There are reported instances of microdrives accommodating LFP recordings [39], but there are none that support sensor modalities, such as cortical screws or depth electrodes, that can be implanted externally to the microdrive structure.

An additional limitation of microdrives is a limitation on chronic implants in general. That is, the size and weight of the structure must be comfortably supported on an animal’s head for freely-behaving experiments. Estimates for the head-weight carrying capacity of song birds, mice, and rats are 2 g, 5 g, and 25 g, respectively [19]. That equates to approximately 10% the animal’s body weight. Microdrives must be designed with these limitations in mind. As such, there are various microdrive designs specifically suited for use in bird studies [19] [20], mice studies [13] [19] [39] [12], and rats [28] [30].

In the following chapter, we will move from an exposition of past microdrive literature to a discussion of the necessary design criteria that a new microdrive must meet in order to progress the field further. This will focus on areas of microdrive design that have not seen too much success. Specifically, this includes multi-site and multi-region targeting, and support of external sensing modalities for improved chronic behavioral studies. This is all in preparation for Chapter Four, where our microdrive design is introduced and its place within the literature can be assessed.
Chapter 3 | Design Criteria for a Better Multi-Site, Multi-Region Microdrive System

In the previous chapter, different examples of microdrive innovations and designs were discussed. While many of the microdrives had individual components that would be advantageous for effective multi-site, multi-region (MSMR) design, there was no one microdrive that integrated all the necessary criteria into a single device. This short transition chapter outlines the design criteria for a microdrive suitable for studying distributed cellular networks in a chronic and freely-behaving small animal/rodent experimental context. In the following chapter our microdrive solution to meeting these criteria will be introduced.

The development of a MSMR microdrive was necessitated by our objective to simultaneously record from cell groups thought to regulate sleep-wake regulatory cycles in rats [40] [41] and to probe the bidirectional coupling between epilepsy and sleep-wake regulation [42]. The nuclei that house these cell groups are shown in Figure 3.1. These nuclei reside in deep and separated brainstem or forebrain structures, approximately 7–10 mm from the cortical surface. The target cell groups are non-collinear and most are not co-localized. Thus, there is no single trajectory that recording electrodes can take on to address all of those targets simultaneously. The challenges of such a recording task are further increased because these structures are small, typically of order...
200 - 300 µm in spherical diameter, and microwire electrodes are only tens of microns in diameter.

Take a moment to imagine a nearly perfect multi-site, multi-region microdrive design. Certainly every person will have a different conception of what the microdrive might look like and how it might function. Each of these imagined microdrives, however, are likely to share many common features. Likely, each device has a small and easy-to-handle driving mechanism. They might also share multiple, independent drive axes. Perhaps these axes can be pointed at different angles and in different directions. These conceptual microdrives have these common features because, at their most basic level, these microdrive need only perform only a few basic functions to be effective multi-site, multi-region designs.

One of the most essential features of a good MSMR microdrive design would be the ability to record stable unit activity from non-collinear or non-co-local cell groups. This is a fundamental requirement for a MSMR microdrive, and as we have already seen it has been done with single [30] [31] and multiple small microdrive structures [34]. The targeting abilities of existing MSMR microdrives are still largely limited by having vertical, or parallelized drive axes, though there is a known exception [43]. They are also impeded by an inability to implant electrodes set distances away from their targets. A single multi-region microdrive structure that could independently angle many individual drive axes and implant those electrodes at specific depths...
would greatly facilitate the chronic study of large neural circuits. Here is an opportunity for marked innovation within the microdrive field.

Taking the exercise one step beyond a simple MSMR microdrive, we might conceive a device that is instrumental for chronic full-systems behavior studies. In order to correlate single-unit activity to relevant behavioral expressions, the microdrive would also have to accommodate additional sensing modalities and therefore connections of additional sensors, such as ECoG screws and EEG electrodes. In an extended sense, the microdrive should also be simple to build, consistently accurate, and affordable. How well all of these criteria are implemented together would define the success or failure of our conceptual device. The following sections outline specific details of design criteria that a novel microdrive must address in order to make a significant impact in an already crowded and revered technological field.

3.1 Electrode Implantation and Targeting

A key design feature that an effective MSMR microdrive must implement is straight-forward implantability of its electrodes. Implantability is at the core of microdrive methodology; construction and recordings are simply preludes to and consequences of a successful drive implantation. Here, the term implantability implies both accurate insertion of electrode bundles into brain and subsequent driving of electrodes through tissue. An additional design features is that the device must be both reliable and easily reproducible. The implant methodology would also have to support multi-site and multi-region targeting.

There are two standard methods by which electrode bundles can be implanted and subsequently driven: one, wires converge into a guide cannula that sits superficially in the dura mater, and the wires are driven through tissue from the surface of cortex; or two, bundles are implanted into tissue with the help of guide cannulas and are set at a specified driving distances from targets. The vast majority of microdrives employ the first method. The primary reason for approaching implantation in this fashion is that many microdrives are designed to be used in rodent hippocampal or cortical studies. These brain regions are relatively superficial and every substructure within them can be targeted along a vertical trajectory. For most experiments, this technique is sufficient. The microdrive – cannula superstructure could also be angled to allow for non-vertical trajectories. Driving from dorsal brain surface would not be advantageous for deeper
targets, though, as accuracy and reliability become a major issue.

The second method is an attractive idea for targeting deeper brain structures, such as brainstem, where driving the electrodes from cortex would be cumbersome, time consuming, and less accurate. The implementation, however, is not as straight-forward as it may seem. Early problems arise in simply trying to set the electrode depth a specific distance away from the target with stereotaxic accuracy. To demonstrate, consider an example case: a microdrive structure with a rigidly-attached single electrode bundle, which sits inside of a protective cannula, as seen in Figure 3.2. The wires are fully connected to an EIB at the top of the microdrive. The bottom of the cannula marginally extends past the tips of the electrodes, such that the electrodes are protected by the cannula from the mechanical reaction forces of tissue upon implantation.

![Diagram of ventral implantation of a single microdrive cannula](image)

Figure 3.2: Ventral implantation of a single microdrive cannula. An illustration of how a cannula could be implanted at a set depth in brain. The objective is to implant the electrodes, which sit at the bottom of the cannula, a distance $\Delta$ from the target $X$. The cannula is rigidly attached to the microdrive body. Thus, the entire structure must be manipulated to maneuver the cannula. To achieve the correct implant depth, the position of the bottom of the cannula must be tracked with respect to the target in a common coordinate reference frame. Directions for the coordinate axes are as follows: Dorsal (D), Ventral (V), Anterior – Posterior (AP).

The objective is to position the tips of the electrodes a distance $\Delta$ from the center of a target nuclei, designated by $X$ in the sagittal brain section, with full anatomical coordinate (dorsal-ventral
(D-V), medial-lateral (M-L), anterior-posterior (A-P)) accuracy. In order to do this, the entire microdrive structure would have to be attached to a 3-axis manipulator or stereotaxic unit. The bottom-center of the cannula would have to be zeroed at a reference location, such as bregma, in order to align the anatomical and target coordinate bases. Next, the whole structure would be lowered along the D-V axis through a craniotomy. For depth of the cannula would be tracked with respect to the reference point. Once the D-V coordinate reaches $\Delta$ away from the target, the cannula and microdrive structures would be secured into place to the skull.

The described methodology is realistic for a microdrive with a single guide cannula. The system could even have multiple independently-drivable electrodes going down the guide cannula and the implant could be angled. The technique breaks down, however, with any greater number of guide cannulas if the individual targets have different D-V coordinates or require varied $\Delta$ distances.

What would be needed in order to achieve implantation of multiple electrode bundles at set depths along independent drive axes is either of the following methods: a microdrive system with guide tubes pre-angled on the structure to match the desired drive trajectories; stereotaxically-placed drive axes that are hooked up to drive mechanism after implantation; or a generalized system that decouples implantation of skull-fixed guide cannulas with subsequent placement of electrode bundles.

The first MSMR system has been incorporated into the design of a not-yet-published hyperdrive-like device on the Open Ephys website (open-ephys.org) [43]. This set up requires electrodes to be driven from the cortical surface, though, and, as such, is not ideal for deep-structure targeting. Targeting multiple structures in very distant regions of brain from one another would also present a significant challenge.

The second method would be very inconvenient and difficult with a large number of microwires with which to make connections. There would also be a high risk of failure due to any gluing or other manipulation of delicate implant bundles that would need to take place in order to connect the drive axis to a driving mechanism on top of an anesthetized animal. One design that might work are implants that have flexible lead cables that can be easily plugged into a connector one the drive axis has been secured to the driving mechanism.

The third system uses a tube-in-cannula setup and is the best option for our specific design criteria. This system is used for intracranial pharmacological injections [44], as well as for static recording implants. There are no reports of this system being used as part of a microdrive device,
though. The targeting and guide cannula implantation would be the same for a microdrive. The only major difference and challenge would be in handling the multiple drive bundles that all connected to the microdrive structure. This challenge could be overcome by providing each bundle enough length to be maneuvered into position inside the guide cannulas and then lowering the microdrive structure and further sliding in the drive bundles.

The tube-in-cannula implant method could also be very useful in a MSMR microdrive scheme. In this setup, the guide cannula is surgically implanted independent of the drive bundles. As such, the drive trajectory can be arbitrarily angled simply by angling the guide cannula. Using simple algebra, the ventral depth of the electrode bundles can be known when it is fully seated in the guide cannula.

Figure 3.3: Sensitive structures to avoid. Brain structures that are on the midline, such as DR, are not as easily targeted as lateral structures, such as PPT, which can be targeted with vertical trajectories. This is due to the location of many sensitive anatomical structures that also are on the midline and that should not be damaged for chronic experiments. Labels are: ventricle (V), pineal gland (P), and sagittal sinus (Sinus). Modified from Figure 100 of Paxinos and Watson, 6th ed. [45].

Independent drive axis angling is especially useful for avoiding sensitive obstacles in brain, such as ventricles and arteries. Such structures must not be damaged by implants for the chronic survival of the animal. This becomes especially relevant for the sleep-wake network, a co-planar portion of which is shown in detail in Figure 3.3. Note that the DR falls on midline, with the ventricle, pineal gland, and sagittal sinus directly above. Unlike the vertical targeting feasible for
PPT (PTg in Paxinos and Watson, 6th Ed. [45] notation), DR must be targeted at an angle to avoid damaging the sensitive midline structures. There are no microdrives available that support the ability to independently angle drive axes that could achieve both a vertical approach to PPT and an angled approach to DR. Many researchers do end up damaging sensitive structures on the way to midline targets.

3.2 Microdrive Structure and Enclosure

An effective MSMR microdrive would likely be designed in the context of achieving large-network neurophysiology. This suggests monitoring multiple types of electrical activity while an animal is in a freely-behaving state. In other words, an effective drive design would accommodate the implantation of additional sensors, such as ECoG screws and EEG depth electrodes, as well as the mounting of peripheral electronics, such as an EIB and amplifier. Consequently, details of the microdrive structure, meaning the body as it would sit on top of an animal, must be carefully tailored to support these features.

Most microdrives, however, do not tailor their design to implement this criteria at all. This is in spite of the importance of extra electrophysiological signals that are useful in studying behaviors from a systems neuroscience perspective. The majority of microdrive bodies converge down to single craniotomies or have enclosed bottoms that limit or prevent the placement of additional sensors. Ground screws for electrical recordings are typically placed outside the microdrive structure and are protected with dental acrylic.

In coordination with the tube-in-cannula system for electrode targeting, an effective MSMR microdrive structure would have to be open to allow for guide cannulas to be placed anywhere on the cranium. This requirement also provides available space to implant cortical screws, depth electrodes, and other sensors. Instead of having a closed bottom, the microdrive body would look more like a stadium that had an open center and structures along the perimeter. As is with many other microdrives [13] [34], head-caps can be 3D-printed in highly-customized shaped to protect the microdrive and the microdrive itself can be customized to support EIB and amplifier mounting.
3.3 Manufacturability, Customization, and Cost

Plenty of microdrives are designed in-house by independent research labs, while many drives are also commercially available. In-house designs that are typically reported can either be overly complex in their descriptions or require tooling of metal parts, which can be inconvenient. Commercial designs are expensive and come with the added difficulty of trying to use a general-purpose microdrive for a very specific experimental use. An ideal microdrive would be simple to construct, able to be customized for many particular experiments, and would cost significantly less money than the commercially-available designs that companies offer.

Rapid manufacturing, or 3-D printing, is a good choice for making a microdrive that meets the above demands. Stereolithographic printing is widely available these days and high-resolution plastic prints can be done at a low price. A typical print, using material volumes on par with those of standard microdrives, would only cost between 15 to 50 dollars. Furthermore, the computer-aided-design (CAD) model from which the microdrive body would be printed could be easily modified. This provides options for customizing the microdrive design to meet the specific demands of a particular experiment. An additional benefit of a 3-D printed drive would be that plastic is light weight, as opposed to metal, which is sometimes used in drive designs. Thus, there would be less weight on the animal’s head. Lastly, a printed microdrive would need only a small set of preparation steps, such as hole-tapping and electrode loading, to have it ready for experimental use. Overall, if designed and implemented correctly, a 3-D printed body could be a very cheap, simple to use, and flexible option for microdrive neurophysiology.

3.4 Conclusions

Reliably implementing a design that can address the criteria listed in this chapter is the next critical step in the progression of core microdrive technology. There is a growing demand in neuroscience, as a whole, to study multiple neuronal populations involved in behavioral circuits, especially chronically in freely-behaving animal models. A multi-target, multi-region microdrive that can position electrodes along arbitrarily-angled drive axes could effectively meet those demands. However, as is evident in the literature, this is not an easy goal to accomplish. Such a microdrive would have to focus its entire design and methodology around supporting those
abilities. As will be seen in Chapter Four, that is exactly how the microdrive presented in this work was designed.
Chapter 4  
A Multi-Site, Multi-Region Microdrive with Independent Drive Axis Angling

In Chapter Three, the design criteria necessary for an effective multi-site, multi-region microdrive were discussed in detail. In this chapter, we present our solution for meeting these criteria with a single and harmonious microdrive system. The first parts of this chapter are dedicated to explaining the core innovation of our design: the flexible drive axis. The second part of this chapter focuses on additional elements of the microdrive that include the driving mechanism, overall microdrive structure, and headmounts for implementing chronic recording experiments. The primary purpose of this chapter is to show and explain our novel microdrive and the system we developed to overcome the engineering challenges of multi-site cellular-level recordings in distributed neural circuits. The full details of microdrive construction, however, are elucidated in the subsequent chapter.

4.1 Flexible Drive Axes

The core innovation of our microdrive, and the technology that enables multi-region targeting in a way that no other microdrive can implement, is the flexible drive axis. A diagram of the flexible
drive axis, along with a conceptual depiction of its use for multi-position targeting, is shown in Figure 4.1. The flexible drive axis decouples drive axis alignment from electrode implantation. This allows each electrode bundle, which snakes through the constrained geometry of a long drive axes, to take on an independent and well-defined trajectory. As alluded to in the previous chapter, other microdrives require that their entire structures be angled to accommodate non-vertical drive trajectories. Even designs specifically tailored to multi-region targeting are either limited to defining trajectories from a single craniotomy [43], or cannot independently angle their separated guide cannulas or shuttles that carry individual drive axes [30] [31] [34].

Figure 4.1: **Flexible drive axis.** The core innovation of our microdrive is a flexible drive axis that decouples the positioning of a guide cannula, which sets the drive trajectory, with the placement of the electrode implant. 2a. Diagram of the flexible drive axis. The length of the body tube (orange) is fixed with respect to the skull-fixed guide cannula (yellow) and the microdrive body (gray rectangle). Further, the placement cannula (blue), which is rigidly attached to the body tube, is tightly fit inside the guide cannula. The overall effect of this setup is that the electrode bundles must move out of the placement cannula along a straight trajectory. 2b. Depiction of bundles achieving different drive trajectories when placed inside guide cannulas. The bottom of the body tubes stop at the top of the guide cannulas; this sets the depth of each implant. Base figure from Figure 100 of Paxinos and Watson, 6th edition, Copyright Elsevier [45].

Our system avoids these constraints by the method shown in Figure 4.1b. First, a guide cannula is stereotactically implanted into brain and then fixed to the skull. The orientation and ventral position of the guide cannula will define the angle and depth of the drive axis. Afterward,
the drive bundle is manually placed into the guide cannula. Once in position, the end of the placement cannula, where the tips of the electrodes emerge, will be a set distance away from the target.

In our system, the guide cannula and placement cannula, both made from polyimide tubing, were chosen to have as small a difference between their inner and outer diameters as possible. This is important for ensuring concentric alignment of the electrodes along the drive axis. For example, the spacing between our placement and guide cannula is approximately $13 \, \mu \text{m}$ (see Chapter Five for tubing material, sizing, and drive axis construction). This means that the angular deflection of the placement cannula from the center of the guide cannula is very close to $0^\circ$. For the typical extension of the placement cannula past the guide cannula, which is around 4 mm, the deflection is approximately $0.003^\circ$. Thus, the electrodes are very likely to move along the drive trajectory set by the guide cannula.

A surgical system was co-developed alongside the design of the flexible drive axis to implement accurate trajectory angling and depth setting of implants for our microdrive. As mentioned in the previous chapter, this methodology is familiar in the pharmacological injection and static implant literature, but has not before been incorporated into a microdrive. The system has three basic requirements in order to work: knowing the absolute lengths of the objects being implanted, aligning the stereotactic axes to the final drive axis trajectory, and choosing a driving distance $\Delta$ for the electrodes.

All of the targeting calculations were designed in the context of using atlas-referenced coordinate systems as defined by Paxinos and Watson, 6th edition [45]. We use a virtual intersection of midline, which we reference as the animal’s midline suture, and the interaural line as our axes origin. Recall that the objective of our process is to stereotactically implant a guide cannula at a specific ventral position along an arbitrary trajectory angle, such that the bottom of the implant will stop at a set depth when inserted into the guide cannula. Shown in Figure 4.2 are the calculations for this process. The vectors that point between the origin, a user-defined external fiducial mark on the cranium, and the target in the original coordinate system are calculated. Once the vector between the fiducial mark and the target is known, a change of bases is performed to align the stereotax to trajectory of the drive axis. This allows the absolute to-target distance between the origin of the new coordinate bases (the fiducial mark) and the target to be tracked. The reader is referred to Section 6.2.1, and specifically Figure 6.4, for a detailed example of how
Figure 4.2: **Stereotactic targeting vector diagram.** The distance to a target from a user-defined external fiducial mark can be calculated using vector subtraction. The vector $X_T - IA$ is known. The vector $X_F - IA$ is measured using a stereotax. The difference between those two vectors is $X_T - X_F$ in the interaural reference frame.

This alignment method works in a surgical context.

The realignment of the stereotax with the desired drive trajectory defines the angling of the guide cannula. The second piece of the method is to implant the guide cannula at a specific ventral position in order to have the drive bundle end at a known depth. This is easier with the realigned bases, because the user can work in absolute lengths and not have to consider the angle of the stereotax in the calculations. The core elements for setting the implant depth are shown in Figure 4.3.

In order to set a driving distance $\Delta$ for the electrodes, two simple measures, $R$ and $\alpha$, must be known. $R$ is the length of the placement cannula and $\alpha$ is the length from the top of the guide cannula to the tip of a stereotactic stylus. The stylus (S.T. unit) is effectively a surgical shuttle for implanting the guide cannula using stereotactic targeting. We used a standard tapered tungsten electrode (FHC, Bowdoin, ME), which can be easily manipulated on most stereotax systems, as a stylus. On the S.T. unit is a 6.3 mil (1 mil = 1/1000 inches) polyimide tube that acts as a spacer that 8.9 mil guide cannula slides over. This spacer improves centering of the...
Figure 4.3: Setting the implant depth. Electrodes can be implanted at an absolute driving distance $\Delta$ away from a target X along any arbitrary trajectory. A stereotactic unit (S.T Unit) carries a guide cannula that will be fixed to the skull. The top of the guide cannula sets the stopping point of the implant. The difference between the length of the placement cannula (R) and the length from the top of the guide cannula to the tip of the S.T unit ($\alpha$) is known. The ventral depth of the guide cannula that would set the bottom of the placement cannula $\Delta$ away from X is guaranteed by implanting the S.T unit at a distance ($\zeta$) away from the target.

guide cannula with respect to the stylus tip.

The key to setting $\Delta$ comes from using the same sized tubes for both the guide cannula and
body tube in the flexible drive axis. As was depicted in Fig. 4.1 and also seen in Fig. 4.3, the
bottom of the body tube must stop at the top of the guide cannula in its final positioning. Thus,
along any absolute axis, if the tip of the tungsten stylus is stopped at a distance $\zeta$ from the target,
the bottom of the placement tube will end exactly $\Delta$ away when inserted into the guide cannula.
These parameters are combined into a single equation shown below:

$$ \zeta = R - \alpha + \Delta $$

Equation 4.1 uses the difference between lengths $R$ and $\alpha$ to track the position of the S.T. unit
tip with respect to the length of the placement cannula. The user-set driving distance $\Delta$ is then
added to this difference in order to track the tip with respect to the target. The equation outputs
the distance-to-target $\zeta$ that the S.T. unit must stop in order to correctly position the guide
cannula. After the guide cannula is fixed to the skull, the placement cannula can be inserted. The
bottom of the placement cannula will stop a distance $\Delta$ away from the target, which is exactly
when the body tube meets the top of the guide cannula.

What was just described was the design and surgical realization of the flexible drive axis in
completion. To our knowledge, this is the first reported instance of a methodology like this being
used in a microdrive system. The system is robustly designed and can accommodate a wide
range of trajectory angles and $\Delta$ distances. The logistics of implanting at varied depths for more
superficial targets are discussed in Chapter Six.

4.2 Drive Elements and Design

Our microdrive utilizes variations on classical tubing structures, recent innovations in driving
mechanisms, and modern rapid-prototyping technologies in its design. The resulting structure is
easy to fabricate as well as inexpensive, relative to commercial microdrives. For visual reference
as each of the drive elements are introduced and discussed, pictures of two completed microdrives
are shown in Figure 4.4. The main pieces to notice in the pictures are the individual drive bundles,
the 3D-printed microdrive bodies, and the electrode interface boards (EIB) that sit on top of the
microdrive structure. There are two design types shown in Fig. 4.4. The design on the left is
the original low drive-position-density design that was used for the majority of this work. The
version on the right is the more recent high-density design. Additional components that are a
part of the full microdrive system, such as the headmount and amplifier, are not shown in these pictures. They will be discussed later in this section.

Figure 4.4: Built microdrives. Examples of constructed low-density (a, b) and high-density (c, d) microdrive designs. Each drive has springs and screws that are aligned with individual drive positions along the outside of the body structure. Each drive position contains an individual and independent drive axis. An electrode interface board (EIB) sits on top of the body structure for making electrical connections between the electrodes and an amplifier. The high-density design has 14 usable drive positions; the low-density design has six.

4.2.1 Drive Bundles and Driving Mechanism

A diagram of the flexible drive bundle is shown in Figure 4.5. Like many other microdrives, our set up uses a telescoping tube-in-tube structure. Here, a shuttle tube, which is maneuvered up and down by a drive shuttle, carries electrodes through a longer body tube, which is fixed to the microdrive body. Our tube structure is different, however, in that it also has a placement tube coming out of the bottom of the body tube. As mentioned previously, this is a necessary piece for the decoupled guide cannula system. Another major difference is that the second fixed-point
in the tube length, which is needed to constrain the electrode bundle along a defined path as it moves through the tubes, is not another point on the microdrive body. Instead, as shown in Fig. 4.1a, the second fixed-point is the guide cannula.

Figure 4.5: **Drive axis diagram.** The flexible drive axis is made of a telescoping tubing structure that defines a path for the electrode bundle as it is driven through the axis by a drive spring. The shuttle tube carries the electrode bundle, and is attached to the head of the drive spring and moves through the body tube. As the drive spring is moved up or down with the turning of a drive screw, the shuttle tube and electrodes move with it. The body tube is fixed to the microdrive body with cyanoacrylate and is very long to allow the placement tube to reach the guide cannula that is fixed to the cranium. The placement tube is fixed to the bottom of the body tube and is implanted into the guide cannula. Petroleum jelly space-fills the placement cannula to prevent fluid and protein from getting into the drive axis and binding the electrodes.

The drive mechanism used in our microdrive was adapted from the FlexDrive [13]. As discussed in the literature review, this modern driving mechanism is a variation on the classic keyway shuttle design employed by so many other microdrives. The advantages of the spring-based shuttle
are that the springs are very light and are not a physical component of the microdrive body itself. The latter point is useful because one needs to add springs only where they are needed (Fig. 4.4). A representation of the flexible-spring driving mechanism and our specific adaptation of the design is shown in Figure 4.6. The left part of the diagram depicts the flexible spring being driven up and down by the turning of a screw. The spring is fixed between a wedge in the microdrive body and the neck of the screw. As a result, the spring must bow outwards and move down with the screw head because it is rotationally constrained. This is how the spring driving mechanism translates rotational motion (i.e., the turning of the screw) into linear motion.

Our specific modifications on the original FlexDrive design are shown in the right part of the diagram. We use a rectangular structure with a polyimide tube bridging between the ends of the spring head. This rectangular design is necessary for the higher-density microdrive structure where there is a very small amount of space between drive positions. The polyimide bridge is used to fix-and-center the shuttle tube so that it is concentrically aligned with the top of the body tube. This alignment is important for preventing kinking of the shuttle tube as it moves through the body tube.

4.2.2 Microdrive Body and Auxiliary Components

The microdrive body is the nexus for all the various drive elements and auxiliary components that make up the complete microdrive system. The high-density version of the microdrive body is shown in Figure 4.7. The microdrive body, as well as every other 3D-printed object for this work, were designed using AutoCAD (AutoDesk, San Rafael, CA). The 3D printed structure has four main subcomponents: the drive positions, the spring wedge, the EIB screw columns, and the headmount feet.

The microdrive has a 2 x 7 array of 2.5 mm drive positions that are designed to hold a 1/4" 000-120 screws. Each position has a 0.66 mm hole that is then tapped with 000-120 threads. The array is angled at 20° so that the long drive bundles cross over the midline instead of pointing straight down. This is helpful during implantation, as guide cannulas of the drive axes are located across the midline from their respective screw positions. Having a large array of drive positions on the body provides flexibility for where the drive bundles can be mounted. Targets can be distributed almost anywhere along the rostral-caudal axis, so having the ability to load bundles closer to the targets can make implantation much easier.
Figure 4.6: **Driving Mechanism.** The driving mechanism for the microdrive is a modified version of the spring drive used by Voigts et al. [13]. A thin piece of spring steel, which has a shaped head that fits around the neck of a 000-120 screw, is fixed between a wedge in the microdrive body and a screw. The spring is rotationally constrained. Thus, as the screw moves down the spring must bow outward and the spring head moves down with the screw head. The spring is rectangular to accommodate a high-density array of drive positions on the microdrive body. A small polyimide tube is fixed to the head of the spring to provide a centered point to attach the shuttle tube. This is for better alignment of the shuttle tube and body tube as the spring head pushes the shuttle tube through the body tube.

The array is also raised up a few millimeters from bottom of the microdrive structure. This is critically important during implantation, because the forceps that are used to maneuver the drive bundles into the guide tubes can only reach from within the bottom gap between the headmount base and the array. Additionally, each position has a ledge that is depressed 1 mm from the top and extends 1.8 mm toward the center. Each ledge has a non-circular hole, called the drive axis hole, where the body tube can be fixed to the microdrive body. Altogether, the ledge feature allows the top of the body tube to extend upward enough for cyanoacrylate to safely penetrate down into the hole without getting into the body tube and without the tube extending too far up and limiting the drive stroke.

As mentioned earlier, the wedge on the microdrive body is designed to fix the bottom of the flexible drive springs in place (Fig. 4.6). The wedge is 1 mm in height and is effectively an extension of the drive position array. The wedge’s 20° angle is sufficient to initially set the spring
Figure 4.7: Microdrive body design. The four primary features on the microdrive body are: the drive positions, the screw column, the base feet, and the spring wedge. The arrays of drive positions have tap holes from 000-120 threads and are angled at $20^\circ$. Each individual drive position is 2.5 mm in width and includes a drive axis hole for fixing the drive axis bundles to the microdrive body. The two screw columns have 00-90 tap holes and are spaced apart by 20 mm. The feet of the microdrive are where the body is secured to the headmount base. The 1 mm tall spring wedge is on the outside of the drive position array and is angled at $20^\circ$. This provides a smooth arc from the drive springs. All non-angle dimensions are in mm configuration with a natural bend and to maintain a smooth arc as the spring is pushed down and outwards. Lastly, there is enough clearance between the wedge and the position array to evenly space-fill with epoxy and fix the bottom of the springs into place.

The last two features of the microdrive body are the EIB screw columns and the headmount feet. The screw columns are wide-shaped structures designed to hold the EIB on top with a 00-90 screw and to limit torsions along the length of the microdrive body when it is put under load. The center-to-center distance of the columns is 20 mm. The diameter of the holes is 0.88 mm. The tops of the columns are extended high above where the drive springs and bundles sit to
provide room to thread and rivet the microwires during construction (see Chapter Five for more details). The headmount feet have 0.97 mm diameter holes that are later enlarged to be 00-90 through holes. The microdrive is secured to the base of the headmount with two screws. The width of the feet, along with the size of the 00-90 torx screw used to secure the drive down, are both wide enough to provide stability to the drive during chronic use; the drive does not wobble under freely-behaving motions of animals.

The microdrive and all its sensitive components have to be protected during chronic recordings as the animals are freely-behaving. The solution for this is a head enclosure, which we call a headmount, that is fixed to the animal’s head. The headmount fully encloses the microdrive, EIB, and amplifier. Shown in Figure 4.8 are the CAD designs for the headmount. The headmount is divided into two components: the base and the cap. Both of the pieces are 3D printed and only weigh several grams. The base gets fixed to the animal’s skull during surgery with dental cement. The microdrive body and cap are mounted on the base with 1/4” 00-90 and 3/16” 00-40 screws. The side and back (caudal) walls of the base are slopped to provide better access when placing the drive bundles. The cap is mostly an empty shell that conforms to the general shape of the microdrive with an amplifier connected on top of the EIB. Other less tall cap designs are used for periods when the animals do not have an amplifiers on their heads.

The last peripheral components of the microdrive system are the EIB and the amplifier. These two auxiliary components are mounted on top of the microdrive structure like most other microdrive systems. The EIB is a printed circuit board that is used to connect the electrode leads to channels of the amplifier. We used Advanced Circuits (Aurora, CO) as the PCB manufacturer. The EIB is designed for an Omnetics 36-pin nanostrip connector (Omnetics Connector Corp, Minneapolis, MN) to be mounted at its center; many commercial amplifier systems are compatible with this choice of connector. The commercial amplifiers used for all of the experiments in this thesis are Intan RHD2132 or RHD2216 amplifier/accelerometer boards (Intan Technologies, Los Angeles, CA). Other amplifiers could also be used with the same EIB or with slight modifications of the EIB.

A CAD assembly of the complete microdrive setup, which includes the amplifier and headmount, is shown in Fig. 4.9. Mock tubes and springs have been added for a better representation of the system configuration. The amplifier was imported from a SolidWorks model of the RHD2132 board created by Intan Technologies and the Omnetics connector was imported from a SolidWorks
Figure 4.8: **Headmount design.** The headmount is used to carry and protect the microdrive and auxiliary components while on top of a freely-behaving animal’s head. The headmount is split into two components: the headmount base and the headmount cap. The headmount base has a 4-40 tap hole for the cap screws and a smaller 0.66 mm hole that gets enlarged and tapped with 00-90 threads for the microdrive feet screws. The base has a 15.01 mm x 11.46 mm opening for access to the cranium that allows placement of multiple guide cannulas and other electrophysiological sensors. All dimensions are in mm.

model created by Omnetics Connector Corp. Altogether, the system weighs approximately 14 g, which is light enough for a rat to chronically support on its head.

### 4.2.3 Open-Structure Platform

Another key feature of our microdrive system, which incorporates elements from both the headmount base and microdrive body design, is the large open center within the setup. The headmount base has a 15.01 mm x 11.46 mm rectangular opening. The width roughly conforms to the width between outer skull ridges and the length extends a few millimeters past the anatomical landmarks lambda and bregma. This opening serves two advantages: access to the full cranium
for positioning of guide cannulas and drive axes, and a large area to place auxiliary physiological sensors, such as cortical screws or depth electrodes. Such auxiliary sensors are critical for studying complex behaviors like sleep-wake regulation because they provide additional information about the animal’s state of vigilance.

Most other microdrive systems have large bodies that funnel down into a smaller chamber around a craniotomy, which severely limits the ability to implant any additional sensors. To our knowledge, this is the first microdrive to intentionally incorporate the ability to place extra sensors, besides microwires and perhaps a ground screw, within a single protected structure.

4.3 Conclusions

Here, a novel multi-site, multi-region microdrive system was introduced. The design incorporated long, flexible tube structures with decoupled guide cannulas to create independent drive axes that can be arbitrarily angled and implanted at set depths. The structure of the microdrive body was specifically designed to accommodate this novelty. The result is a harmony across features of the
device. This feature coordination, ease of implementation, and simplicity of design and materials makes a strong argument for our microdrive as an effective solution to meeting the challenges of making a multi-site, multi-region microdrive.

It is important to realize that many of the same techniques, structures, and designs presented in this chapter can also be employed with use of static implants or other variations on the microdrive system. The angle orientation and depth setting will work for any implant with a decoupled guide cannula system. An acute experiment with results will be discussed later in this narrative that supports these claims. Additionally, the microdrive body is readily modifiable to accommodate different spatial setups, EIBs, or amplifiers. So long as the basic elements of the microdrive setup and the surgical technique to implement the drive axes are present, the system is quite robust to changes to meet individual experimental demands.
Chapter 5

Microdrive Construction

This section provides step-by-step build instructions in the order we currently use for drive construction. Many of the construction processes were developed to align with standard established practices of the microdrive field. However, a lot of the processes are also highly specific to this particular microdrive. A note to the builder: construction of this drive takes time and patience. The most important step in the entire construction process is to perpetually use delicacy and caution when handling the microdrive. The system is very fragile and has moving parts; any one misstep, such as placing glue in the wrong spot or dropping the drive, may ruin several days worth of hard work.

5.1 Microdrive Construction Procedures

5.1.1 Microdrive Body Construction and Preparation

5.1.1.1 3D Printing

Microdrive bodies are printed using a PolyJet 3D printing process with an Objet260 Connex (Stratasys, Eden Prairie, MN) printer. The PolyJet process utilizes digital material, which is a combination of several photopolymers, such as Tango Black and Vero Clear, to provide a range of flexibility and hardness to the end body material. Vero is the most rigid blend of cured photopolymer available for the PolyJet process. The microdrive is designed to be as rigid as possible, as to minimize motion artifacts into electrical recordings when the animal is
freely-behaving. Thus, Vero Clear, or a photopolymer with a cured hardness equivalent to Vero Clear, such as Vero White, is used for printing.

Headmount caps and bases are printed using a uPrint SE (Stratasys) 3D printer. This printer uses ABS thermoplastic in a standard extrusion process. External support material is removed by hand using a dental pick set; walls of the headmount cap are too thin to withstand the sonicated acid bath typically used for removing support material.

5.1.1.2 Microdrive Body Preparation: Cleaning and Hole Tapping

![Microdrive body hole cleaning and tapping](image)

Figure 5.1: Microdrive body hole cleaning and tapping. There are four different holes that require clearing on the microdrive body: the drive screw holes, the drive axis holes, the EIB column holes, and the corner holes. This process is done with a subset of size #71, #64 drill bits and 00-90, 000-120 taps. a. A diagram of the four different holes and the drill bit and/or tap size that are used to prepare them. b. A photo of the collets, drill bits, and taps used to prepare the microdrive body at the center. c, d. Clearing the drive screw and drive axis holes with a #71 drill bit. e. Tapping the drive screw hole with a 000-120 thread. f Enlarging the corner hole with a #64 drill bit. g, h. Tapping the corner and EIB column holes with 00-90 threads.

Once printed, microdrive bodies are prepared for screw and spring mounting, and electrode bundle loading. Shown in Figure 5.1 are steps of the body cleaning and preparation process. The bodies are first cleaned of external printing support material via soft-bristle brushing with a Dremel 3000 Rotary Tool (Dremel, Racine, WI), water rinsing, and pressurized air blow drying.

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Then, the bodies are cleared of excess material within the drive screw and drive axis holes with a size #71 and/or #64 drill bit.

Holes intended for 00-90 sized screw threads are initially cleared out using first a size 71 drill bit and then a size 65 bit; the latter is the tap-hole drill bit for the 00-90 threads. This process is done to ensure that no excess material is left in the holes and so that clear threads are made in the plastic during tapping. Next, a 00-90 tap is used to thread the EIB column and corner screw holes on the drive position array. The corner holes are intended for a custom stereotactic holder that is used during microdrive placement (see Fig. 7.3). Spring-shuttle holes are threaded with a 000-120 tap. Every hole should be tested for working unstripped threads with screws before they are committed to functional use.

5.1.2 Springs

5.1.2.1 Spring Cutting

Springs are cut from .002" blue spring steel shim stock (McMaster-Carr, Cleveland, OH) using a water jet cutting process on an Omax JetMachining abrasive waterjet (Omax, Kent, WA). The assembly design was made in AutoCAD as a two-dimensional outline; design was exported as a Drawing Exchange Format (.dxf) file. The kerf of the water jet cut is approximately 0.020". This had to be taken into account in the design of the spring body. Springs are thinly coated with pure white beeswax to prevent corrosion from exposure to the water jet cutting process; uncoated pieces of steel may become rusted after cutting. Pieces of steel are secured with masking tape to an aluminum backing plate during cutting. The water jet runs at a setting appropriate for cutting tool steel.

5.1.2.2 Spring Mounting and Preparation

The following steps are for mounting the springs into the drive position array and forming the bridge on the spring heads used to center the shuttle tubes. A summary photo is shown in Figure 5.2. These steps require the application of epoxy, which takes approximately 24 hours to fully cure. As such, these steps take the most time in the building process. During this waiting period, the wire bundles and drive axis structure can be constructed.

Individual springs are cut out from an assembly and then mounted into the wedge of the
Figure 5.2: **Spring Mounting.** Springs are mounted onto individual drive positions.  

**a.** A photo of the spring assembly, microdrive body, and 1/4" 000-120 screws. **b.** Screws are secured into the drive positions and individual springs are mounted in front of the drive position in the wedge. **c.** The spring is bent and the spring head is hooked at the neck of the screw, directly below the screw head. **d.** Epoxy is applied in the wedge to fix the bottom of the spring. Some epoxy is also applied on the back of the wedge at the lip. **e.** Small drops of epoxy are applied to the arms of the spring heads in preparation for mounting polyimide tubes. **f.** Small polyimide tubes are mounted onto the spring heads to form a bridge used to center the shuttle tubes.

The microdrive body near the drive position for which they are intended. The spring necks are bent and the spring heads are hooked to pre-loaded drive screw. The springs are centered, secured with a small amount of Loctite heavy duty epoxy (Henkel Corp., Westlake, OH) onto the bottom of the spring and within the gap of the wedge and left to fully cure.

To form the bridge used to center the shuttle tubes, as previously described in Figure 4.6, a small piece of 8.9 mil polyimide tubing is cut and epoxied to both arms of the spring head. The tube should be long enough to span the gap between the two arms, but not large enough to
Figure 5.3: **Fabrication of electrode bundles.** a. Electrode bundles (n = 8 microwires) are prepared using Neuralynx Tetrode Assembly Station. b. Two lengths of 18 µm insulated NiChrome wire are unspooled to approximately 50 cm and the right ends are taped down using Scotch tape. c. The left ends of the wires are folded over to meet the right ends and are taped down. This loop has four electrode leads. d. The apex of the loop is folded over to meet the four wire ends at the right and then are taped down. This loop has eight electrode leads. e. The loop of the microwire bundle is threaded onto a notch in the horizontal bar of the tetrode assembly station. The bottom microwire ends are clamped with an alligator clip. The bundle is pulled taut and the alligator clip is rotated 70 turns forward and 35 turns backward. This tightly twists the microwires together, which provides mechanical rigidity to the bundle. f. The insulation on the microwires is fused together with heat. A heat gun is held several centimeters away from the bundle and low-setting heat is applied along the length of the bundle, below the loop notch, for approximately 5 seconds on three different sides of the bundle.

interfere with driving of other springs.

### 5.1.3 Microwire Electrode Bundle Construction

Microwire bundles are constructed using 18 µm (bare) diameter formvar-insulated nichrome wire (A-M Systems, Sequim, WA). Standard protocols for tetrode fabrication were referenced for the construction of both tetrodes (n = 4) and multiwire (n = 6, n = 8) bundles [46] [47] [48]. Shown in Figure 5.3 are summary photos of an example bundle construction. In brief: lengths of 50 cm
wire are unspooled and laid straight in groups of one or two with one end taped down with Scotch tape to the work surface of a Neuralynx Tetrode Assembly Station (Neuralynx, Bozeman, MT). The opposite (top) ends of the wires are then folded over, together, to meet the tape and are subsequently taped down with another piece of tape. The folding process is repeated once more to form a group of four or eight wires. In the case of six wires, one wire of full length is folded once, taped, and a second 25 cm strand is added with one end taped so that upon folding the top end there will be six wire segments in total.

After the wire group is made, the pieces of tape are lifted from the work surface and the top wire loop is fed over the groove of the assembly station’s horizontal rod. The tape is then cut, loosening the grouping of the wires. The two ends of the wire groups are then secured with a soft-coated alligator clip that has a magnetic base to attach to the rotating platform on the station. If the wire groups are difficult to evenly bring together, a small amount of water on the tip of a finger can be run down the length of the wires to bring the group close together before clipping. Wires are then twisted together for 70 turns forward and 35 turns backward. Insulation is then melted slightly using a heat gun aimed roughly 5 cm from the wires. The heat gun is set on a low setting and should always be aimed below the notch of the loop. Heat is applied to the bundle length for approximately 5 seconds on multiple sides. Finally, the clip is removed, the crushed ends are cut off, and the completed microwire bundle is stored in a clean and secure box.

5.1.4 Drive Bundle Construction, Parameter Measurement, and Loading into the Microdrive

5.1.4.1 Construction

There are four main pieces that make up individual drive bundle implants: the electrode wire bundle, the shuttle tube, the body tube, and the placement tube. The overall structure is a telescoping tube-in-tube design that allows a mobile shuttle tube to carry electrodes along the drive axis. The drive bundle has already been shown in Chapter Four (Fig. 4.5). This section provides instructions on how to measure, make, and load each drive bundle. The diameters (1 mil = .001") and lengths of these tubing elements, as well all other tubes used in the microdrive system, are included in Table 5.1. Note: each implant must be made in full prior to loading the complete implant onto the microdrive body. For reference, the term “loading” refers to: fixing
Figure 5.4: **Drive axis construction.** The three tubes that make up the flexible drive axis are the shuttle tube (ST), body tube (BT), and placement tube (PT). 1. The ST and PT are made from 6.3 mil diameter polyimide tubing and are cut to lengths of 6 – 10 mm and 11 – 12 mm, respectively. The BT is cut to a length of 17 – 18 mm. 2. The bottom of the BT is marked with black permanent marker. After drying, the PT is inserted into the BT by several millimeters and is fixed into place with cyanoacrylate. The exposed PT length is measured under a microscope and is R. 3. The ST is placed onto the electrode bundle somewhere below the loop notch. Next, the electrode bundle is threaded through the BT-PT composite structure. The bottom of the ST tube feeds several millimeters into the top of the BT. Electrodes are cut with a razor blade near the bottom of the PT. After a clean cut, the ST, BT-PT, are moved down, together, to cover the ends of the electrodes. Then cyanoacrylate is applied at the top of the ST to secure the electrode bundle in place. 4. The drive axis is dipped into heated liquid petroleum jelly to space-fill part of the PT.
the shuttle tube to a spring head, rigidly attaching the top of the body tube to a drive axis hole, and riveting individual electrode microwires to the EIB.

<table>
<thead>
<tr>
<th>Element Name</th>
<th>Tube Diameter (mil)</th>
<th>Tube Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shuttle Tube</td>
<td>6.3</td>
<td>6 – 10</td>
</tr>
<tr>
<td>Body Tube</td>
<td>8.9</td>
<td>17 – 18</td>
</tr>
<tr>
<td>Placement Tube</td>
<td>6.3</td>
<td>11 – 12</td>
</tr>
<tr>
<td>Guide Cannula</td>
<td>8.9</td>
<td>5 – 5.5</td>
</tr>
<tr>
<td>Stopper (S.T Unit)</td>
<td>8.9</td>
<td>2 – 3</td>
</tr>
<tr>
<td>Spacer (S.T Unit)</td>
<td>6.3</td>
<td>2 – 3</td>
</tr>
</tbody>
</table>

Table 5.1: **Microdrive system tubing.** Tube names and associated diameters and lengths that are used in the microdrive system.

Outlined in Figure 5.4 are the steps for constructing an implant prepped for a \( n = 8 \) wire electrode bundle. Slight modifications, namely the addition of a 4.5 mil tube inside the placement tube, must be made for \( n = 6 \) and \( n = 4 \) electrode bundles because they are smaller. First, a shuttle tube, body tube, and placement tube are cut using fine curved surgical scissors (Fine Science Tools, Foster City, CA) from polyimide tubing stock (A-M Systems, Sequim, WA). One end of the body tube is marked with black permanent marker and allowed to dry for several minutes. This is a visual queue to identify when the body tube is seated on the top of the guide cannula with the bottom of the placement tube at the correct ventral depth.

Next, on a clean surface and under a 4x stereo microscope, approximately 3 – 4 mm of the placement tube is fed into the body tube. Using a 30 gauge needle, an extremely small amount of Loctite thin liquid cyanoacrylate (Henkel Corp., Westlake, OH) is applied at the joint of the two tubes and the structure is allowed several minutes to dry. The new body-placement tube composite is then measured prior to proceeding to the next step (see 5.1.5.1 for details). At this point, all the pieces are prepared and can be put together to make the complete implant.

With all the pieces ready, the electrode bundle is threaded through the shuttle tube. The shuttle tube sits a few millimeters below the notch of the bundle loop at the top, but with enough slack to ensure threading across the underside of the EIB. It is important to move the tubes slowly over the wire bundles to prevent bending or kinking of the wires. Next, the tube structure is carefully slid over the wires next until the top of it meets the bottom of the shuttle tube. Approximately 1 – 5 mm (depending on the initial length of the shuttle tube) of the shuttle tube is fed into the body tube to leave only 5 mm exposed. This length spans the fixed point on the bridge of the spring head and the top of the body tube.
With the tubes on and mostly in place, the microwire bundle is cut with a fresh stainless steel razor blade on a hard glass microscope slide. The cut should be made as close to the bottom of the placement tube as possible, such that the tubes do not need to be slid down much afterward. If the cut is not clean (most of the time this is due to flattening of the wires or the tips are not well exposed), all the tubes can be moved up a little to prepare room for another cut. Following a good cut, the shuttle tube and body-placement tubes are moved down together to cover the ends of the wires. The wires should sit approximately 100 – 200 \( \mu \)m from the bottom of the placement tube. Next, a very small amount of thin liquid cyanoacrylate is applied at the top of the shuttle tube to glue it with the wire bundle. It is critical not to apply a excess amounts of glue because the shuttle tube or wires must not adhere to the body tube.

Once the glue is dry, the construct is carefully picked up and the complete length of the exposed placement tube is dipped into 10 ml of liquid petroleum jelly (Vaseline) heated to approximately 100 \( ^\circ \)C. The petroleum jelly space-fills the inside of the placement tube to prevent proteins and other substances from infiltrating and binding the electrode bundles to the inside walls of the polyimide tube. Petroleum jelly is a common material used to fill craniotomies in microdrive systems [8] [12] [34] [49].

### 5.1.5 Comments Concerning Tube Lengths

The lengths of each tube element are specific both to the geometry of the microdrive and the experiments for which the implant(s) are to be used.

The shuttle tube must extend the length from the spring head down into the top of the body tube and be secure in the body tube by at least 1 mm; 5 – 10 mm is an appropriate length to provide enough driving distance (approximately 3 – 3.5 mm) without risking the shuttle tube coming out of the body tube. The shuttle tube can extend several millimeters into the body tube, so long as it does not collide with the placement tube while driving.

For experiments to monitor the sleep-wake regulatory system (Fig. 3.1), the body-placement tube structure was a total length of approximately 25 mm when glued together. This is due to several factors that include: the height of the microdrive when it is secured onto the headmount base, the very deep brain nuclei that were being targeted, and the need for slack and maneuverability of the body tube during implantation. If the body-placement tube composite is too short, the implant depth will not be set correctly; if it is too long, the risk of the body tube
kinking during implantation increases significantly. Adjustments from the 25 mm length should be straightforward to test for different experimental designs.

5.1.5.1 Tube Measurement

As mentioned in the previous subsection, the prepared body-placement tube construct must be measured prior to creating the full implant. Specifically, the length of the exposed placement tube must be known. This measure, also known as R, (Fig. 4.3) is essential for the depth of the implant to be set correctly during surgery and microdrive placement (Eq. 4.1). As an aside, while the measurement can be made after constructing the implant with wire bundles in place, it is much easier and safer to work with just the tube structure.

To make the R measurement, an individual tube structure is placed onto a glass microscope slide under a microscope capable of making line-length measurements with high spatial resolution ($\pm 10 \mu\text{m}$) and measure the length from the bottom of the placement tube to the bottom marked end of the body tube. We used an Olympus Confocal Microscope (Olympus Corporation) at 4x magnification with Stereo Investigator software (MBF Bioscience). After making the measurement, the R-value is recorded and the full implant can now be constructed.

5.1.5.2 Loading

To complete the following loading and wiring steps, the microdrive body is secured onto a custom microdrive holder that makes manipulation easier. The holder, which includes a headmount base to attached the microdrive, is shown in Figure 5.5. The holder is made from a medical urine sample cup that is cut approximately in half, sanded at the bottom to be level, and that has two 8-32 screw holes in its lid. The holder has several important features: a center hole in the lid that allows the long implant constructs to extend below the drive bottom in a protected area; it provides the user six degrees of freedom when handling the microdrive and trying to load implant tubes or rivet microwires; the headmount base can be unfastened from the two screws which hold it down for easy and safe handling.

The constructed implant tube is loaded into its designated microdrive position by hand under a stereo microscope. Caution should be taken to ensure no electrode wires are protruding out of the placement tube before feeding the structure through the drive axis hole on the microdrive. The implant is positioned to where the top of the shuttle tube meets the polyimide bridge of the
Figure 5.5: **Microdrive holder.** A custom holder used to secure the microdrive and protect the drive axes during handling. The holder is made from a modified medical sample cup. Two 8-32 screws hold down a headmount base to which the microdrive is secured. The cap has a hole cut out in the center to allow the drive axes to extend below the drive bottom in a protected area.

spring head. A small amount of medium liquid cyanoacrylate is applied onto the polyimide bridge to secure the shuttle tube at its center. Next, thin liquid cyanoacrylate is applied to the drive axis holes to create a fixed point between the body tube and the microdrive. Extra caution should taken to ensure no glue creeps inside the body tube, otherwise the shuttle tube will adhere to the body tube and the system will not drive. After all the cyanoacrylate dries, very small amounts of epoxy are added at the notch where the shuttle tube and polyimide bar meet for extra positional security.

### 5.1.6 Wiring to Electrode Interface Board

The final step to loading the microdrive is to make electrical connections between the microwires and the electrode interface board. The overall objective is to thread single microwires through the bottom-side of individual vias on the EIB. First, the EIB is secured onto the top of the microdrive body using 00-90 torx screws. Placing a spacer between the tops of the screw columns and the EIB board is helpful for providing extra room to maneuver during the riveting process. We use
Figure 5.6: **Completed microdrive construction.** Photos of a fully constructed microdrive with three drive axes.

an array of two 25-mil pitch male-male MillMax (Mill-Max Manufacturing Corp, Oyster Bay, NY) connector pins, which fit nicely around the diameter of the 00-90 screw shaft.

The next step should be done under a stereo microscope to better see the microwires. Using fine scissors, the loop of the electrode bundle is cut at its apex. Next, individual microwires are threaded into vias one at a time. Individual wires can be separated from the others in the bundle with surgical forceps or by gently arcing groups of wires. Using the six degrees of freedom allotted by holding the custom holder, the microwires are fed across the bottom of the EIB and thread through open vias. If the bundle position is on the right-side, its wires should be threaded to a via on the left-side of the EIB. This configuration protects the microwires.

Once the wires are in position, electrical connections are made to the EIB by riveting the wires using small gold EIB rivets (Neuralynx, Bozeman, MT). We use a custom riveting tool for this process. The tool is a modified pair of forceps that has one of its tips longer than the other. The custom riveting tool allows for the application of pressure to the top of the rivets while the bottom jaw applies upward pressure to the bottom of the EIB. After every microwire is riveted, the microdrive is ready for electrodeposition of gold onto the tips of the electrodes. This process is detailed in the following section. After electrodeposition, a small amount of epoxy is applied to the bases of the gold pins to secure them to the EIB. A completely loaded and riveted microdrive is shown in Figure 5.6.
5.2 Microwire Electrodeposition

The final process before implantation is electrochemical deposition (electrodeposition) of gold onto the nichrome microwires. The purpose of this process is to reduce the impedance of the electrodes from > 1 MΩ to values of approximately 250 kΩ. Lower impedance electrodes can improve the quality of electrophysiological recordings by decreasing background noise and increasing signal-to-noise ratios. The general methodologies which follow are derived from the tetrode literature [46] [47] [48]. The basic electrodeposition steps are to iteratively deposit gold onto microwires with short durations of constant deposition current and test impedance until the desired values are reached. Careful handling of the microdrive structure is essential during this processing as the tips of the microwire bundles will be exposed for a lengthy period of time.

Details of the electrodeposition procedure are as follows:

1. The tips of microwire bundles are exposed approximately three turns in preparation for cleaning and electrodeposition. Bundles should be cleaned in a 30 ml ultrasonic bath of acetone for approximately 30 seconds. The bundles are dipped in and out from the bath several times during the 30 seconds. Acetone is an effective solvent for petroleum jelly. Several millimeters of the the placement tube is also submerged into the acetone to clean the tube and to create a small petroleum jelly-free pocket at the end of the tube. The pocket ensures that deposited electrodes do not get covered in petroleum jelly upon final retraction prior to implantation.

Rinse the microwires and tubes with de-ionized (DI) water in preparation for the next step.

2. A 30 ml medicine cup is filled with non-cyanide gold solution (Sifco ASC) and gold is deposited onto the electrodes using software and hardware of your choice. The remaining steps are with regards to the in-house process that we use.

Microwires are connected as working electrodes in a three-electrode configuration with platinum reference and counter electrodes inside the gold solution. A custom galvanostat/-potentiostat circuit board and Labview (National Instruments, Austin, TX) script is used for both electrodeposition and impedance testing. To achieve electrical connections between channels on the circuit board and the microwires, a custom EIB access board was fabricated using Autodesk Eagle (Autodesk, San Rafael, CA) software. The access board serves as an
interface between the Millmax connectors of the electrode processing board and the male omnetics connector on the EIB. A full set up of the deposition process is shown in Figure 5.7.

Figure 5.7: **Electrodeposition setup.** A photo of the three-electrode galvanostat set up used during electrodeposition and impedance testing. Platinum-deposited silicon wafers are used as counter and reference electrodes. The microwires are the working electrodes. All three electrodes are placed inside gold solution. Electrical connections between the microwires and the galvanostat circuit are made through a custom access circuit board.

3. The deposition step is initially run at a current of $-2 \mu A$ for 30 seconds. For the 18$\mu$m diameter wires, this corresponds to a current density of $-7.9 \text{mA mm}^{-1}$ through each wire. Impedance of active electrodes is tested with the same reference and counter electrodes inside the gold solution. Connected and deposited electrodes should show impedance of approximately 1 MΩ or less at a test frequency of 1000 Hz with several nA of applied current. Any channels that are disconnected or did not deposit correctly will show impedance on the order of 10 MΩ.

4. Deactivated channels are disconnected for the next deposition increment. The deposition is run again for 20 seconds with the same current parameter and then impedance of all active channels is measured. Typically, the impedances should be close to the desired range by this point. Electrodes that show significantly lower impedances than other channels should be temporarily deactivated upon the next deposition increment. This will prevent dendritic outgrowth of gold on any single low-impedance electrode. These channels can be reactivated once other electrodes have comparable impedance. If the impedance of any channel falls significantly below the 250 kΩ target, apply $2.0 \mu A$ for approximately 5 – 10
seconds to remove some of the deposited gold and increase the electrode impedance.

5. Deposition and impedance testing steps should be repeated until the electrodes show impedances of approximately 250 - 300 kΩ. If any bad electrode processing channels are suspected, try switching the positions of the microwires to known working channels of the deposition system and perform this process with just those channels active.

6. After all desired electrodes are successfully deposited, the electrode bundles are rinsed in DI water, then ethanol. Finally, the wires are retracted back into the placement tube in preparation for implant.

5.3 Conclusions

This chapter included step-by-step instructions for building our microdrive design. The typical time it takes to build one device, from start to finish, is about two days. Many of the steps, such as microdrive body preparation, electrode bundle construction, and drive axis construction, can be done in bulk to have stock for several microdrives. This can reduce the overall build time and effort.

In the following two chapters, the experimental implementation of our microdrive design is presented. Chapter Six includes acute experiments used to validate the flexible drive axis design and its implant methodology. Chapter Seven provides results from chronic microdrive implants in freely-behaving animals.
Chapter 6

Acute Placement Experiments: Validating the Drive Bundle Implantation Technique

In the previous chapters, our multi-site, multi-region microdrive was introduced and its construction detailed. These next two chapters discuss experiments, from methodology to results, that help to validate the microdrive design. This chapter, in particular, is dedicated to an acute surgical experiment that demonstrates the implementation and accuracy of the drive axis system.

As discussed in Chapter Four, the surgical method developed for the drive axis system sets the angle and implant depth for each drive axis. A fundamental advantage of the method is that everything is performed with a standard stereotactic system and standard stereotactic surgical techniques. To demonstrate the method, several distinct brain nuclei that are visually recognizable in a stained histological slice were targeted. Targets groups were chosen within the same anterior-posterior plane, but with dramatically different implant angles. The experiment was designed such that the surgical steps used and results would closely resemble those of a chronic microdrive experiment. The one difference was that individual single-electrode implant bundles were used instead of the full microdrive axis structure. The mock drive axes were direct analogs to the microdrive bundles.
6.1 Experimental Design

6.1.1 Implant Structure

Figure 6.1: **Single-electrode implant bundle.** Drive axis implants for the acute experiments were designed using the same basic features as the microdrive implants. A single 50 µm wire was fixed inside a telescoping structure made of 4.5 mil, 6.3 mil, and 8.9 mil diameter polyimide tubes. The wire extended approximately 1 – 1.5 mm past the bottom of the 4.5 mil spacer. The R length was the distance between the bottom of the 8.9 mil body tube and the tip of the wire.

Shown in Figure 6.1 is the implant design that was used in the acute experiments. The main difference was the use of a single 50 µm gold-coated stainless steel wire instead of a twisted microwire bundle and a 4.5 mil diameter spacer tube inside the placement tube. The wire tip extended approximately 1 – 1.5 mm past the bottom of a 4.5 mil tube. This extension distance was designed to make the electrode tract more distinct from the end of the placement tube in the histology. The 4.5 mil tube was used to concentrically-align the wire with the placement tube.

For the microdrive implants, a unique R-length is measured for each drive axis. For these acute experiments, the R-length was made to be approximately the same for each implant. This was done using an 8.9 mil polyimide tube “standard” that was cut and measured to be 9.27 mm.
The standard was placed on each implant and it extended the length between the bottom of the body tube and the tip of the electrode wires. When the end of the standard was flush with the body tube and electrode tip, the body tube was fixed into place. The R-lengths of each set of implants was subsequently measured prior to each experiment. For the experimental results shown in section 6.3 of this chapter, R values were 9.25 mm ± 0.033 mm for the first experimental results and 9.26 mm ± 0.036 mm for the second. The uncertainty in length is a sum of the measured uncertainty in R as well as the ± 0.01 mm uncertainty in our stereotax system. Note that additional positional uncertainty is inherent in stereotactic surgery where external fiducial marks are used to identify relative positions with respect to internal brain targets according to a brain atlas. There is also inherent variability of brain targets between animals.

6.1.2 Target Selection

Figure 6.2: **Acute experiment targets.** The three targets used for the acute experiments are outlined in red. Targets were chosen for their extreme lateral or midline locations and for their recognizability under standard Nissl staining methods. Left: ectorhinal cortex (Ect); upper midline: dorsomedial periaqueductal gray nucleus (DMPAG); lower midline: pre-Edinger-Westphal nucleus (PrEW). Adapted from Figure 80 of Paxinos and Watson, 6th Ed. [45].

The targets for this experiment were chosen for their recognizability and distinct anatomical locations. The three targets used were: ectorhinal cortex (Ect), the pre-Edinger-Westphal nucleus (PrEW), and dorsomedial periaqueductal gray nucleus (DMPAG). Shown in Figure 6.2 is the
marked-up atlas section (Interaural = 3.36 mm), adapted from Paxinos and Watson, 6th Ed. [45], from which the targets and their coordinates were chosen. Ect is recognizable because of its location directly above the rhinal fissue, which can be easily seen on histological sections along a large portion of the rostral-caudal axis. DMPAG and PrEW were selected because they both are on midline (medial-lateral coordinate = 0), and are directly above and below the cerebral aqueduct, respectively. These structures also appear distinctly using a standard Nissl stain.

Figure 6.3: Acute experiment target trajectories. Structures were targeted with radically different trajectory angles to demonstrate the accuracy of the drive axis implant system. Angles were calculated using the ML and DV coordinates of each target and a hypotenuse made from the target point to the desired entry points on the cranium. Adapted from Figure 80 of Paxinos and Watson, 6th Ed. [45].

This experiment was designed to convincingly demonstrate our drive axis implant methodology. As such, we chose to target the listed structures from radically different trajectories. Outlined in Figure 6.3 are the intended implant trajectories for each nucleus. The Δ distance, which represents the to-target driving distance for electrodes in equation 4.1, was chosen to be zero. Thus, the electrode tips were intended to end directly in the target nuclei. Angle calculations were performed with basic trigonometry using DV and ML lengths corresponding to the drawn hypotenuses. Table 6.1 lists each target and trajectory angle with associated interaural coordinates that were used in the experiment.

The method was initially designed to accommodate targeting deep brainstem structures. The
<table>
<thead>
<tr>
<th>Targets/Coordinates (mm)</th>
<th>AP (IA)</th>
<th>ML</th>
<th>DV (IA)</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ect</td>
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<td>-7</td>
<td>3.6</td>
<td>29.35°</td>
</tr>
<tr>
<td>DMPAG</td>
<td>3.36</td>
<td>0</td>
<td>4.8</td>
<td>16.08°</td>
</tr>
<tr>
<td>PrEW</td>
<td>3.36</td>
<td>0</td>
<td>3.4</td>
<td>31.22°</td>
</tr>
</tbody>
</table>

Table 6.1: **Acute experiment target coordinates.** AP, ML, and DV coordinates, with respect to the interaural (IA) reference, and the associated trajectory angles for each target.

Overall system is robust enough, however, to allow for targeting of more shallow structures. The critical design criteria is to implant the guide cannula such that its bottom ends past the dura mater and the top is above the cranium surface for fixation. For our acute experiments, the guide cannulas were approximately 5 - 6 mm in length. The target coordinates for DMPAG (DV = 4.8 mm) are over 1.0 mm more shallow than either Ect or PrEW in the acute experimental design. Recall from equation 4.1 that the two parameters $R$ and $\alpha$ are used to determine the distance-to-target parameter $\zeta$ of the tungsten stylus. For DMPAG, with the particular constant $R$ value used across targets, $\zeta$ had to be smaller than for the deeper targets. Thus, $\alpha$ had to increase in order to achieve a smaller $\zeta$ distance. Tables of targets, R-lengths, and $\alpha$ value used will be shown alongside specific histological results. The $R$, $\alpha$, and $\Delta$ parameters could all be adjusted in tandem to accommodate targeting both very superficial cortical structures and extremely ventral structures.

### 6.2 Acute Surgery: Procedure and Protocol

All animal work was approved and performed under the oversight of the Pennsylvania State University Institutional Animal Care and Use Committee. Specific details regarding surgical steps are outlined in Appendix-B. Steps that are specific to the acute surgery procedures are listed in this chapter.

Animals (300 - 375 grams) were put under anesthesia, prepped, and placed securely and level within the earbars. Following an incision to expose the cranium, the skull was cleaned and craniotomies for the targets were drilled in accordance to Table 6.2. Next, tracts of india ink were made at (0 ML, 0.5 AP, 0 DV) and $\pm$ 21.8° to help level the brain during later sectioning. Finally, the guide cannula implantation, which is explained in the following subsection, was performed.
Figure 6.4: **Guide cannula implantation method.** The steps in this method are taken to calculate the vector between an external fiducial mark $X_F$ and the target $X_T$ with respect to an interaural reference origin (IA). 1. The stereotax unit (S.T unit) is moved to an external mark, which has known IA coordinates, on the earbars. Then the S.T unit is moved to an IA-referenced position on the surface of the cranium. 2. The S.T unit is moved to $X_F$ and the vector $X_F$ - IA is calculated. 2a. If the target trajectory is at an angle $\Theta$, the stereotax is realigned at $X_F$ by $\Theta$. 3. $X_T$ - $X_F$ is calculated as the to-target vector from $X_F$, the S.T unit is moved to the target craniotomy and lowered ventrally until it is at a calculated distance $\zeta$ from the target. The guide cannula is then fixed to the skull and the S.T unit is removed.
Table 6.2: **Acute experiment craniotomy coordinates**. AP, ML, and DV coordinates, with respect to the interaural (IA) reference, of craniotomies for each target and the india ink tracks. The symbol * indicates no coordinate; the depth of the craniotomies depends on individual variations of the skull at their locations.

<table>
<thead>
<tr>
<th>Targets/Coordinates (mm)</th>
<th>AP (IA)</th>
<th>ML</th>
<th>DV (IA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ect</td>
<td>3.36</td>
<td>-3.4</td>
<td>*</td>
</tr>
<tr>
<td>DMPAG</td>
<td>3.36</td>
<td>-1.5</td>
<td>*</td>
</tr>
<tr>
<td>PrEW</td>
<td>3.36</td>
<td>4</td>
<td>*</td>
</tr>
<tr>
<td>India Ink</td>
<td>0.5</td>
<td>4</td>
<td>*</td>
</tr>
<tr>
<td>India Ink</td>
<td>0.5</td>
<td>-4</td>
<td>*</td>
</tr>
</tbody>
</table>

### 6.2.1 Guide Cannula Implantation

Shown in figure 6.4 is an outline of the stereotactic-targeting steps for implanting the drive axis guide cannula. Recall from the discussion in chapter 4 and Figure 4.2 that the surgical method uses atlas-referenced coordinates to find the to-target vector distance between a user-defined external fiducial mark somewhere on the cranium and the target in brain. To get the vector $\mathbf{X}_T - \mathbf{X}_F$, both the target and external fiducial mark must be known in reference to an atlas-defined reference. In our case, the reference is formed by the virtual intersection of the animal’s midline suture and the interaural line. We use a small black-ink dot placed on the cranium as an external fiducial mark.

With the overall objective in mind, and following along with 6.4, the surgical steps for implanting the guide cannula are detailed in the list below. We used an Angle One stereotactic instrument (MyNeuroLab.com) for tracking our stereotax position. Procedures specific to this stereotax system are omitted for a better-generalized overview of the surgical technique. For our exact steps using the Angle One, please see the excel document A.1 in Appendix A.

1. The tip of the stereotax unit (S.T unit) (see Fig. 4.3 for details on stereotax unit) is moved to the interaural reference mark on the earbars. This mark should have a known DV coordinate with respect to IA. In our case, the reference is a cross that is AP-aligned to the tip of the earbars with a half-width of 3.05 mm. Thus, the cross is at an IA-referenced (AP, ML, DV) coordinate of $(0, *, 3.05)$, where * indicates an arbitrary value.

   Next, the S.T unit is moved to a known anatomical reference position (the green dot) on the animal’s cranium. We used a point along the midline suture coplanar with the interaural line. The cranium reference will have net-IA coordinates of $(0, 0, 3.05 + \delta)$, where $\delta$ is the
2. The anatomical reference is used to determine IA-referenced coordinates of an external fiducial mark that the surgeon makes on the cranium. From the green dot, the S.T unit is moved to the center of the external mark. This provides the net-IA coordinates of the fiducial mark. Determining $X_F$ - IA only needs to be done once for the specific stereotax unit being used.

If necessary, the stereotax bases are realigned to the desired trajectory angle with the tip of the S.T unit centered on the external fiducial mark.

3. The vector $X_T - X_F$ is calculated by subtracting the IA coordinates of the target with those of the stereotax unit at the external fiducial mark. From here, the S.T unit is moved to the craniotomy and is implanted along the aligned target trajectory. The absolute to-target stopping distance, $\zeta$, is calculated from equation 4.1.

4. When the tip of the S.T unit is at a distance $\zeta$ from the target, the guide cannula is fixed to the skull with dental cement. The adhesive must be dry before removing the stereotax stylus from the guide cannula. Finally, the drive axis implant is slowly inserted into the guide cannula until the body tube is seated flush with the top of the guide cannula.

### 6.2.2 Surgery Conclusion

After the implants were hand-placed into the skull-fixed guide cannulas, a protective cap, made from a modified paper wax cup, was fixed to the animal’s heads with dental cement. This cap protected the implants from being moved or damaged during perfusion.

At the end of the surgery, the animal was deeply anesthetized and transcardially perfused sequentially with (a) physiological saline, (b) 4% sucrose paraformaldehyde solution, and (c) 10% sucrose paraformaldehyde solution. Animal was decapitated and the head was placed in a 30% sucrose paraformaldehyde solution for approximately 24 hours to further fixate the tissue. Afterwards, the brain was extracted from the bottom of the cranium. This was to ensure that the implants stayed in position as the brain was removed. Prior to histological sectioning, the brain was further cryoprotected by immersion in 30% sucrose paraformaldehyde solution until it sank.
6.3 Histology

Histological sections were made following fixation and cryoprotection of the brains. A microtome was used to cut 60µm slices of brain. Slicing began caudal to the ink tracts and the brain was leveled to get both tracts in a single slice. Sections were placed into cell wells filled with phosphate buffer solution. Sections were mounted onto glass slides and dehydrated for approximately 24 hours. The sections were then Nissl-stained and cover-slipped for imaging.

Targets and targeting parameters from two different experiments are shown in Tables 6.3 and 6.4. Associated histology for these experiments are seen in Figures 6.5 and 6.6.

Accurate targeting of Ect and PrEW structures from the first experiment is shown in Figure 6.5. Each section contains the lowest observed points of the electrode tracts. The ends of the electrode tracts were compared to the targets as seen in atlas sections. In this experiment, DMPAG could not be targeted because dental cement got into the craniotomy and underneath the guide tube during fixation. The reason for this is that the $\alpha$ parameter was not large enough to adjust for the shallow target. As a result, the guide tube did not go into brain at all. This problem was fixed in the following experiment.

<table>
<thead>
<tr>
<th>Targets/ Targeting Parameters</th>
<th>$R$</th>
<th>$\alpha$</th>
<th>$\zeta$</th>
</tr>
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<tbody>
<tr>
<td>Ect</td>
<td>9.25</td>
<td>5.52</td>
<td>3.73</td>
</tr>
<tr>
<td>DMPAG</td>
<td>9.25</td>
<td>5.52</td>
<td>3.73</td>
</tr>
<tr>
<td>PrEW</td>
<td>9.25</td>
<td>7.44</td>
<td>1.81</td>
</tr>
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</table>

Table 6.3: Animal one: acute experiment parameters. The measured $R$ and $\alpha$ lengths used for the first acute experiment animal. The $\Delta$ distance was set to zero. The to-target stopping distance $\zeta$ was calculated from Equation 4.1.

<table>
<thead>
<tr>
<th>Targets/ Targeting Parameters</th>
<th>$R$</th>
<th>$\alpha$</th>
<th>$\zeta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ect</td>
<td>9.26</td>
<td>7.02</td>
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<tr>
<td>DMPAG</td>
<td>9.26</td>
<td>8.28</td>
<td>0.98</td>
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<tr>
<td>PrEW</td>
<td>9.26</td>
<td>7.02</td>
<td>2.24</td>
</tr>
</tbody>
</table>

Table 6.4: Animal two: acute experiment parameters. The measured $R$ and $\alpha$ lengths used for the second acute experiment animal. The $\Delta$ distance was set to zero. The to-target stopping distance $\zeta$ was calculated from Equation 4.1.

Placement of implants into DMPAG and PrEW targets from the second experiment is shown in Figure 6.6. The electrode tracts end immediately above and below the cerebral aqueduct. The PrEW implant is directly within the small target structure, too. Unfortunately, Ect did not end
Figure 6.5: **Animal one: acute experiment results.** Histological results from the first acute experiment animal. The most ventral parts of the located Ect and PrEW electrode tracts are circled in red. The intended targets are outlined in black on histological plate 82 of Paxinos and Watson, 6th ed [45]. The DMPAG targeting failed in this experiment because the α length was too small, which caused the guide cannula to not be implanted properly.

in the correct location. There are several possible explanations for this. The first is that the lateral ventricles were somehow filled during the perfusion process. The brain for this animal took over a week to sink; the normal time for sinking is three days. As such, the expansion of the ventricle could have pushed the implant and tissue away from an accurate trajectory, since the majority of the implant normally is right above the ventricle. Another possible reason is a surgical error in angling or placement.

Compared to the histological sections taken from Paxinos and Watson, which were ideal samples taken from intact brain, there should be some expected differences among certain structures, such as the ventricles.
Figure 6.6: **Animal two: acute experiment results.** Histological results from the second acute experiment animal. The most ventral parts of the located DMPAG and PrEW electrode tracts are circled in red. The intended targets are outlined in black on histological plate 82 of Paxinos and Watson, 6th ed [45]. The Ect targeting result is not shown in this figure. An enlarged right ventricle can be seen on the slices. The left ventricle was also enlarged in more anterior slices where the Ect tract was located, which may have caused the inaccuracy in targeting that structure.

### 6.4 Conclusions

The results from the two experiments are a strong demonstration that our surgical method is accurate and works across widely different implant trajectories. The placements that worked were accurate to within the uncertainties listed for each implant set. This high-resolution spatial accuracy is critical when targeting small structures, such as PrEW or many of the cell groups in the sleep-wake regulatory network. Note: as with any high-precision targeting, this method susceptible to failing by having any inaccuracies in the stereotactic setup, such as misalignment of the stereotax frame or poor leveling of the animal. Lastly, targets were all chosen to be within
the same AP-plane simply for aesthetic purposes. There are generally no coordinate constraints on targeting, other than those imposed by the stereotax system, or in the case of our microdrive, the boundary formed by the headmount base. This technique can be used with confidence for both microdrive drive axes placement as well as for placement of static implants.
Chapter 7  
Chronic Microdrive Experiments

This chapter includes details related to the chronic experiments that necessitated the development of our multi-site, multi-region microdrive. Unlike the acute placement experiments, the methodology, protocols, and results from these chronic microdrive experiments are much more involved. The ultimate goal of these experiments was to simultaneously record from multiple sleep-wake-related cell groups in freely behaving small rodents. At the time of this writing these experiments are still ongoing.

The microdrive design took many iterations to get to the refined product presented in this work. Even our choice of amplifier had to change after many animal experiments to get reliable and clean-enough data to analyze. Additionally, animal work is often very challenging and unpredictable. Individual animal experiments lasted two to three months on average. As of writing, the end goals of simultaneous recording of wake-active, NREM-active, and REM-active cell populations had not been achieved. Yet, the results that are detailed in this chapter, which include many single-unit recordings from different drive axes, animals, and relevant sleep-wake structures, prove that our microdrive does work for its intended purpose.

The chronic microdrive experiments had three major design components: surgery and implantation, recording and data analysis, and histology. The following section outlines each of these design components in detail and includes protocols, procedures, examples, and results wherever relevant.
7.1 Experimental Design

Figure 7.1: Microdrive experiment targets. Individual drive axes were implanted at multiple sites and regions to target brainstem sleep-wake cell groups. Electrodes targeted the PPT structures bilaterally (interaural = 0.96 mm) and the DR (interaural = 1.20 mm) at an angle. Brain atlas sections were adapted from Figure 100 of Paxinos and Watson, 6th ed. [45].

For the experiments reported here, two sleep-wake regulatory structures were targeted for observation: DR and PPT. Part of our objective was to demonstrate a microdrive that could target greater than two non-collinear brain structures. As such, DR was targeted with a single angled implant and PPT was targeted bilaterally with two implants, as shown in Figure 7.1. Included in Table 7.1 are specific details related to the target coordinates and implant trajectories. The to-target driving distance $\Delta$ for the electrode bundles was set as 1 mm.

<table>
<thead>
<tr>
<th>Targets/Coordinates (mm)</th>
<th>AP (IA)</th>
<th>ML</th>
<th>DV (IA)</th>
<th>Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right PPT</td>
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<td>2</td>
<td>3.6</td>
<td>0°</td>
</tr>
<tr>
<td>Left PPT</td>
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<td>-2</td>
<td>3.6</td>
<td>0°</td>
</tr>
<tr>
<td>Left DR</td>
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<td>3.6</td>
<td>21.33°</td>
</tr>
</tbody>
</table>

Table 7.1: Microdrive experiment target coordinates. AP, ML, and DV coordinates, with respect to the interaural (IA) reference, and the associated trajectory angles for each target.
Screw electrodes were implanted for monitoring cortical activity, as well as for amplifier reference and ground. Pairs of 50 µm wires with a 300 µm tip stagger were implanted in hippocampus to monitor field potentials. These accessory implants were used for better discrimination of sleep-wake behavior in animals [50]. Screws were made by soldering seven-strand Teflon-coated stainless steel wire (A-M Systems, Sequim, WA) to 00-80 stainless steel screws. Depth electrodes were made from gold-coated 316 stainless steel wire (A-M Systems, Sequim, WA) electro-deposited with iridium-oxide. Pairs were made by securing two wires together inside a 4.5 mil diameter polyimide tube with leads exposed by approximately 500 µm. Include in Table 7.2 are the coordinates for each accessory implant. A diagram of the overall surgical implant setup can be seen in Figure 7.2.

<table>
<thead>
<tr>
<th>Targets/Coordinates (mm)</th>
<th>AP (IA)</th>
<th>ML</th>
<th>DV (IA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left-Anterior Screw</td>
<td>11</td>
<td>-3</td>
<td>*</td>
</tr>
<tr>
<td>Right-Anterior Screw</td>
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<td>3</td>
<td>*</td>
</tr>
<tr>
<td>Left-Middle Screw</td>
<td>7.5</td>
<td>-4</td>
<td>*</td>
</tr>
<tr>
<td>Right-Middle Screw</td>
<td>7.5</td>
<td>4</td>
<td>*</td>
</tr>
<tr>
<td>Left-Posterior Screw</td>
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<td>-4</td>
<td>*</td>
</tr>
<tr>
<td>Right-Posterior Screw</td>
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<td>3</td>
<td>*</td>
</tr>
<tr>
<td>Left Depth Electrode Pair</td>
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<td>Right Depth Electrode Pair</td>
<td>5</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Left PPT</td>
<td>0.5</td>
<td>-2</td>
<td>*</td>
</tr>
<tr>
<td>Right PPT</td>
<td>0.5</td>
<td>2</td>
<td>*</td>
</tr>
<tr>
<td>Left DR</td>
<td>1.5</td>
<td>-2.5</td>
<td>*</td>
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</table>

Table 7.2: Chronic implant craniotomies and depth electrode targets. AP and ML coordinates for the implant craniotomies. The symbol * indicates no coordinate; the depth of the craniotomies depends on individual variations of the skull at their locations. The depth electrode pairs also includes the DV coordinate of its target position.

7.2 Survival Surgery: Procedure and Protocol

All animal work was approved and performed under the oversight of the Pennsylvania State University Institutional Animal Care and Use Committee. Specific details regarding surgical steps are outlined in Appendix-B. Steps that are specific to the survival surgery procedures are listed in this chapter.

Animals (300 - 375 grams) were put under anesthesia, prepped, and placed securely and level within the earbars. Once in place, the animals were given a 5 mg kg⁻¹ injection of dexamethasone for long-lasting anti-inflammatory effects. After an incision had been made, the craniotomies for the implants were drilled according to coordinates in Table 7.2. Craniums were then coated with
OptiBond UV-cured dental adhesive (Kerr Corp, Orange, CA) in preparation for later attaching headmount bases. Next, screws and depth electrodes were implanted. Following this, guide cannulas for the flexible drive axes were implanted.

The implantation method of the guide cannulas for individual microdrive bundles was the same as the acute placement experiments (see Section 6.2.1 and Fig. 6.4). Once the guide cannulas were fixed in place with Ortho-Jet dental acrylic (Langdental, Wheeling, IL) to the skull, petroleum jelly-filled polyimide tube plugs were inserted. The plugs were made from 6.3 mil and 8.9 mil tubes in the same fashion as the placement-body tube structure. The 6.3 mil tube extended approximately 6 mm below the 8.9 mil stopper tube, as to just barely pass out of the bottom of the 5 mm guide cannulas. The 8.9 mil stopper tube was approximately 3 mm in length. These plugs helped prevent cerebral spinal fluid (CSF) and/or blood from coming out of the guide cannulas and clogging of the tubes. These plugs could stay in for extended periods of time, upwards of one week, between initial surgery and implantation of the microdrive bundles.

Following placement of the plugs, headmount bases were secured onto the craniums with dental acrylic. Then the headmount caps were screwed on. The animals were assisted in waking up with an 0.1 mg kg$^{-1}$ injection of atipamezole and extubated when they began to breathe on...
their own. Animals were placed in a heated incubator to recover.

### 7.2.1 Microdrive Placement

![Manipulator placement tool](image1)

**Figure 7.3: Manipulator placement tool.**

*a.* A CAD model of the placement tool. The tool is designed to screw into a corner position on the microdrive body. The head of the arm provides contact points on three sides of the microdrive body, which adds stability during manipulation. 

*b.* A photo of a 3D-printed placement tool that has been heated and given a 90° bend at the center of the arm. A 8-32 screw is placed in the through hole at the tool base for attachment to a stereotactic rod.

Microdrive placements consisted of three main parts: insertion of placement tubes into guide cannulas, lowering and securing of microdrive body onto the headmout base, and riveting of accessory electrode leads. We used #5 fine-tip forceps (Fine Science Tools, Foster City, CA) to manipulate the placement cannulas. The microdrive body needed to be stabilized and finely maneuvered to facilitate the placement process. For the procedure, a custom microdrive placement tool, which is seen in Figure 7.3, was 3D printed. On one end the placement tool is screwed into a screw position on the corner of the microdrive bodies. On the other end is a hole that holds an 8-32 screw to allow the placement tool to be secured to a metal rod with a ball-joint pivot that connects to the stereotax. The end product was the ability to move the microdrives with six degrees of freedom at 10 µm AP-ML-DV spatial resolution. A 90° bend was made in the placement tool by heating the thermoplastic to better accommodate our stereotax setup.

An example microdrive placement is shown in Figure 7.4. If this procedure did not happen at the time of surgery, the following steps were taken: following approximately seven days of post-operation care, the animal was inducted under 4% isoflurane and then injected with the
Figure 7.4: Microdrive placement procedure. Photos of steps during the microdrive placement procedure. a. Petroleum-jelly plugs are first removed from the guide cannulas and leads of accessory electrodes are taped down to the side of the headmount base. b. The animal is secured lightly in the earbars. The microdrive is attached to the custom placement tool, which can be manipulated by the stereotax. c. The microdrive is lowered and the placement cannulas of the drive axes are inserted into the guide cannulas. d. As the microdrive is lowered further, placement cannulas are pushed further into the guide cannulas using forceps with tape on the tip. e, f. Once the drive axes are in place, the bottom of the body tube is seated on the top of the guide cannula, as indicated by the contrast between the black marker and the light-orange guide cannula. The microdrive is then secured onto the headmount base with the 00-90 foot screws.

standard ketamine/ xylazine mixture. The animal was placed lightly in the earbars to secure it in the stereotax frame; no cranium leveling was necessary. Temperature was monitored externally with a temperature probe sitting underneath the animal’s stomach. Heart rate and oxygen percentages were monitored with a pulse oximeter. If the animal began to wake up, a small
amount of isoflurane, typically 0.5% - 1.5% was administered through a nose cone.

The drive bundles are very long. As a result, they then can manipulated into the guide cannulas fairly easily. This is because they can move large distances near the cranium to meet the guide cannulas without applying too much stress at the fixed point on the microdrive body. This configuration also provides ample space to maneuver the forceps. Implant manipulation must be done very delicately, however, as the thin polyimide tubes are readily kinked. Application of excessive force at the tips of the forceps may permanently deform the tubes, which may impede electrode driving. The tubes may also kink if they experience too high a bending stress. As such, forming sharp angles near or less than 90° anywhere along the length of the tube should be avoided.

The procedure steps were as follows: first, petroleum-jelly plugs were removed from the guide cannulas. Next, placement cannulas were guided partially into their respective guide cannulas. The implants for the DR and LPPT drive axes were inserted first. To accommodate this, the microdrive was offset laterally left of the midline by a few millimeters. The RPPT drive bundle was then inserted; the microdrive was moved medially to accommodate the bundle reaching the guide cannula. Once placement cannulas were all partway into their guide cannulas, the microdrive was slowly lowered ventrally. During lowering, the bundles bowed outward small distances due to the extra slack in their axes. As this happened, the placement cannulas were further pushed into the guide cannulas with the forceps. We found that modified forceps that have a small area of tape at the tips to be very helpful in pulling on the placement cannulas without squeezing and kinking the polyimide tubes. Occasionally, the placement cannulas just slid in further through the guide cannula simply by forces from lowering the microdrive.

The placement cannulas were in position once the bottom of the body tubes met the tops of the guide cannulas. This was easy to recognize by the black marker visual cues at the boundary of the body tubes and guide cannulas. From this point, the microdrive body was carefully secured to the headmount base with 00-90 screws and the microdrive holder was unscrewed and removed. Next, accessory electrode leads were riveted to the EIB. This included one screw for ground and another for reference. Finally, the headmount cap was screwed on and the animal was assisted in waking up with an 0.1 mg kg\(^{-1}\) injection of atipamezole.
7.3 Chronic Recordings

The second component of the microdrive experiments was performing and analyzing chronic recordings of animals while they were freely-behaving. This process consisted of two subparts: electrode driving and in-cage recordings. By design, electrodes were implanted at a $\Delta$ distance of 1 mm from the sleep-wake cell groups in DR and PPT. As such, electrodes had to be slowly driven to the target coordinates over the course of days and weeks. Once electrodes were estimated to be within relevant brain structures, animals were continuously recorded from in their home cages. Recordings were typically analyzed in between driving sessions to provide better estimates of where the electrodes were and what they were recording, based on the physiological data. The processes and protocols of these experiments are detailed in the following subsections.

7.3.1 Electrode Driving

Electrode driving is a significant part of any microdrive experiment. The driving should be done in the context of a strict protocol that ensures animal safety, equipment safety, recording stability, and experimental reliability.

For microdrives with manual driving mechanisms, driving sessions often require that the animals be restrained or anesthetized to protect sensitive microdrive equipment that is exposed during the procedure. This can be stressful for the animals, so it is important to monitor the animal’s health and to handle them with care. We used rats in our experiments, which are more difficult to restrain than mice due to their size. As such, our driving sessions were done with the animals anesthetized. Driving sessions were spaced apart at least one day to prevent over stressing the animal or overexposure to isoflurane.

The setup for driving sessions is shown in Figure 7.5. Animals were inducted at 4% isoflurane in an induction box. Once their posture relaxed, they were transferred onto a custom cradle with a nose cone and the isoflurane concentration was reduced to 2.5%. The cradle was enclosed by a fine copper mesh to shield signals from ambient electrical noise. The headmount cap was removed and an amplifier was plugged into the EIB for recording using Intan RHD2000 Evaluation System (Intan Technologies, Los Angeles, CA) software. Data were acquired at a rate of 30 kS/s.

Driving sessions were broken up into two parts: pre-driving and driving. The pre-driving phase involved checking electrode impedances and noting meaningful observations related to
neural activity on electrode channels. The driving phase involved driving electrode bundles and monitoring neural activity. Electrode bundles were initially driven one turn of the drive screws (one turn = 212 µm) during the microdrive placement procedure. When driving, electrodes were moved slowly in maximum increments of 1/2 turn and no more than one full turn per driving session. This protocol was implemented to reduce the foreign body response and improve recording stability and reliability. Electrodes were moved in 35 µm (1/6 turn) increments when low-amplitude unit activity was detected to try and better isolate the unit(s). A driving history across sessions was kept to track the position of electrode bundles in tissue. An example note file from a driving and freely-behaving recording session is included in Appendix A.

As part of some driving sessions, especially when electrodes were near target structures, the isoflurane concentration was slowly reduced to bring the animals to light-anesthesia or near-arousal conditions. Anesthesia can significantly reduce neural activity and it was important to know whether our electrodes would observe unit-activity prior to hooking the animals up inside their cages for freely-behaving recordings. This procedure was especially useful for monitoring activity of wake-active cell groups in DR, which dramatically increased their firing rate and signal amplitude.
when the animals would become aroused. Example data from this procedure is shown in Figure 7.6. In most cases, increases in unit activity could be observed before the animals started to fully wake up and move. When the animals did begin to wake up, isoflurane was increased to 3% - 3.5% and the animal was gently restrained until their posture relaxed again.

**Figure 7.6:** *Lowering anesthesia during a drive session.* A five minute file was recorded while the animal was brought to a very light anesthetized state by gradually lowering the concentration of isoflurane. From the bottom: firing rate, in Hz, of a detected single-unit. As the animal became lighter, the firing rate gradually until a large increase at the end when the animal woke up; raster plot with the maximum amplitude of detected units; X, Y, and Z accelerometer channels. Transient increases in unit activity are time-locked with small signal changes in the accelerometer data, which indicates twitching or small movements. At the end of the file, the animal was fully moving its head; 1 to 55 Hz bandpass-filtered ECoG data from a bipolar pair of cortical screws; 250 Hz high-pass-filtered data from microwire electrodes from which the unit was detected.

### 7.3.2 Freely-Behaving Recordings

Animals were attached to commutators and continuously monitored on video for freely-behaving recordings. In most cases, animals were transferred to their home cages following a driving session. Recording sessions generally occurred when electrodes were in target structures. A diagram of the experimental setup and an example photo of an animal being recorded from in its home cage is shown in Figure 7.7.

The setup included a Moog AC6373 slip-ring commutator (Moog Inc, Buffalo, NY) and a 3’ lightweight SPI interface cable (Intan Tech, Los Angeles, CA). The low-torque commutator allowed the cable connections from the animals’ heads to rotate as they moved around while
maintaining electrical connection between the amplifier and computer. The SPI cable was light enough that no counter-balance was needed to relieve weight off the animals.

7.4 Neural Activity and Histology

Perfusion and histology were performed in the same manners as were described in Chapter Six, except that there were no India ink tracts made in these animals and slices were stained using both Nissl and nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase. NADPH-diaphorase is immuno-histochemically similar to nitric oxide synthase (NOS) and will stain neurons that are NOS-positive. This stain can is used to identify cholinergic-positive neurons in the brainstem tegmentum structures [51].

The rest of this section will include histological and data evidence from animals (n = 3) that support the validity of our multi-site, multi-region microdrive design. Data were analyzed using Matlab (Mathworks, Natick, MA) software. Histological micrographs were taken with an Olympus Confocal Microscope (Olympus Corporation) at 4x magnification using Stereo Investigator (MBF Bioscience) software.
7.4.1 Simultaneous Targeting of Three Separated Brain Structures with Unit Activity

Figure 7.8: **Three drive axes histology.** a – c. Electrode tracts from right PPT, left PPT, and DR drive axes in 60 µm tissue slices from a single animal. The dashed red line indicates the intended implant trajectory. Scale bars in a – c represent 500 µm. d, e. Close up of the ventral most parts of the PPT electrode tracts. The red circles the tracts and parts of the PPT nucleus. The right PPT tract went directly through the PPT structure. The left tract was slightly lateral, but still within the PPT. Scale bars in d – e represent 250 µm. f, g. An isolated single unit from electrodes on the DR bundle. Electrodes were slowly advanced in 35 µm increments until the unit was clearly distinguished from the background activity. f. One second of channel recording showing. g. The averaged spike waveform from a full minute of data.

Histological sections showing targeting of bilateral PPT and DR structures from a single animal, along with an example isolated unit from an electrode on the DR bundle, are shown in Figure 7.8. The electrode tracts for each drive axis closely match their intended trajectories. Tissue tearing and blood trails demonstrate that electrode bundles moved from their initial implant sites 1 mm away to and through their target structures.
The isolated unit from the DR bundle was obtained during an early driving session. Anecdotally: the isolated unit was observed with a relatively small amplitude after initially driving the electrodes approximately 106 µm. From there, the electrode bundle was slowly moved in 35 µm increments until the spikes became clearly distinguished from the background noise. A full minute of data was analyzed to extract the single-unit waveform. Spikes were negatively thresholded at seven standard deviations of the baseline activity with an epoch of −1 ms to 3 ms around the threshold crossing. This result demonstrates that angling of the flexible drive axes does not have an effect on the ability to advance electrode bundles with high spatial resolution.

Figure 7.9: Units along electrode tracts. Sample neuronal discharges detected on three drive axes, from a single animal, at different spatial locations as electrodes were driven through tissue over the course of two months. Each colored asterix corresponds to a specific recording date and approximate spatial location along the electrode tracts. Scale bars represent 250 µm.

Shown in Figure 7.9 are observed units at various points along electrode tracts from each drive axis. The units were recorded over the course of two months. Typically, each advancement of the electrode bundles yielded new observable units. The approximate spatial locations for each unit
at particular dates were determined by adding the cumulative driving distances those electrodes had been moved from the bottom of their placement cannulas. The transition points between the placement cannulas and where the electrodes emerged was clear by the size differences within the tracts. This transition is not shown in the left and right PPT tracts in Fig. 7.9 because it appeared in other, more rostral or caudal, slices. This transition can be seen on the DR tract, however, where the tissue tear is wider near the top and narrows before the blood tract. This experimental result is the first demonstration of simultaneous unit recordings from a microdrive with three independent, multi-site drive axes at different trajectory angles.

### 7.4.2 Unit Activity and Behavior Correlates

Figure 7.10: **Wake-active DR unit.** A five-minute file that includes unit, ECoG, and EEG neural activity to demonstrate that wake-active behavior is a correlate of the detected unit. From the bottom: firing rate of the detected single unit; raster plot of the maximum peak amplitude from thresholded single units; accelerometer data. The animal was sleeping where the accelerometer signals are flat; spectral power (dB) of a bipolar pair of hippocampal depth electrodes. Increased power in the 1–8 Hz range indicates sleep; EEG from cortical screw and depth electrodes. The signal amplitude decreases when the animal wakes up.

Putative wake-active unit activity from the DR structure of a freely-behaving animal is shown in Figure 7.10. Included in the plots (from bottom to top) are: a firing rate histogram and
raster plot of a unit thresholded at five standard deviations from the baseline signal, traces of the three accelerometer axes of the amplifier, a spectrogram of hippocampal EEG power (dB), and neural activity from a pair of cortical screws and depth electrodes. The five-minute file was selected to demonstrate transition of the animal from sleep to wake. MEMS accelerometers are used for improved discrimination of sleep-wake behavior in small rodents [52]. In the recordings, the unit is quiet during periods of no head movement, which can indicate that the animal was asleep. Further evidence of a sleep-wake transition comes from the spectral power and EEG signals. During quiet periods, the spectral power has increased 1–8 Hz signal with high-amplitude cortical and hippocampal oscillations, which indicates that the animal is sleeping. When the accelerometer voltages change and the unit starts firing, the low-frequency power drops along with the EEG signal amplitudes. By the end of the recordings, the unit becomes less active, the accelerometer channels show no head movement, and the low-frequency range hippocampal power and EEG-amplitudes begin to increase again.

Putative REM-active unit activity from the PPT is shown in Figure 7.11. The figure includes: neural activity from electrode channels that were in the PPT structure, accelerometer data, ECoG and EEG signals from screw and depth electrodes, ten seconds of the hippocampal EEG that shows 6-Hz theta activity, and the averaged waveform of the detected unit. The unit increases its firing rate as the animal transitions from wake to NREM sleep, which is indicated by high 1 – 4 Hz EEG power, and from NREM to REM sleep. Once there is clear theta activity in the hippocampus, which is a hallmark of REM sleep, the unit is at its maximum firing rate. The triphasic average unit waveform resembles those of cholinergic cells of the PPT and the firing rate increased during NREM and was maximum during the putative REM bout, which agrees with previously described REM-on cells in freely-behaving rats [53]. This result further demonstrates that our microdrive system is unique in its ability to accommodate these types of behavior analyses using sensor modalities outside of just microwire electrodes.

One advantage of using a microdrive for chronic recording experiments is the ability to observe and characterize unit activity across many spatial locations within a target. This can greatly increase overall yield of observed neuronal subtypes and activity compared to static electrode implants. Shown in Figure 7.12 are many different single-unit waveforms from the DR of a single animal recorded from multiple channels over several weeks. Electrodes were driven upwards of 212 µm between consecutive dates. Each unit was recorded from the animal while it was
Figure 7.11: **REM-active PPT unit.** A fifteen-minute file that shows unit activity from a PPT electrode channel during a period of sleep. Top figure, from the bottom: firing rate in Hz of a unit detected with a seven standard deviation threshold; a raster plot of maximum peak values of the detected unit; x, y, and z accelerometer channels. The channels are offset from one another to better see the separate axes traces; hippocampal depth electrodes and screw EEG bandpass filtered from 1 Hz to 55 Hz; a fourier power spectrogram of the hippocampal depth electrodes. Bottom left: 6-Hz theta rhythm from 475 seconds to 485 seconds during one of the bouts where the unit firing rate increased. Bottom right: the averaged waveform of the detected unit.

freely-behaving in its home cage. Many of the neurons seem to have wake-active behavioral dependence (unit 7a, Fig. 7.10), and the discharge profiles of units 4b, 7a, and 7b have general characteristics that match known wake-active unit profiles of DR neurons [54]. At the time of writing, electrodes are continuing to record from locations further ventral into the DR structure of this animal and histology is not available.
Figure 7.12: Various unit waveforms from neurons in the DR structure of a single animal. Unit activity was acquired over the time course of several weeks. Every unit was recorded while the animal was freely-behaving in its home cage. Units were thresholded at 5 or 7 standard deviations from the baseline activity.

7.5 Conclusions

The results from the three chronic microdrive experiments shown in this chapter validate our microdrive as a tool that can be used to study multi-region behavioral circuits in freely-behaving animals. Demonstrated in the histological results was accurate electrode targeting from three independent drive axes. Additionally, observed units from multiple electrode locations on each electrode bundle proved our system as the first reported microdrive to achieve recordings from three separate, arbitrarily-angled drive axes. Furthermore, our system is able to incorporate additional sensory modalities that enable complex behavioral analysis of during free-behavior. Overall, our microdrive is a suitable tool for behavioral-systems and distributed-circuits neurophysiology.
Chapter 8  
Discussion, Analysis, and Applications

The multi-site, multi-region microdrive presented in this thesis was designed to overcome existing challenges of studying neural circuits with distributed cellular nodes. The core innovation to the design was the flexible drive axis, which incorporated a long, flexible tube structure with a decoupled guide cannula. This system provided our microdrive the ability to independently position the trajectory angles and implantation depths of many individual drive axes. Additionally, the flexible nature of the drive axis allowed for simultaneous targeting at separated implant sites. As a result, our microdrive was able to record from three non-coplanar, non-collinear targets of the sleep-wake circuit in rats.

To our knowledge, this is the first reported microdrive design capable of achieving multi-site and multi-region targeting with independently-angled drive axes. While the work presented here validates our design and its implementation, there are still challenges to address and overcome in order to further improve the system as a whole. The remainder of this final chapter is dedicated to the discussion of these challenges, as well as assessing the relevance of our design to other systems/ circuits neuroscience research and a discussion of future research goals for our microdrive in the context of our sleep-wake behavioral study.
8.1 Challenges of the Current Design

There were several significant challenges with the microdrive design throughout its development. Many early challenges, such as material selection, electrode binding, surgical methodology, and recording noise were addressed in previous iterations of the design. Others issues, however, still persist in the current version that is presented in this work. These challenges include: ease of implantation and recording reliability/ stability.

One of the primary problems faced in the microdrive experiments was the loss of drive axes during implantation. This happened, most often, due to kinking of the placement or body tubes as they were being manipulated into place. Either too much pressure was exerted onto the tubes by the forceps or the axes were bent too acutely when being placed. This problem has been mitigated in recent implantations with the implementation of modified forceps, as mentioned in chapter 7. Those forceps, however, are not an ideal solution; the tape surface is larger than necessary and cumbersome to manipulate around other drive axes, and the adhesive wears out after single use. An improved tool will be necessary as we aim to include more drive axes in future experiments and space to maneuver the forceps underneath the microdrive structure is decreased.

Another significant challenge is achieving reliable and stable recordings on the majority of electrodes across repeated experiments. Throughout our experiments, the success of recordings across separate axes varied. For example, one of the animals with wake-active DR recordings had no recordings from electrodes on its PPT axis. In many cases, this was due to electrodes not driving before the implementation of petroleum jelly to prevent electrode binding. In current animals, though, this is still a persistent issue. One possibility is that the speeds at which the placement tubes are inserted into the guide cannulas are too fast, which can cause increased mechanical trauma and glial scarring around implants [35].

One method to recover recordings from “bad” electrode bundles is to drive them forward from areas with no recordings [13]. A to-target drive distance of 1 mm, however, may be too close to the target structures, and some tissue may experience distal chronic tissue effects from initial implant trauma. Thus, driving electrodes onward from a dead-zone while in the targets may have no useful effects. Increasing $\Delta$ to somewhere between 1.5 mm to 2 mm would lessen the likelihood that structures of interest are affected by the initial implantation and could allow electrodes to recover activity by incremental advances of the drive bundles.
8.2 Utility for Systems/ Circuits Neuroscience Research

There is a trend in neuroscience to move toward understanding behavioral expressions from a systems and circuits perspective [55] [56]. Whole-cell population recordings are only one piece of a larger picture that includes anatomical connectomes, transcriptomes, single-synapse connections, and many other network measurements and constructions [56]. Yet, having a tool to facilitate the study of multi-region neural circuits in a chronic and freely-behaving experimental context is instrumental in understanding the overall picture.

There are many example circuits that our microdrive could be of use in studying. The sleep-wake (SW) regulatory circuit is one example, as mentioned throughout this work. Our microdrive could be used to assay the waveforms and behavioral correlates of units in the freely-behaving state that only recently been characterized fully in head-fixed rodent [54] [57]. Another example is a recently discovered circuit involving the zona incerta regulation of superior colliculus projections to the posteromedial thalamus (POm) and parafascicular nucleus (PF) that is believed to influence learned behavior and sensorimotor information in the striatum [58]. This multi-site, multi-region circuit could be studied for the first time in the freely-behaving state with our microdrive.

8.3 Future Direction and Goals

Our microdrive was purposed to study simultaneous observe cell populations thought to be implicated in the sleep-wake regulatory circuit and to study how chronic seizures may perturb normal sleep-wake dynamics. To date, we have targeted and recorded from two of the structures using three drive axes. The immediate next step is to increase the number of simultaneously targeted structures. The laterodorsal tegmental nucleus (LDT), which promotes rapid-eye movement (REM) sleep, and the locus coeruleus (LC), which promotes wake activity, are two additional brainstem structures that we aim to observe. Another planned target is the ventrolateral pre-optic nucleus (VLPO). VLPO promotes non-rapid eye movement (NREM) sleep and is in the hypothalamus.

Targeting very small ventral structures, such as VLPO, could require the use of tetrodes instead of the larger microwire bundles that were used in observing activity in PPT and DR. Having several microwires along the same drive axis would allow simultaneous recordings from
multiple sites within the same structure. This would help prevent significant lesioning of the VLPO structure as electrodes are driven through it. As such, our microdrive design would benefit from the ability to drive multiple tetrodes independently along the same drive axis.

This is done commonly on many microdrives, but the decoupled guide cannula system of our drive axis system makes implementing this feature more complicated. Currently, the placement cannula of the drive bundle fits tightly into the guide cannula. This creates the second fixed-point needed for the drive axis. The driving of multiple tetrodes would require the convergence and fixation of multiple placement cannulas into a single axis. Placing a group of multiple guide cannulas, one for every placement cannula, is not a good idea because the structure be too large and could not be implanted using our surgical method. Instead, a funnel structure that is wide at the top and narrows into a smaller diameter axis could work. This same design could be used to add drivable optical fibers and fluid cannulas to the system.

8.4 Final Remarks

Overall, the work presented in this thesis demonstrates the implementation of a multi-site, multi-region microdrive that was designed specifically to accommodate chronic neurophysiology for distributed neural circuits. Our design is not limited by the same challenges of trajectory angling, number of axes, and maximum region separation that other MSMR microdrives face. Instead, the core innovation of our design, the flexible drive axis, enabled accurate targeting of multiple separated brain structures at arbitrary angles. Further, because of the open-platform structure of the microdrive and headmount base, our system can accommodate additional sensor modalities necessary for chronic behavioral studies. Our design and its success marks a turning point in microdrives and the role that these tools can have in systems and circuits neuroscience.
Appendix A

Experimental Documentation

This Appendix includes examples of documents that are repeatedly used in the surgical procedures and chronic microdrive recordings. These examples follow the general structure that is used in all experiment-specific versions of this documentation.

A.1 Example Excel Worksheet for Targeting

The generic steps for implantation of the guide cannulas were explained in Chapter 6. In this section is the Excel worksheet that we use for our AngleOne (MyNeuroLab.com) stereotax readout system. The general procedure is the same as the steps already discussed. The Excel sheet takes user inputs, such as the target coordinates and the measurement of the external fiducial mark, and outputs calculations of the vectors $X_F - IA$ and $X_T - X_F$. The worksheet also outputs the value $\zeta$ based on user $R$, $\Delta$, and $\alpha$ inputs. The one difference is that the AngleOne system has a “To-Target” mode that tracks the AP, ML, and DV distance from a zeroed origin point and a user-defined target. This tracking can be done in the realigned bases that are used for targeting at an angle.
Figure A.1: **Excel worksheet for guide cannula implantation.** The specific steps for guide cannula implantation are detailed in the right-hand column. First, the user inputs target coordinates $\vec{X}_T$. Next, the vector $\vec{X}_F - \vec{1}A$ is calculated by first moving the stereotactic unit to an IA-reference on the earbars, then moving to the midline suture, which is at a known position with respect to IA. From here, the distance from the anatomical IA-reference to the user-defined external fiducial mark is calculated. The readout on the AngleOne are input into the ML, AP (IA), and DV (IA) cells for “Distance from IA Reference to External Fiducial Mark” and the vectors $\vec{X}_F - \vec{1}A$ and $\vec{X}_T - \vec{X}_F$ are output by the worksheet. After inputing $R$, $\Delta$, and $\alpha$ parameters, the worksheet outputs $\zeta$. From the external fiducial mark, the stereotax is angled (if necessary) and the AngleOne readout is zeroed. Finally, the system is switched to “To-Target” mode, the target coordinates are entered, the stereotactic unit is moved to the craniotomy and is implanted until the DV coordinate is $\zeta$ from the target, which is $(0, 0, 0)$ in To-Target mode.

### A.2 Chronic Recording Observation and Electrode Driving Notes

Our chronic recording experiments include documentation that tracks the cumulative driving distances of electrodes from every drive axis implant. Also included are real-time observations.
during driving sessions and home cage recording sessions. Figures A.2 and A.3 are snapshots of a single example document that outlines the general format we use in our chronic recording documentation. At the of the document are the cumulative driving distances, which include the total distance driven from the last driving session. Next in the document is a map of the amplifier channel numbers and their corresponding implants. The Pre-Driving section and Driving sections are used for driving sessions. These sections include the isoflurane concentration being used at various times, impedance tests of electrodes before and after electrode driving, and observations about activity on amplifier channels before and after electrode driving. The Driving section also includes notes about which electrodes are being moved, by how many fractions of a turn, and at what time. This makes correlating newly-discovered neural activity easier during data analysis. After this, the cumulative distance driven is updated so that it can be copied into the next driving session’s recording notes document. Last is a section for activity observations on channels while the animal is freely-behaving in its home cage.

Figure A.2: Example chronic experiment recording notes. Included in this snapshot are: the cumulative driving distances of each electrode implant, an amplifier channel map for each implant, and the Pre-Driving procedure and observations section.
Figure A.3: **Example chronic experiment recording notes, continued.** Included in this snapshot are: the Driving section, an updated cumulative driving distance record, and observations of channel activity and behavior while the animal is in its home cage.
Appendix B
Surgical Procedures

Included in this Appendix are details of the surgical procedures used for the acute and chronic experiments. Any experiment-specific steps that are not common between both surgeries are explained within the individual experiment chapters.

All animal work was approved and performed under the oversight of the Pennsylvania State University Institutional Animal Care and Use Committee. Animals (300 - 375 grams) were first inducted under 4% isoflurane. Once the animals were unresponsive, a 50 mg kg\(^{-1}\): 6 mg kg\(^{-1}\) mixture of ketamine: xylazine was administered to induce a general plane of anesthesia. Following loss of consciousness, the animals heads were shaved and the animals were intubated. Intubation tubes had a small amount of lidocaine on the outside. Intubation was used for oxygen delivery throughout the surgeries; isoflurane (0.5% - 2.5%) was added when necessary to keep the animals under anesthesia. Animals were connected to a ventilator, and temperature controlled with a heating pad and rectal probe (Physiosuite, Kent Scientific, Torrington, CT). Oxygen percentages and heart rates were monitored with a SurgiVet (Smiths Medical PM, Inc, Norwell, MA) pulse oximeter system and recorded periodically into a log book. Animals were then placed securely in earbars of a stereotax u-bar frame with instrumented readout (Angle One, MyNeuroLab.com). Scalps were then prepped with iodine and local 4 mg kg\(^{-1}\) injections of bupivacaine administered subcutaneously around the skull. Two injections of 8–10 ml of saline were injected subcutaneously before incision and approximately two–three hours into surgery to prevent dehydration. Incisions were made to expose the craniums. From here, craniotomies for the implants were drilled.
Bibliography


