Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Greetings everyone, and welcome back to the NPTEL course on micro sensors, implantable devices, and rodent surgeries for biomedical applications. In our previous lecture, we explored the typical building blocks involved in generating biphasic pulses. Today, we will dive deeper into these components and their functionality.

As a quick recap, let's revisit the slide outlining the topics we've covered so far. We discussed the rationale behind brain stimulation, examined the various parameters involved in biphasic pulse generation, and emphasized the importance of considering these parameters during the design process. We also touched upon the implementation of such a system on a rat model and gained a basic understanding of current mirrors.

Now, let's assume you've programmed the microcontroller to deliver a current of 200 microamperes. We'll explain the architecture in more detail shortly, but for now, understand that the current will flow from VDD (the power supply) to the ground. If we ensure that transistor M1 operates in saturation mode, the current will also flow through the brain, completing the circuit.

We've incorporated three electrodes into this setup, but once you grasp the underlying concept, you can easily extend it to multiple electrodes. Observe how we've programmed the GPIO (General Purpose Input/Output) pins of the microcontroller to act as select lines for the SPDT (Single Pole Double Throw) switches S4, S1, S2, S3, and S5. These select lines control the pole positions of the switches.

In the current configuration, CaOM4 (Cathode Output 4) is connected to NC4 (Normally Closed 4). This connection creates a closed path, or a short circuit, between CaOM4 and NC4, offering a low on-resistance, typically in the range of 20 to 30 ohms. Conversely, CaOM4 and NO4 (Normally Open 4) are not connected, resulting in an open circuit with a high resistance in the mega-ohm range.

Now, let's focus on the current flow. Assuming current is generated up to a certain point in the circuit, it encounters two possible paths. It can either flow through the first branch or the second branch. The first branch includes the on-resistances of two switches. The second branch, however, leads to an open circuit due to the disconnected switch, presenting a mega-ohm resistance.

Let's analyze branch 2 in more detail. If this branch offered minimal resistance, a significant portion of the current would naturally flow through it, following the path of least resistance. This fundamental principle governs the current distribution in the circuit.

Now, let's switch the color of our pen and continue our exploration. We've established that one branch offers mega-ohms of resistance. Next, we will examine the resistance offered by the other branch and understand how this influences the overall current flow in the circuit.

By carefully analyzing the resistance values and connections in each branch, we can predict the current distribution and ensure that the desired current reaches the target electrodes for effective brain stimulation. Understanding these circuit fundamentals is essential for designing and implementing robust and reliable implantable devices for biomedical applications.

Now, let's examine the path available for the current. We have the current source, represented by the arrow, and two branches. Between these branches, we have the brain or the load, symbolized by the brain image. Let's connect E2 (Electrode 2) and E1 (Electrode 1) with a 1 kilo-ohm resistor. For a rat, the maximum brain impedance typically observed is around 1 kilo-ohm.

Tracing the path from point P1 on the right side, we see that the current can flow through a series of connections, following the arrow marks all the way. In contrast, the other branch presents a mega-ohm impedance due to the open circuit.

So, what will the current's direction be? How will it flow? Remember, the current originates from VDD and seeks the ground path. In this scenario, with one branch offering a kilo-ohm resistance and the other a mega-ohm resistance, the current will predominantly flow through the branch with the lower resistance, that is, from E2 to E1. This direction is crucial, especially in Case 1, where the switches are configured in this specific manner.

Now, let's move on to Case 2, where the switch positions are slightly altered. In Case 1, COM1 (Common 1) was connected to NC1 (Normally Closed 1), while in Case 2, COM1 is connected to NO1 (Normally Open 1). Similarly, COM2 is connected to NO2 in Case 1 and NC2 in Case 2. We make these changes to reverse the current flow, aiming to generate a negative pulse by directing the current from E1 to E2.

With this new configuration, the current still flows through the transistor, but now it faces two different branches. One branch leads to NO2, which is not connected to COM2, creating a high-resistance path in the mega-ohm range.

Let's explore the other branch. Following the arrow marks, we can see a clear path from E1 to E2 and then to the ground. If we place the same 1 kilo-ohm resistor across E1 and E2, this branch will offer kilo-ohm resistance, while the previous branch still presents mega-ohm resistance. As we know, current favors the path of least resistance. Consequently, a significant amount of current will flow through the E1 to E2 branch, while negligible current will pass through the other branch.

The direction of current flow is now reversed, flowing from E1 to E2. This signifies the generation of a negative pulse. In contrast, Case 1 generated a positive pulse with current flowing from E2 to E1.

Furthermore, if you observe the network, you'll realize that the same network generates both the positive and negative pulses. This significantly increases the likelihood of these two pulses being nearly identical. Let's explore why this is advantageous.

Imagine you have a current source or sink. You could use a single network (Network 1) to generate a biphasic pulse across the brain. Alternatively, some designs employ two separate networks (Network 1 and Network 2) to generate the positive and negative phases of the biphasic pulse. However, using two distinct networks introduces a higher probability of amplitude mismatch between the pulses.

This potential mismatch arises because the two networks might have different impedances. We can't guarantee that both networks will offer the same resistance to the incoming current. Consequently, the current mirroring might be imperfect, leading to variations in the amplitudes of the positive and negative pulses.

The single-network design we presented earlier mitigates this issue. By utilizing the same network for both phases, we ensure a high degree of similarity between the positive and negative pulses, or cathodal and anodal pulses, as you might call them.

This consistency is vital for maintaining charge neutrality, a critical aspect of brain stimulation. Charge neutrality means that the net charge delivered to the brain should be zero at the end of the biphasic pulse. In other words, the positive and negative pulses should have equal intensity. If one pulse generates 200 microamperes, the other should also generate 200 microamperes.

While we can control the "on time" of each pulse to ensure equal charge delivery, it's equally important to minimize any discrepancies in current amplitudes. Ideally, they should be exactly the same. For instance, if one pulse generates 200.7 microamperes, the other should also be 200.7 microamperes.

Besides charge neutrality, this design offers another advantage. By cleverly programming the pole positions of the switches, we can redirect the current flow. Instead of flowing from E1 to E2, we can make it flow from E2 to E3, or vice-versa. Let's designate E1 and E2 as pair 1 and E2 and E3 as pair 2. Achieving this current redirection requires careful programming of the switches.

Now, if we ensure that the GPIOs generate the correct logic signals, either 1 or 0, we can control the pole positions of the switches and, consequently, the direction of current flow. With this simple configuration, we can generate two biphasic pulses, one across pair 1 (E1 and E2) and another across pair 2 (E2 and E3).

The beauty of this design lies in its scalability. We can replicate this same network and connect it to the transistor, allowing us to generate biphasic pulses across additional pairs, such as P3 and P4. We can continue adding more networks, enabling stimulation across P5 and P6, and so on.

However, it's important to note that within each network, you can only choose one pair at a time. Simultaneous stimulation across P1 and P2, for instance, is not possible. This limitation stems from the shared current path within each network.

Nevertheless, current mirroring allows us to stimulate multiple pairs concurrently by utilizing different networks. Each network can independently generate biphasic pulses across its designated pair of electrodes. This flexibility expands the possibilities for multi-site stimulation and complex stimulation patterns.

Now, let's explore the typical architecture of such a system. At its core, we have a microcontroller, which serves as the brain of the operation. This microcontroller can be connected to a Bluetooth Low Energy (BLE) module via UART (Universal Asynchronous Receiver/Transmitter) communication. The BLE module enables wireless communication with a Graphical User Interface (GUI) developed using MATLAB.

MATLAB offers excellent tools and built-in functions for creating GUIs for Bluetooth Low Energy applications. You can leverage these functions to design a user-friendly interface for controlling and monitoring the stimulation parameters.

The electronic system, including the microcontroller, BLE module, and other necessary components, is typically housed in a backpack worn by the rodent. This backpack communicates wirelessly with the GUI at the user's end.

Through the GUI, you can select the desired electrode pair for stimulation and program the GPIO pins of the microcontroller accordingly. The system is powered by a battery, often a 3.7-volt LiPo battery, and a charge pump is used to generate a regulated 5-volt supply. This regulated voltage ensures greater control over the current amplitude that can be generated.

Finally, the current mirroring stage comes into play. This stage replicates the programmed current from the microcontroller across multiple electrode pairs, enabling simultaneous stimulation at different sites.

In essence, this architecture combines microcontroller-based control, wireless communication, and current mirroring to create a versatile and user-friendly brain stimulation system. It allows researchers to precisely control stimulation parameters, target specific brain regions, and monitor the effects of stimulation in real-time.

This system's flexibility and scalability make it adaptable to a wide range of research applications, from investigating the neural mechanisms of behavior to developing novel therapeutic interventions for neurological disorders. By understanding the architecture and underlying principles, you can leverage this technology to advance your research and contribute to the growing field of neuromodulation.

And as I explained earlier, using the same network, we can achieve a similar outcome with E2 and E3. This demonstrates the versatility of a single network to generate biphasic pulses across multiple load pairs. You can even extend this concept further by adding more networks, each capable of stimulating a different pair of electrodes, such as P3 and P4, or P5 and P6. This parallel configuration allows for the generation of multiple currents simultaneously, significantly enhancing the system's capabilities.

However, it's important to reiterate that while each network can stimulate either electrode within its assigned pair, it cannot stimulate both simultaneously. The current mirroring mechanism within each network necessitates this limitation.

Now that we've explored the theoretical aspects, let's examine a typical architecture of a wireless biphasic pulse generator. The central component is a microcontroller, which acts as the control center. It connects to a Bluetooth Low Energy (BLE) module via UART communication, enabling wireless interaction with a MATLAB-based Graphical User Interface (GUI). MATLAB provides convenient built-in functions for developing BLE-compatible GUIs, streamlining the process of creating a user-friendly interface for controlling the stimulation parameters.

The entire electronic system, comprising the microcontroller, BLE module, and other essential components, is typically packaged into a backpack worn by the rodent. This backpack establishes a wireless link with the GUI, allowing for remote control and monitoring of the stimulation.

Depending on the chosen electrode pair, you can program the GPIO pins of the microcontroller to control the switch configurations. The system is powered by a battery, often a 3.7-volt LiPo battery, and a charge pump generates a regulated 5-volt supply. This regulated voltage ensures consistent and reliable current generation across a wider range of amplitudes.

Finally, the current mirroring stage replicates the programmed current from the microcontroller across multiple electrode pairs, enabling simultaneous stimulation at various sites.

Before deploying this system in animal models, thorough benchtop testing is essential. We connected a 1 kilo-ohm resistor across E1 and E2 and observed the resulting current flow. We tested a range of current amplitudes, from 40 microamperes to 1.25 milliamperes, with a load of 2 kilo-ohms.

The results showed that even with a 1 milliampere current amplitude, the voltage across the resistor slightly exceeded the expected 2 volts (based on Ohm's Law: $V = IR$). The maximum observed error was around 8.38%, which is understandable given the use of a basic current mirror configuration.

Ideally, if you pump in 1 milliampere, you should get 1 milliampere out. However, due to the inherent characteristics of the current mirror, we observed a slightly higher current, around 1.08 milliamperes. This discrepancy arises from the current equation, which includes a dependence on Vds (drain-source voltage). While maintaining identical Vds values for both transistors in the current mirror would theoretically result in equal currents, achieving this in practice is challenging.

More complex current mirror configurations, such as the Wilson current mirror, can improve accuracy but require additional transistors. In our application, where slight variations in current amplitude are tolerable, the basic two-transistor current mirror suffices.

We also tested the system with a resistor and capacitor in parallel across E1 and E2 to mimic brain tissue impedance. Even with this more complex load, the waveforms remained relatively undistorted, maintaining charge neutrality.

Once benchtop testing was successful, we proceeded to deploy the system in animal models. We targeted the motor cortex, a brain region responsible for generating signals that control movement. By stimulating specific areas within the motor cortex, we can elicit corresponding movements in the animal.

In a similar vein, we conducted experiments to observe forelimb movement in rodents. We targeted a specific region in the motor cortex dedicated to forelimb control. By electrically stimulating this region, we could elicit forelimb movement even when the rat was under anesthesia.

For this application, we designed an electrode interface board to connect the electrodes to the electronic system. We used a tungsten wire as the electrode, inserting it to a depth of 1.8 mm in the forelimb region of the motor cortex. We obtained all necessary electrical clearances for this procedure from the Central Animal Facility at the Indian Institute of Science.

During the experiment, we varied the current amplitude from 40 microamperes to 120 microamperes. We observed that at lower currents, around 40-45 microamperes, the forelimb movement was minimal, with a small swing. As we increased the current to 80-90 microamperes, the swing amplitude reached saturation. Further increases beyond 120 microamperes did not result in any additional increase in swing amplitude.

To ensure precise delivery of the stimulation pulses, we employed a series resistor in the circuit. The voltage across this resistor was monitored using an oscilloscope, allowing us to confirm that the pulses were reaching the brain tissue, which acted as the load in this setup.

The complete setup consisted of the electronic system, the electrode interface board, the tungsten wire electrode, and the oscilloscope. Additionally, we incorporated an HM10

BLE module for wireless communication and control, indicated by a red LED. A green LED was also present for visual feedback.

This experimental setup allowed us to systematically investigate the relationship between stimulation parameters and forelimb movement in anesthetized rats. By varying the current amplitude and observing the corresponding changes in swing amplitude, we could establish the optimal stimulation parameters for eliciting robust forelimb movements.

Furthermore, the use of an oscilloscope to monitor the voltage across the series resistor provided valuable information about the actual current delivered to the brain tissue. This ensured that the stimulation was within safe and effective limits, minimizing the risk of tissue damage or adverse effects.

The incorporation of the HM10 BLE module added another layer of sophistication to the setup, enabling wireless communication and control. This feature facilitated remote monitoring and adjustment of stimulation parameters, enhancing the convenience and flexibility of the experiment.

Overall, this study demonstrates the successful implementation of a wireless biphasic pulse generator for stimulating the motor cortex and eliciting forelimb movement in rodent models. The combination of careful electrode placement, precise current control, and real-time monitoring allowed us to achieve reliable and reproducible results.

These findings have important implications for the development of brain-computer interfaces and other neuromodulation therapies. By understanding the relationship between stimulation parameters and motor responses, researchers can design more effective and targeted interventions for individuals with movement disorders or paralysis.

The green LED indicates that the PCB (Printed Circuit Board) is receiving power from the battery. When the blue LED lights up, it signals the start of the stimulation. The entire setup, including the electronics and battery, is neatly enclosed within a backpack that is comfortably fitted onto the rat. The rat's head is securely fixed in a stereotactic apparatus, allowing for precise targeting of brain regions using the XYZ coordinate arm. A tungsten wire electrode, held by a specialized holder, is carefully lowered into the right hemisphere of the rat's brain, specifically targeting the region responsible for controlling the left forelimb.

As the stimulation begins, pay close attention to the oscilloscope (CRO) display and the rat's left forelimb. You should observe distinct pulses on the CRO, coinciding with movements in the left forelimb. This visual correlation confirms that the electrical pulses are effectively reaching the brain and eliciting the desired motor response. We validated our setup and parameters by comparing our results to those reported in a published paper, ensuring that the current parameters we used were within the expected range for eliciting forelimb movement.

Once the functionality is confirmed, the entire system, including the electrode interface board and the HM10 BLE module (identified by the red LED), is encapsulated in dental acrylic for protection and biocompatibility. The microcontroller used in this setup is a PSoC 5LP, which offers flexibility and programmability. The FPC connectors provide convenient interfaces for programming and debugging, while the header accommodates the Bluetooth module.

The ALD1107 transistor array serves as the current mirror, replicating the programmed current across multiple electrode pairs. The SPDT switches enable precise control over current flow direction, and the MAX682 ensures a stable 5-volt power supply for the system.

This setup provides several advantages, including wireless control, real-time monitoring of stimulation parameters, and the ability to elicit controlled forelimb movements. Additionally, it is adaptable for various behavioral experiments, even in awake and freely moving rats.

Once the rat has recovered from the implantation surgery, the backpack can be attached, and various behavioral experiments can be conducted. The specific experiments will depend on the research application, whether it involves studying the effects of deep brain stimulation, investigating the neural mechanisms of motor control, or exploring other areas of neuroscience.

It's worth noting that this project exemplifies the collaborative nature of biomedical research. Different team members contribute their expertise in areas such as 3D printing for custom casing design and PCB design using specialized software. This interdisciplinary approach fosters innovation and enables the development of sophisticated tools and techniques for addressing complex biomedical challenges.

While we focused on intercortical microstimulation in this demonstration, the principles and techniques presented here can be extended to other brain regions and applications. The system's versatility allows for the exploration of various research questions in neuroscience and neural engineering, both in anesthetized and awake animal models.

In summary, we have showcased the development and implementation of a wireless biphasic pulse generator for brain stimulation. This system, with its precise control, real-time monitoring, and wireless capabilities, provides a powerful tool for researchers to investigate brain function and develop novel therapeutic interventions.

Thank you for your attention. We look forward to seeing you in future sessions where we will continue to explore exciting advancements in the field of neural engineering and implantable devices.