RoboRoach Experiment #1 - Neural Interface Surgery

What will you learn?
You will learn about the anatomy and nervous system of a cockroach. You will also learn the proper surgery and husbandry techniques for experiments on insects. After the surgery, you will then use your RoboRoach circuit to briefly influence the cockroaches movements by microstimulation and you will observe the subsequent adaptation of the cockroach in response to the microstimulation.

Introduction
Are you a teacher or parent that wants to teach a student about advanced neurotechnologies? You are in luck! After 3 long years of R&D, the RoboRoach is now ready for its grand release! We are excited to announce the world's first commercially available cyborg! With our RoboRoach you can briefly wirelessly control the left/right movement of a cockroach by microstimulation of the antenna nerves. The RoboRoach is a great way to learn about neural microstimulation, learning, and electronics!
Time: 1.5 - 2 hours  
Difficulty: Advanced*

*This experiment is appropriate for college students. Also, high school students with adult supervision.

Background
The RoboRoach is an educational DIY cockroach cyborg kit. It includes one PCB (printed circuit board), a.k.a. “the backpack”, which carries the Bluetooth Low Energy wireless receiver/transmitter (so you can communicate to it via a smartphone), a few indicator LEDs (light-emitting diodes), and other circuit components (resistors and capacitors). A small coin cell battery is also provided to power the PCB’s components. Last but not least, the kit includes 3 sets of electrodes to implant three adult cockroaches. One end of each electrode set is a connector to plug in the RoboRoach backpack and the other end is a 0.003" silver wire (smaller than the thickness of your hair) that serves as the conductive electrical element that interfaces with the sensory nerves of the cockroach. There are 3 electrodes in each set; one for the left antenna, one for the right antenna and one for the ground. Since electricity needs a closed circuit to flow, the ground wire is necessary to provide a return pathway for the electrical stimulation current. The ground can go anywhere in the cockroaches body, but we use the dorsal side of the thorax, near the flight muscles (these cockroaches can’t fly), as a ground location of minimal damage to the insect.

So, wait a second... What is a cyborg?
The word “cyborg” is an abbreviation of “cybernetic organism” and is defined as a living organic
being that has abiotic artificial parts. Coined in the 1960s by neuroscientist Manfred Clynes, it described the idea of a cyborg as a "new frontier" that would be "a bridge...between mind and matter."¹ Since then the rapid rate of technological growth has brought on all different types and definitions of cyborgs. Direct neural interfaces like the ones you will learn about with the RoboRoach experiment, have been being developed since the early 1970s.² This type of brain-computer interface, or BCI for short, has been researched, developed, and utilized by scientists and doctors to learn about our nervous systems and to help treat people who've lost critical biological functions such as their sight, hearing and motion with neuroprosthetics. Take for example the cochlear implant, which has been used to return hearing to over 220,000³ people worldwide. Here’s a moving video of a young woman, Sloan Churman, who was born deaf and lived without hearing for the first 29 years of her life until this day when her cochlear implant was first turned on: [http://www.youtube.com/watch?v=LsOo3jzkhYA]

By the way, we’re not the only ones who are creating cyborg invertebrates to learn about the electrophysiology and assist in the development of neurotechnologies. Starting in the late 90’s, two groups of researchers (University of Tokyo⁴ and the University of Michigan⁵) achieved rudimentary control of cockroach turning. The American work was funded by DARPA with the ultimate goal to have “hybrid robots” that would be able to do disaster relief monitoring and reconnaissance, on the premise that insects are more power efficient than tiny robots. The work was published in some small trade journals, received some press, but went largely unnoticed.

More recently, a group at Cornell University⁶ was able to identify different signals to control the wings of a moth and scientists at University of Arizona⁷ have built a robot that is controlled by the nervous system of a moth [http://www.youtube.com/watch?v=dSCLBG9KeX4]. Research at Berkeley⁸ has resulted in the control of the flight of a giant beetle [http://www.youtube.com/watch?v=hFguLwUT5lg] and researchers at North Carolina State University⁹ are also experimenting with microstimulation of cockroach sensory nerves to develop a “Kinetic-base System for Autonomous Control of Terrestrial Insect Biobots” [http://www.youtube.com/watch?v=jhuyDuV-ERQ].

Wow! ...but why cockroaches?
Backyard Brains’ favorite invertebrate for the RoboRoach is of the kingdom Animalia, phylum Anthropoda, class Insecta, order Blattodea, family Blaberidae, genus Blaberus, and species Discoidalis. Since it’s full biological classification is quite a mouth full we will call them Discoid cockroaches. We found Discoids to be the best for the RoboRoach for several reasons. First of all, they are readily available in the U.S. because people breed them as feeder insects for pet reptiles and amphibians. Also, they are easy to take care of and handle (they are much slower than the cockroaches native to the U.S.). Last but not least, they are large enough to carry the RoboRoach backpack.
Cockroaches have neurons similar to ours, but they have much fewer numbers of them. Cockroaches have about 1 million neurons, while we have have 100 billion! This allows us humans to have cool human talents like speaking/understanding languages and designing neural stimulation circuits. A cockroach also has a decentralized nervous system, with ganglia (small brains) running down its body. However, a cockroach does have a “bigger” ganglion in its head, which can be considered a brain. Even though our nervous systems are very different from a roach’s, the structure and function of our individual neurons is quite similar and this allows us to learn about our brains by studying theirs.
Unless you name is Gregor Samsa, your central nervous system looks like the one on the left (photos are not to scale nor are the

Cockroaches have two antennae on their anterior end. These antennae assist to guide the cockroaches through the world by sensing touch (as small as moving air) and smell. These tiny “hair-like” sensors are connected directly to neurons that communicate messages to the cockroach brain.
The neurons communicate just like ours by sending information down their neural pathways in the form of electrical activity called action potentials, or as we neuroscientists call them, “spikes”.

They also have two cerci located on their posterior end. These are sensors similar to the antenna and also have specialized wind and vibro-auditory sensory receptors that send “spikes” to the cockroach ganglia, which has been honed by evolution, to very quickly initiate escape responses if they sense approaching stimuli. This enables cockroaches to have very fast behavioral responses and why cockroaches can dart away so quickly just when you open the
By implanting the tips of your tiny electrodes into the roach’s antennae, you create a neural interface. Since the electrode wire is electrically conductive, you can then send an electrical signal similar to the one that the roach’s antennas’ sensory neurons naturally create. The result: the neurons in the antennae will be stimulated to fire neuronal messages (spikes!).
These “spikes” will then travel to the roach’s ganglia and stimulate the natural sensio-motor reactions. This is how you can control your cockroach’s movement. Basically stated: if we stimulate the neurons on the right antenna, the cockroach will react by turning left. If we stimulate the neurons on the left antenna, the cockroach will turn right.

The science of neural interfaces you will be studying today is very similar to the cutting edge neural engineering going on in hospitals and universities around the world. For example, you can try stimulating at 55 Hz, which is roughly the same stimulation that neural engineers use when stimulating the subthalamic nucleus as a treatment for Parkinson’s disease.

**Preparation for the RoboRoach Surgery**
Are you ready to try your hand as a cockroach brain surgeon and make your very own temporary cyborg?

The surgery itself takes about 30-45 minutes. An important aspect for a good scientific surgery is proper preparation, thorough documentation, and consideration of the well-being of the animal. So, considering both set-up and cleanup time, plan for at least an hour for the full operation.

Anyone can do the RoboRoach surgery, but like everything in life, it takes practice and patience to master! That’s why your kit comes with 3 sets of electrode arrays, enough for you to prepare 3 RoboRoaches to learn and improve from repeating the experiment. Additional RoboRoach electrodes are available through our web store [https://backyardbrains.com/products/] or you can take the DIY route and build your own electrodes [build instructions coming soon]. We recommend that you carefully read through this guided experiment entirely before you start the surgery to prepare your human brain as well!

Also, we’d like to remind you that the RoboRoach is an educational tool to be used with
cockroaches to learn about neural interfaces. Please respect and abide by your presiding government’s laws and regulations when it comes to animal research. If you have ethical concerns or questions, please refer to our ethics guideline and discussion: [http://wiki.backyardbrains.com/Ethical_Issues_Regarding_Using_Invertebrates_in_Education].

What you'll need:

- The “RoboRoach Surgery Procedure” (below). This is also available as a(n):
  - PDF [download link]
  - Instructional video [http://www.youtube.com/watch?v=5Rp4V3Sj5jE] NOTICE: This video is for the RoboRoach beta, which is essentially the exact same procedure EXCEPT that the electrode connections have changed places on new Bluetooth RoboRoach. If you have the new RR, the ground is the center electrode, NOT the left electrode. On the new RR, the left electrode is on the left and the right electrode on the right.
- A printed “RoboRoach Surgery Worksheet” [download link]
- Your brain, some patience, and a deft hand
- Approximately 1 hour of your time
- 1 “RoboRoach Surgery Kit” (available through our [webstore] or you can source the tools on your own)
  - 150 grit sandpaper
  - Loctite Super Glue Gel Control (note: we’ve found this stuff works the best, other super glues may not substitute)
  - Cotton swabs
  - Silly putty
  - Small-diameter needle
  - Toothpicks
  - Dissection scissors
  - Tweezers or forceps
  - Magnifying glass (or upgrade and impress your friends with a [link to RoachScope])
  - Low-temp hot glue gun & glue cylinders
  - Popsicle stick
  - Small amount of flour
- Cup with of ice water (you’ll want enough ice to keep the water very cold but enough water to be able to submerge the roach). Ice water is always 32 degrees fahrenheit, so long as there is still ice in the water
- Paper towels
- Clock with minutes hand
- Work lamp, don’t try this in the dark!
- 1 RoboRoach electrode array
- ...and of course: 1 large, healthy adult Discoid cockroach
  - IMPORTANT: Adults can be identified by a black dot on the pronotum (the
exoskeleton “hood” above the roach’s head) and the presence of wings. They will no longer molt. Therefore, affixing a connector to its head permanently is fine. NOTE: if you glue an electrode connector to a juvenile cockroach (no wings), it will not be able to split its exoskeleton when molting and will die. Do not do this surgery on juvenile cockroaches.

Procedure
Step 1: Prepare your workspace. Choose your cockroach. Document!
Lay out all the necessary supplies and tools listed above. Plug in your hot glue gun and if it has a temperature setting, set it to low. Fill in the initial information on your RoboRoach Worksheet - Surgery Setup Record.

Step 2: Attaching the electrode array to the roach
2.1) Anesthetize your cockroach by submerging it in the ice water. Note the start time on your Surgery Worksheet - Procedure Record. From now on, for ease of reading we will not include a reminder at every step to update your worksheet but you should promise to be a good scientist and document your experiment!

Since the roach is a cold-blooded animal (ectothermic, not producing its own heat through metabolism), reduction in temperature results in reduction of nervous & metabolic function effectively “anesthetizing” the insect\textsuperscript{10}. It typically takes 2-5 minutes for a cockroach to “go to sleep”, just watch for the roach to stop moving and reacting to stimuli like a touch on its leg.
2.2) Once fully anesthetized, carefully remove the roach from ice water with forceps and place the cockroach on your table. With sandpaper, lightly sand the center of the pronotum to roughen the waxy chitin. Be careful not to push too hard when sanding, you don’t want to hurt the roach! Hemostat forceps come in handy here to grasp the pronotum (their exoskeleton “hood”). This is similar to sanding your fingernails -- it's a hard substance without nerves.
Sand until the pronotum feels “slightly rough” to the touch. This will allow the super glue to stick securely.

2.3) Wipe the pronotum with wet towel to remove debris from sanding, then fully dry with paper towel. Put a dab of superglue on the sanded area. Be sure not to touch the glue with your fingers, you want it to stick to the roach, not to you!
2.4) Carefully place the black connector on the glue with the electrodes facing the anterior direction (towards the antennae). Be sure to orient it squarely with the body, the pins should be parallel to the length of the body. The connection should be strong in 1-2 minutes.

2.5) Place the roach back into the ice for 1-2 minutes to ensure it is continually anesthetize (or until no response to stimuli is observed).

Step 3: Implanting “Ground” electrode in Thorax
3.1) Take the cockroach out of water, place on table belly down, and carefully splay one wing to the side. Use silly putty to hold the wing down and stabilize the roach. Use a cotton swab to dry thorax and then lightly sand (because this is a glue step) the thorax.
3.2) Use your needle and lightly poke a small hole on in the exoskeleton of the thorax of the cockroach just behind the head. Avoid the center line as that is where the esophagus is located.

The flight muscles on the sides can tolerate a small poke (this species of cockroach cannot fly
anyway, so it does not use these muscles much). Use a careful amount of force, enough to penetrate the hard chitin exoskeleton but not too much that you push the pin in too deep. A helpful tip, sometimes you can find a small marking on the roach’s exoskeleton (the back of the cockroach has 5-8 small black “freckles” you can use as references for insertion points). Make the hole here and it will be easier to locate when implanting the electrode.

3.3) With fine forceps (or tweezers) carefully insert the center electrode 1 mm (0.039”) into the hole you just made. It helps to straighten the tip of the electrode as much as possible before inserting.
3.4) Using a toothpick apply a small bead of the super glue to the electrode just above where it enters the tissue.

Then using forceps, carefully “park” the electrode 1-3 mm (0.039” - 0.118”) into the insertion hole. The goal is for the superglue to enter the body, because when it comes in contact with the internal saline, it will polymerize quickly and securely. Super glue (cyanoacrylate) was actually used in the Vietnam War as quick battlefield solution to closing wounds of soldiers.
If you need to secure it further, add an extra small bead of super glue above the insertion point, then replace the wing to it’s resting place. After the glue has set, a light tug can test if the ground electrode is secure.

3.5) Place cockroach back in ice bath for 1-2 minutes to maintain anesthetization.

Step 4: Implanting right antenna electrode
4.1) Take the cockroach out of the ice water and lay it dorsal side down (turn it on its back). Then use forceps to carefully splay the antenna out and cut the cockroach left antennae to ~1/8 – ¼ inch (~3 - 6 mm).
4.2) Taking the right electrode, “park” the electrode 1 mm (0.039”) inside the right antenna (not all the way in).
Like you did earlier, dab a bead of super glue just above where the electrode is in the antenna (parked).

4.3) Then use forceps to park the electrode such that the super glue bead partially enters into the antenna, ~2 - 4 mm (0.078" - 0.157"). If needed, add a bit more super glue at the top after the wire is inserted, but avoid excess glue. The super glue should polymerize immediately once it is inserted into the antenna. The point is to get super glue just inside the inner ring of the antenna; otherwise it will fall out easily. Don’t touch glue with fingers because it will stick to you!
4.4) Place cockroach back into ice bath for 1-2 minutes to maintain anesthetization.

**Step 5: Implanting left antenna electrode** → Repeat Step 4, but replace “right” with “left”
Step 6: Tidying up the slack in the wire
6.1) It is important that the “wire slack” be cleaned up. Using your fingers and forceps, carefully organize (fold back) the wire slack on top of the connector. Try to ensure as little slack wire exists between antenna and connector. Cockroach legs are very strong and can pull electrodes out if they get a firm hold or get it snagged. Also ensure “exposed” parts of silver wire are not touching.
6.2) Prepare the end of a small flat surface such as a popsicle stick (you can also use a small ruler or back end of your forceps) by wetting it and then dipping it in flour.

6.3) To hold excess wire in place, use a hot glue gun and place a dab of hot glue on top of the wires.
6.4) Quickly after applying the hot glue, use your “floured” flat edge and smush down hot glue. The purpose of the flour is to prevent the hot glue from sticking to your smushing tool.
Make sure the wires are all tidy on the header, sometimes adding a bit of extra super glue to secure loose portions of the wires must be done.

**Step 7: Surgery is complete! Clean-up time**

7.1) Put roach back in its terrarium and provide food and water. Recovery can take up to 2-4 hours. When preparing for demonstrations we typically do the surgery the night before, to allow the cockroach a full night to recover. The cockroach will be ready for the experiment by the following morning.
7.2) Put your tools away and discard of soiled materials. Don’t drink the water! Clean your surgery table and tools. Always remember to wash your hands!


Step 8: Testing your RoboRoach
The following morning, you can test the success of surgery by recording the neurons with a Spikerbox and/or stimulating the RoboRoach (via the RoboRoach app) and observing any behavioral response. To begin testing take your RoboRoach out of its terrarium. You may find cooling down the RoboRoach in the fridge for a couple minutes may make it easier to plug in the connector.

8a) To listen and/or see the electrical activity of the neurons in the antenna surrounding your recently implanted electrodes, plug in a male connector to the female connector on the roach’s head. Then attach two probes, one to the antenna connection and the other to the ground. Plug your probe set into your Spikerbox and turn it on.
You should hear nice spontaneous activity. Note: Sometimes the spikes are so high amplitude it clips out the speaker on the SpikerBox and you won’t hear anything. If this occurs, plug in an external speaker or headphones to hear your spikes. You can also do some experiments with this prep. Blowing on the roach causes broad neural activation. Try this setup on both the left and the right antenna, if you hear spikes then your surgery was successful. You can return the cockroach to its home terrarium to recover and until you are ready to begin your experiment.

8b) To test the microstimulation of the antennae, first plug the male connector of the RoboRoach PCB into the female connector on the roach’s head. If you haven’t already, download the RoboRoach app in the iOS App Store or the Google Play App Store (Update 12/20/2013: The Apple App Store has not approved the RoboRoach app for iOS. We are working hard to get this figured out. For now, we can provide early users a test version of the app, although the number of “testers” we can have is limited. The Android version of the RR app available now for phones with Android 4.3 (Jelly Bean) or later. For questions or requests, email hello@backyardbrains.com). Next, press the small black button on the left edge of the PCB, this will wake up the microcontroller so that you can connect with it using the RoboRoach app from your Bluetooth Smart-compatible device. Using the default stimulation settings, swipe either left or right on the screen and watch for any behavioral responses. If the cockroach turns towards the direction that you swiped, your surgery was successful. Good job! Your RoboRoach is ready for the next experiment. If there is no observable behavioral response, review you surgery record, learn from the experience and try again when you are ready. Don’t get discouraged, failure has a bright side. It provides opportunities to improve and is an important result when utilizing the scientific method. Albert Einstein (perhaps it was Thomas A. Edison) said it best, “I have not failed, I have just found 10000 ways that don’t work.”

Experiment, Learn & Share
- Check out the other RoboRoach Experiments!
  - Test out your new cyborg and learn about the behavioral effects of Neural Microstimulation with our second RR experiment. [coming soon!]
Learn how to make your own RoboRoach electrodes. [coming soon!]

- Share your Surgery Experiment experience with us.
  - Send us your results and worksheet to bill@backyardbrains.com
  - Tell us any tricks and/or tips you found helpful.
  - Let us know if you have any trouble, we are more than happy to help!
- Show and share your new neuroscience knowledge with your friends and family!

Tips & Tricks
- Keep a dry piece of paper towel handy and separate from the one you use to dry off the roach after removing from the ice bath.
- Keep your glue hole clean so you can be precise with the superglue.

Failures & Fixes
- Failure: GND electrode came loose
  - Fix: Verify that your roach is still anesthetized. Trim the tip of the wire to remove any glue or crumpled portion. Next take a lighter to the tip to burn off ~1-2mm of the insulation (will take a steady hand and careful eye). Then with your scissor trim off the bead of silver that forms on the tip of the electrode. Now, poke another hole, preferably away (opposite side of thorax) from the failed insertion point and redo step 3.
- Failure: Antenna electrode came out
  - Fix: Verify that your roach is still anesthetized. Trim the tip of the wire to remove any glue or crumpled portion. Next take a lighter to the tip to burn off ~1-2mm of the insulation (will take a steady hand and careful eye). Then with your scissor trim off the tiny bead of silver that forms on the tip of the electrode. Now, trim off any glue on the antenna and try again.
- Failure: Connector broke loose from pronotum
  - Fix: If the wires haven’t been damaged, a dab of super glue can fix this, otherwise, you will have to start over. To prevent this from happening next time, make sure the connector is sanded and cleaned of dust or water so the glue sticks well.
- Failure: Hot glue broke loose from the top of connector
  - Fix: If the wires haven’t been damaged, a dab of super glue can fix this, otherwise, you will have to start over. To prevent this from happening, make sure the connector is sanded so the glue sticks. Also, be sure to clean and dry the top of the connector before applying the glue. Additionally, since hot glue dries quickly, pack it down with the popsicle stick (with flour on the end) immediately after applying the glue.
- Failure: Cockroach moves slightly during procedure
  - Fix: Add more ice to your ice bath and allow the cockroach a few more minutes in it for full anesthesia. Also, prepare the tools you need for the next step while the cockroach is in the ice bath so that you can be as quick and efficient as possible on each step.
FAQs

- Where do I get more batteries?
  - Go to https://backyardbrains.com/products/roboroachbatteries

- Where do I get more electrodes
  - Go to https://backyardbrains.com/products/roboroachelectrodes

- What smartphones are Bluetooth Low Energy Compatible?
  - Supported iOS Devices: iPhone 4s+, iPod 5th generation+, iPad mini, iPad 4th Generation+.
  - Supported Android Devices: Motorola Droid Razr M; Nexus 4, 5, 7; Samsung Galaxy S3+ (many others when BLE is officially released)

  - It is free to download but currently in the review process for iOS.
  - The Android version will be coming out soon.

- Where can I get cockroaches?
  - If you live in the United States, we can send you a sturdy box of a dozen, healthy discoids, or discoid/cranifer hybrids. Cockroaches are the happiest when they aren’t in a shipping box for a long period of time. Therefore, we ship cockroaches out on Monday or Tuesday via 2-3 day USPS priority mail.
  - Go to https://backyardbrains.com/products/cockroaches

- Why?
  - 1 in 5 people will have a neurological disorder in their lifetime (11)
  - Providing affordable educational tools and experiments such as the RoboRoach can spread awareness and knowledge of neurological disorders and the current neurotechnologies.
  - Inspiring and exciting people to learn more about how their nervous system works and to pursue neuroscience and/or fields in STEM.

- What can you learn with the RoboRoach?
  - Neural control of Behaviour: First and foremost you will see in real-time how the brain responds to sensory stimuli.
  - Learning and Memory: After a few minutes the cockroach will stop responding to the RoboRoach microstimulation. Why? The brain learns and adapts. That is what brains are designed to do. You can measure the time to adaptation for various stimulation frequencies.
  - Adaptation and Habituation: After placing the cockroach back in its terrarium, how long does it take for him to respond again? Does he adapt to the stimuli more quickly?
  - Stimuli Selection: What range of frequencies works for causing neurons to fire? With this tool, you will be able to select the range of stimulation to see what works best for your prep. Is it the same that is used by medical doctors stimulating human neurons? You will find out.
  - Effect of Randomness: With the addition of a new “random” mode to our stimulus patterns you can experiment with nonperiodic stimulations. We, as humans, can adapt easily to periodic noises (the hum a refrigerator can be ignored, for
example). So perhaps the reason for adaptation is our stimulus is periodic. Now you can select random mode and see if the RoboRoach adapts as quickly... or at all!

- **Do cockroaches feel pain?**
  - Scientists are still figuring this one out. Since we don’t know for sure, we assume they do and we take all precautions to minimize wounding and the potential for pain. We send small amounts of current to the neurons. This method is called microstimulation, and is used to make nearby neurons fire action potentials or "spikes". This is not an electric shock, nor does it cause pain. We can verify this by the fact that the cockroach can adapt to the microstimulation in a few minutes, and ignore it completely, something that cannot be done with painful stimuli.

- **Can I use the RoboRoach to make my brother/sister/mom/dad/dog/cat/hamster into a cyborg?**
  - No. In the US there are rules, regulations and protocols for neural microstimulation on vertebrates(12). The control of the roach’s trajectory of movement works because the nerves in the antennae are being stimulated. The antennae are directly involved in navigation, and the interface to the antenna is much easier and simpler than navigational systems in other animals.

- **What are the specifications of the RoboRoach?**
  - Total Weight: <4.5g
  - Stimulation Frequencies: 1Hz-200Hz
  - Stimulation Pulse Widths: 1ms-500ms (Max pulse width is dependent on the Frequency)
  - Stimulation Gain: 0 to 100%
  - Stimulation Duration: 5ms to 1000s.
  - Communication Protocol: Bluetooth Smart (Low Energy)
  - Electrode: 0.003" bare, 0.0055" insulated, Silver wire
  - Battery: 16mm 1632 Coin Cell Battery
  - Use Time: 12 hours per battery
6) A. Bozkurt, A. Lal, and R. Gilmour, “Aerial and terrestrial locomotion control of life assisted insect biobots,” in 31st Annual International Conference of the IEEE EMBS, September 2009