**Closed-Loop Neural Control Using Deep Reinforcement Learning**

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BSC and ELB conceived the study, acquired funding, and wrote and reviewed the manuscript. BSC designed the closed-loop algorithms, performed surgeries, acquired data, and designed the protocol. ELB supervised the study

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BSC and ELB hold a provision patent (USPTO: 18/083490) related to the technology described in this protocol.

**Summary**

Closed-loop neural control is a powerful tool for both scientific exploration of neural function as well as a potent clinical tool to mitigate deficits found in open-loop deep brain stimulation. Reinforcement learning (RL) offers the ability to learn subject-specific neural dynamics in real-time. We present a protocol to integrate RL closed-loop control into neuroscience and neuromodulation studies. We also outline the types of research questions that can be addressed through closed-loop RL control.

For complete details on the use and execution of this protocol, please refer to Coventry et al and Coventry and Bartlett1

**Before You Begin**

The protocol herein describes the use of reinforcement learning to learn neural firing patterns and stimulation parameters to drive firing patterns to target states. Critical to algorithm performance is appropriate computational hardware setup and proper surgical preparation to ensure quality neural recordings. This section describes setup of RL algorithms and surgical preparation.

Reinforcement learning is a process by which an artificial “agent” learns optimal actions to take to navigate an environment while maximizing short and long-term rewards by repeated iterations through the environment. To perform reinforcement learning, the user has to define an environment in which the agent acts, an observation space quantifying the current state of the environment, and an action space quantifying actions that an agent can take. RL generates a “policy” function which maps optimal actions to take to maximize current and future rewards given the current state of the environment. In a neuroscience/neuromodulation context, an environment will encapsulate recorded neural signals, such as microelectrode single-unit recordings or surface EEG potentials. The observation space is then a measurement of the current state of the neural response, such as an evoked response from a stimulation or resting state dynamics. The action space encapsulates actions that an external stimulator can take. External stimulation can take the form of electrical stimulation through microelectrodes or naturalistic stimulators such as auditory generators or tactile whisker stimulators. The action space consists of stimulation parameters that are fed to the external stimulator to learn optimal policies.

The most potent forms of RL are actor-critic Q-learning models2,3. Q-learning quantifies the “goodness” of taking action a given state s as:

where is the current state and action respectively, the learning rate determines how fast new information overtakes older information and helps prevent instability in the learning process, the current Q value, the discount factor which determines the importance of future rewards, and quantifying the maximum future reward that can be achieved by taking action . In deep RL, deep neural networks are used to estimate Q-functions. It has been found that single Q-value RL tends to overestimate the value of taking actions, which is solved using two separate Q-neural networks, termed actor and critic, which updates the a policy function and an action-value function used to inform policy functions sperately4. In this implementation, we use a method called twin-delayed deep deterministic policy gradients5 (TD3) which represents a state of the art of actor-critic deep-Q learning in continuous observation spaces.

Our stimulator for this implementation is an infrared neural stimulation (INS) system. INS is optical stimulation method which utilizes coherent, mid-infrared (700-2000 nm) light to drive spatially constrained stimulation in nerves6–9 and neurons10,11. We utilize a custom made, open source INS stimulation system called INSight. INSight build documentation, instructions, and source code can be found at: <https://github.com/bscoventry/INSight>.

**Institutional Permissions**

To ensure both animal health and welfare as well as obtaining the highest quality data, all animal work should be approved by an appropriate institutional animal governing committee with all procedures following the appropriate ethical guidelines. All animal procedures in this protocol were approved by Purdue University Animal Care and Use Committee (PACUC protocol #120400631) and in accordance with AAALAC laboratory animal practice.

**SpikerNet, Deep RL setup**

**Timing: Varying, contingent on the availability of in-house computers**

This step describes the implementation of SpikerNet RL-based neural control algorithms.

1. Acquire an AI enabled computer. The authors suggest a Windows or Linux operating system-based computer with minimum intel i7 or AMD Ryzen 7 8700 CPU. Check respective company documentation for most up-to-date AI processor solutions. A minimum of 16 GB of ram is recommended, with higher providing better processing speed. Both Nvidia GTX/RTX series and Intel ARC A7x GPUs have PyTorch backends and are recommended. However, Nvidia GPUs have the benefit of wide scale implementation in PyTorch over Intel A7x solutions.
2. Install a Python package management software. The authors utilize Anaconda, but other solutions can work just as well.
3. Download the SpikerNet source code from the following repository:
   1. All source repositories can be installed automatically by running the following line in anaconda: . Packages can also be manually installed using Python pip.
   2. Test the SpikerNet distribution by running the following line of code: This runs a toy video game and tests SpikerNet’s RL system and associated packages.
4. Define the algorithm reward function. Reward functions encode the goals of closed-loop control through mathematical expressions. In this implementation, we define a mean-square error (MSE) reward of the form:

where are the measured and target responses respectively. Responses can be any measurable bio signal, such as a peristimulus time histogram (PSTH) with bins, EEG activity with time points, etc. This MSE reward “penalizes” observed responses which diverge from the target as evidenced by relatively small reward values and “rewards” measured values closer to the target with higher reward values. Our implementation measured reward from observed and target firing rate density functions from PSTHs estimated with Bayesian adaptive regression splines(BARS)12.

**Critical:** The choice of reward function is critical to SpikerNet performance. Choosing a reward or target function that is outside of physiological realizability will cause poor algorithm performance.

1. Define action space and action space bounds. The action space represents the range of stimulus parameters that can be chosen by the algorithm. For an electrical stimulator, this might be the stimulator current, pulse widths, and frequencies. Action spaces can be continuous (ie stimulus currents of 100.5, 94.5, 123.4 ) or discrete (ie mapping actions to individual stimulation classes such as electrical stimulator monophasic or biphasic modes). Our implementation uses an INS stimulator with a mixed continuous-discrete action space of number of stimulation pulses (discrete) stimulation laser power (continuous), pulse width(continuous), and interstimulus intervals(continuous). The declaration of action spaces is performed using the OpenAI gym command Box2d with parameter upper and lower bounds as follows:

1. #Setup action bounds: numPulses – number of optical pulses, stim – Optical power (mW), PW – Pulse width(ms), tBetPulses – Interstimulus interval(ms). np – numpy python toolbox.

2. Paramslow = np.array([numPulsesLow,stimLow,PWLow,tBetPulsesLow])

3. Paramshigh = np.array([numPulsesHigh, stimHigh, PWHigh,tBetPulsesHigh])

4. action\_space = gym.spaces.Box(low,high,dtype=np.float16)

Actions are clamped to fall between the declared bounds during search.

**Critical:** The choice of stimulus bounds should be made with stimulus safety considerations in mind. Stimulus limits should be constrained to well below ablation levels to ensure that SpikerNet does not damage neural tissue during search phases. This is contingent to the stimulation paradigm. For INS, we constrain pulse energies to per pulse13–16.

1. Define the observation space. The observation space describes the mathematical space of the observed biosignals. For neural recordings, the observation space is generally continuous. Bounds of the observation space generally follow the properties of the biosignal of interest. For example, PSTH responses from cortex have firing rates that are strictly positive (lower bound 0) with upper bounds generally set above maximum realizable firing rates. EEG responses, alternatively, contain positive and negative voltage values with analyses generally performed on negative and positive peaks. As such, observation space bounds should be aligned with maximum positive and negative values. Declaration of the observation space is similar to action space declaration:

1. observation\_space = gym.spaces.Box(low = obsLow, high = obsHigh)

Our implementation has an observation space that describes firing rate function estimates which has a space of (number of time bins, 1000).

**Note:** The TD3 reinforcement learning algorithm is specified for continuous observation spaces only. A discrete observation space will require a different deep Q learning method, such as double deep-Q networks3.

1. **Optional if using BARS:** Install the python-Matlab engine API to run Matlab scripts in python in real time. Instructions can be found at <https://www.mathworks.com/help/matlab/matlab-engine-for-python.html>

**Step-by-step method details**

**Animal or Subject | Rat Implantation Surgery and Recovery**

**Timing: 72 hours**

The purpose of this step is to instrument the animal with desired recording and stimulation devices. This step will vary based on the experimental question to be addressed with SpikerNet and the target neural networks to be studied. In this protocol, we describe implantation of stimulation optrodes into auditory thalamus and implantation of recording electrode arrays into layers III/IV of auditory cortex. The surgery can be easily adjusted for other neural targets. This step is not required if using SpikerNet for minimally invasive control, such as EEG applications. All implantation procedures must follow aseptic surgical technique.

**Surgical Station Preparation**

**Timing: 24 hours**

1. Assemble surgical tools into an autoclave-safe container. Table 1 describes the necessary tools for implantation.
2. Assemble stimulation and recording electronics and Teflon tweezers into a separate gas sterilization container.

Critical: The vast majority of electronics and Teflon surgical tools. will be critically damaged in autoclave sterilization. These tools should be processed by a gas or plasma sterilization, such as EtO processing.

1. Send surgical tools and electronics for sterilization.

**Note:** Autoclave times are usually 30 minutes-1 hour. Gas sterilization times are usually 24 hours.

1. On day of procedure, retrieve tools and organize surgical tools and electronics so that they are readily available during the procedure. Do not yet open sterile packs.

**Animal Preparation**

**Timing: 45 minutes**

The purpose of this step is to anesthesia and prepare the animal for implantation procedures. This step does not yet require aseptic technique.

1. Prepare a surgical record. Record should contain a short description of the procedure, anesthesia start and stop times, drugs administered, and a log for rat vital checks.
2. Weigh the rat and record to surgical log.
3. Ensure that a heating pad for thermostatic control is placed on the stereotaxic frame.
4. Spray down stereotaxic frame and microscope with 70% ethanol for contact sterilization.
5. Place drapes over heating pad and stereotaxic frame.
6. Pull doses of Buprenorphine and Ketamine/Dexmedetomidine cocktail based on animal weight for analgesia and anesthesia respectively.

**Note:** Ketamine/dexmedetomidine cocktail consists of of ketamine and dexmedetomidine. Buprenorphine doses are . All dosing should be approved by the institutional review board in consultation with veterinary staff.

1. Place rat into induction chamber and induce anesthesia using 5% isoflurane with 1.2 mL/min oxygen flow. Wait until loss of righting reflex.
2. Immediately after loss of righting reflex, administer the ketamine dexmedetomidine cocktail via an intramuscular (IM) injection.

**Critical:** There is a potentially lethal drug interaction between high doses of isoflurane and ketamine. Care should be taken to reduce isoflurane induction time. Animal should be monitored via ECG and pulse-oximetry to monitor potentially dangerous drug interactions. Dexmedetomidine reversal agents (anti-sedan) can be administered in case of critical vital drops.

1. Move the animal to the oxygen manifold and heating pad on the stereotaxic frame. Heat and oxygen (1.2 mL/min) should be administered rapidly after anesthesia induction. Begin monitoring vitals after placement into stereotaxic frame.
2. Monitor animal depth of anesthesia by routine application of toe-pinch responses. Toe-pinch response should be absent minutes after anesthesia injection. Proceed to the next step only when a surgical plane has been met.

**Critical:** Vital monitoring and depth of anesthesia is critical to animal welfare and recording quality post-surgery. ECG, SpO2, and toe-pinch response should be recorded atleast every 15 minutes. Updates of Ketamine can be given to maintain surgical plane.

1. Administer Buprenorphine intramuscularly. Buprenorphine should be administered atleast 30 minutes prior to initial incision to ensure analgesia throughout the duration of the procedure.
2. Apply eye lube with Q-tips to keep eyes lubricated and free of debris for the duration of the procedure.
3. Secure the animal into the stereotaxic frame using hollow ear bars. Hollow ear bars allow for application of auditory stimuli to the ear perioperatively to confirm electrode placement into primary auditory cortex.
4. Shave the top of the animals head using a standard electric razer.
5. Apply a triple wash of betadine interleaved with 70% EtOH using Q-Tips in the shaved region of the head. This serves to sterilize the skin at the incision site.
6. Perform a vital check before moving to surgical procedure.

**Surgical Procedure**

**Timing: 4 hours**

This step describes the device implantation procedure in detail.

1. Don sterile surgical gowns, gloves, and mask. The moment these are donned, the surgeon should consider themselves in a sterile field, taking care to not touch non-sterile tools and surfaces.

**Note:** Institution requirements vary, but a non-sterile assistant can be beneficial for maintaining sterility and help with donning sterile gowns and gloves.

1. Unwrap sterilized tools and place a sterile surgical drape over the animal.
2. Cut a hole in the surgical drape above the incision site using drape scissors.
3. Using a #10 scalpel, made an initial midline incision. The incision should be long enough to reveal bregma and lambda sutures.

**Note:** The procedure should be performed under a microscope for best precision.

1. Retract the skin flaps using a retractor or 4 Kelly forcepts positioned at the four corners of the incision site.
2. Gently remove the periosteum using a Fedi Perio chisel.
3. Monitor and control bleeding using a battery powered cautery.
4. Wash the top of the skull with sterile saline to reveal skull sutures.
5. Mount a Dremel with a sterile 1/8” bit to the stereotaxic frame.
6. Using stereotax microdrives, move the bit to above the bregma suture. Slowly lower the bit towards the surface of the skull, making adjustments in the medial/lateral and anterior/posterior directions so that the bit is situated at the intersection of the bregma suture and the midline suture. Zero the coordinates so that bregma serves as a references point.
7. Lift the bit off of the skull, and move the bit to the intersection of the midline and lambda suture. The medial-lateral and dorsal/ventral directions should read 0, indicating the rodents skull is flat relative to the stereotax. If there is significant deviation in either direction, reposition the rats head in the earbars and repeat steps 10-11 till flat.
8. Move the bit to anterior/posterior and medial lateral coordinates of the stimulation array. In our preparation, the stimulation optrode is placed in medial geniculate body (MGB) of auditory thalamus. This structure is located at -6 mm A/P, -3.5 M/L, and -6 D/V. Start the drill and slowly lower the bit till it makes contact with skull. Continue to slowly advance the drill until the craniectomy is complete. Administration of sterile saline can serve as a lubricant and help the drilling process. After the craniectomy is complete, cover the hole with gel foam soaked with sterile saline to prevent debris from entering the hole.

**Caution:** Care should be taken to ensure that the bit does not pierce the brain. This can be achieved by advancing the drill very slowly, paying attention to resistance in the stereotax microdrive and listening for slight increases in auditory drill pitch, indicative of increasingly thin portions of skull.

1. Drill 4 additional holes into the skull for bone screw insertion. Bur holes should be placed at equidistant locations around the skull to serve as stable, load bearing structures for a secure headcap.
2. Using a hand hex screwdriver, screw three stainless steel bone screws into 3 burr holes. Screws should fit tightly into the burr holes, but should not touch the surface of the brain. Screw a titanium bone screw into the remaining hole. The titanium screw will serve as the recording electrode ground and reference point. We recommend placing this screw at a distance from the recording array to ensure that common mode reference noise does not contain activity from the recorded area.

**Note:** Stainless steel screws can also serve as a ground/reference point, but we find titanium offers a superior ground/reference point for neural recordings with better signal to noise ratios.

1. Reflect the temporalis muscle to expose auditory cortex. The auditory cortex of the rat sits on the side of the head and is covered by the temporalis muscle. On the same side as the stimulation optrode craniectomy, use a scalpel to gently lift the muscle above cranial ridge. Use a Fedi Perio chisel to gently pull the temporalis muscle away from the skull. Gentle back and forth motion will loosen connective tissue. Once a significant amount of muscle is pulled away from the skull, use a Kelly forceps to reflect the muscle and expose the side of the skull.

**Caution:** The facial nerve of the rat runs within the temporalis muscle, and can be severed through the reflection process, causing loss of blinking post surgery. Extreme care should be taken to ensure that the temporalis muscle is not cut and that minimum area of muscle is pulled away from the skull.

1. Drill 4 holes centered at -6mm A/P, -5 mm M/L. Each hole should be separated by 2mm to accommodate a 2x2 mm recording array.
2. Create a craniectomy above auditory cortex by drilling a square connecting each of the 4 reference drill holes. Care should be taken to not accidentally puncture the skull into auditory cortex during drilling. Continue until the drill outline in the skull is very thin, as evidenced by transparent appearance of the skull.
3. Using a small rongeur, pinch the skull between the drill outline and gently pull the flap away from the skull. If the bone is sufficiently thin, this should occur with very little force.
4. Using a 25G needle with a bent tip, gently remove the dura. This is done by gently placing the needle on the surface of the dura, catching a protruding dura portion on the curved needle. The dura is semi white/transparent in appearance, and can be seen on the needle. Once caught, gently pull dura away from the surface of the cortex. Microbleeds from dura vessels can be managed by application of sterile saline and light pressure from a sterile dental spear.

**Note:** Most microelectrodes will buckle and break against the dura. Proper dura removal will ensure easy placement of recording arrays.

1. Cover the auditory cortex craniectomy with sterile saline-soaked gel foam.
2. Mount the zif-clip connector to the stereotaxic frame. Mount the recording array to the zif-clip connector on the stereotaxic frame and move the electrode so that it is parallel to the auditory cortex craniectomy. Advance the away so that it sits 1-2 mm above the gel foam.
3. Using Teflon, non-magnetic tweezers, tie the ground and reference wires to the titanium bone screw. It is best to start with a single wire and tie a slip knot around the titanium bone screw such that the wire makes a firm connection to the screw. Repeat with the second wire.
4. Carefully remove the gel foam from the craniectomy. Place a speaker on the contralateral ear coupled through the hollow ear bar. Start applying auditory gaussian noise bursts (80 dBSPL) through the speaker to the contralateral ear. These bursts serve as reference stimuli to target layers III/IV of auditory cortex.
5. Start a TDT recording program. Advance the recording electrode such that the electrodes are situated on the surface of auditory cortex. Zero the dorsal/ventral coordinate to serve as a reference to the surface of the brain.
6. Slowly advance the electrode in 50-100 steps, reducing stepsize as the electrode penetrates the brain.
7. Advance until consistent, low latency bursts of multiunit and LFP activity is observed. Bursts should be several orders of magnitude above baseline electrical activity. Layers III/IV should putatively lie at 750-1000 from the surface of the brain.
8. Once consistent, low latency activity is confirmed, seal the electrode to the skull by first applying a layer of qwik-sil around the recording arrays to protect the brain from sealing agents. Apply metabond from the electrode to the surface of the skull and the nearest bone screw. A sufficient layer of metabond should be applied to the array to ensure a strong connection of the electrode to the headcap. Care should be taken to not cover the stimulation optrode craniectomy or move the recording electrode during metabond application. The auditory guide tones can be stopped at this point.

**Caution:** A weak connection of the electrode to the headcap could result in instability of the electrode and loss of headcap during the recording window.

1. Remove zif-clip connector from the electrode and zif recording cable. Return the stereotaxic arm to its upright position. Mount the optrode holder and optrode to the stereotaxic frame.
2. Position the optrode above the stimulation craniectomy. Slowly advance the optrode in the dorsal/ventral coordinate till the tip of the optrode touches the surface of the brain. Zero the doral/ventral coordinate to serve as reference to the surface of the brain.
3. Slowly advance the optrode till it sits in the medial geniculate body (-6 mm D/V).
4. Apply metabond to the stimulation optrode, surface of the skull, and the bone screws.
5. Once metabond is dry, disconnect the optrode from the optrode connector and stereotaxic frame. Apply additional layers of metabond as needed.
6. Once metabond is dry and firm, complete the headcap by application of UV-curable dental acrylic. Headcap should be thick enough to provide a strong mechanical adhesion of the implanted devices to the skull.

**Caution:** UV light used in UV-curable dental acrylic is potentially damaging to the subject and the surgeons eyes. Ensure that surgical drapes are covering the rat’s eyes. The surgeon and surgeon assistant should wear UV-opaque safety glasses.

1. Apply a purse string suture around the headcap to ensure proper healing of the surgical window.
2. Once the surgery is completed, administer anti-sedan to reverse dexmedetomidine.
3. Remove the animal from the stereotaxic frame and make a measurement of post-surgery weight.
4. Place the animal on a heated pad in its home cage until volitional movement is observed.
5. Buprenorphine analgesia should be applied every 6-12 hours for 72 hours until rat recovery.
6. The animal should be weighed every 24 hours for 72 hours. Report to veterinarian if a weight loss of 20% post-surgery weight measurement.

**Note:** Long release Buprenorphine can also be used, requiring application every 36 hours. The animal should still be monitored throughout the recovery period.

**Key Resource Table**

|  |  |  |
| --- | --- | --- |
| **Resource or Reagent** | **Source** | **Identifier** |
| **Stimulation and Recording Devices** | | |
| Planar neural recording Array | Tucker-Davis Technologies (TDT) | ZIF2030-32 |
| Optical Stimulation Probe (if using INS) | Thor Labs | CFML22L10 |
| Concentric Bipolar Electrical Stimulation Probe (if using electrical stimulation) | Microprobes | CEA-200-SS |
| RZ-2 Bioamp Processor | TDT | RZ-2 |
| RX-7 Stimulator | TDT | RX-7 |
| **Computational Tools** | | |
| NVIDIA AI-ready graphics processing unit (GPU) | NVIDIA | GTX 10x, RTX 20x, RTX 30x, RTX 40x, RTX Titan |
| Intel or AMD AI ready processor | Intel/AMD | Intel i7, i9 AMD Ryzen |
| Python Distribution | Python Software Foundation | N/A |

**Troubleshooting**

**Problem 1:**

Running SpikerNet test programs returns a Box2D error.

**Potential Solution:**This is a common error for some systems using a continuous environment in genRL. To correct, install the most recent version of box 2D. In an anaconda prompt, run

conda install -c conda-forge gym-box2d

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