Video Article

Simultaneous Electrophysiological Recording and Micro-injections of Inhibitory Agents in the Rodent Brain

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Abstract

Here we describe a method for the construction of a single-use “injectrode” using commercially accessible and affordable parts. A probing system was developed that allows for the injection of a drug while recording electrophysiological signals from the affected neuronal population. This method provides a simple and economical alternative to commercial solutions. A glass pipette was modified by combining it with a hypodermic needle and a silver filament. The injectrode is attached to commercial microsyringe pump for drug delivery. This results in a technique that provides real-time pharmacodynamics feedback through multi-unit extracellular signals originating from the site of drug delivery. As a proof of concept, we recorded neuronal activity from the superior colliculus elicited by flashes of light in rats, concomitantly with delivery of drugs through the injectrode. The injectrode recording capacity permits the functional characterization of the injection site favoring precise control over the localization of drug delivery. Application of this method also extends far beyond what is demonstrated here, as the choice of chemical substance loaded into the injectrode is vast, including tracing markers for anatomic experiments.

Video Link

The video component of this article can be found at http://www.jove.com/video/52271/

Introduction

The inactivation of cortical areas and sub-cortical nuclei is important in the study of functional relations between various brain structures[2-4]. Recent literature has employed loss-of-function chemical or cryogenic techniques to study the role of brain structures[2,5]. In regard to pharmacological microinjections, small volumes of drugs can be administered into a brain region at a controlled rate while minimizing the collateral damage to the surrounding tissue[6,7]. This technique can be used to deliver specific agonists, inverse agonists or antagonists to study the effect of different pharmacological targets on neuronal activity. Such effects can also be studied by measuring changes in neuronal responses from distant locations, allowing researchers to study the relationships between different cortical and subcortical structures.

Here, we demonstrate the assembly of a device, the injectrode, capable of both recording electrophysiological signals and delivering small amounts of drugs at the target location. We demonstrate the capabilities of this system by injecting GABA, a common inhibitor of neuronal activity, in the rat superior colliculus. This region is sensitive to visual stimulation, which allowed us to use visually evoked multiunit activity to confirm injectrode localization. The reversibility of the inactivation was assessed by the recovery of normal neuronal activity following the end of GABA injection.

The ability to monitor multi-unit activity from the injection site allows for the fine tuning of the injection rates and volumes needed to achieve the desired pharmacodynamic response. Therefore, an advantage of this technique is the potential limiting of tissue damage caused by microperfusion, since the smallest effective volumes are injected. The proposed protocol provides a cost efficient method for generating the disposable hardware necessary for conducting experiments where drug delivery and local neuronal activity recording is desired.

Protocol

NOTE: All procedures were performed in accordance with the directives of the Canadian Council for the Protection of Animals and the Ethics review board of the Université de Montréal.

1. Assembly of the Recording-injection Pipette

1. Pull an approximately 7 cm long glass capillary (1 mm outer diameter) using a pipette puller.
2. Break the tip of the capillary and check the aperture under a light microscope. Confirm that the inner diameter is between 30 µm to 40 µm.
3. Insert a 7 cm long silver wire into the glass capillary with approximately 1 cm protruding from the non-tapered end of the glass pipette.
4. Bend the excess filament orthogonally to the glass capillary.

5. Apply a droplet of flexible plastic adhesive on the shaft of a 30 G hypodermic needle.
6. Insert a 30 G hypodermic needle in the glass pipette according to the schematics presented in **Figure 1**.
7. Add a second coating of glue to ensure a proper seal from the junction between the glass pipette and the hypodermic needle.
8. Leave the pipette to dry with the tip facing upward for about 12 hr to ensure proper curing of the glue. The finished result is shown in **Figure 2**.

NOTE: This procedure is done on acute experiments and sterilization of the pipette tip is not required.

2. Animal Preparation

1. Place the rat in an anesthesia box.
2. Induce anesthesia using 4% isoflurane for 5 to 10 min.
3. Place the animal on a stereotaxic table with heating pad and rectal probe to maintain a body temperature of 37 °C. Use a nose cone to maintain anesthesia with 2% isoflurane. Secure the rat's head using ear bars and teeth holder.
4. Apply ophthalmic ointment or eye drops, which include 1% Atropine drops to aid in pupil dilation. To prevent dryness, apply lubricating drops approximately every 30 min.
5. Shave the head and clean it with 10% povidone-Iodine.
6. For local anaesthesia, inject 0.5 ml of 2% lidocaine under the scalp in 2-3 locations by lifting up the skin and inserting the tip of the needle.
7. Confirm adequate level of anesthesia by performing a toe pinch and observing the lack of movement. In addition, monitor the heart rate to ensure that it is within normal values (300 to 400 beats/min).
8. Incise the scalp in a straight line along the median with a #10 scalpel blade to expose both the coronal and sagittal sutures.
9. Reveal the Lambda and Bregma points by pushing aside the tissue that is covering the cranium with a surgical spatula.
10. Level the cranium so that Bregma and Lambda positions are on the same plane.
11. To set the reference point, use a stereotaxic device with a mounted glass tube to set it right above Bregma. This will be the "zero" for the antero-posterior and medial-lateral measurements coordinates.
12. Set the point of interest by moving the stereotaxic mount to the required coordinates, note the stereotaxic coordinates and draw a square around the target area that marks where the craniotomy will be performed.
13. Use a surgical drill with a sterilized drill along the marked square slowly without pressure to slowly remove the bone material. Be careful not to drill too long in the same area, as it will produce heat and cause lesions on the cortex.
14. When the bone delimiting the craniotomy has become sufficiently thin, carefully remove the cranial section with tweezers to expose the cortex.
15. Frequently irrigate the exposed cortex with artificial cerebral spinal fluid to prevent tissue desiccation.
    NOTE: Dura mater removal is unnecessary on the rat as the tip of the injectrode is sturdy enough to penetrate.

3. Filling and Mounting of the Injection System

1. Fill the 5-10 µl microsyringe by aspiration with mineral oil.
2. Fill the hypodermic needle with the fixed glass pipette with a solution of 0.5% Chicago Sky Blue (CSB) and 300 µM γ-aminobutyric acid (GABA) or a solution of 2% lidocaine with 0.5% CSB [9]. Dilute all solutions with saline.
    1. In the case of an abundant substance, fill using a regular syringe and use the usual precautionary techniques to avoid the formation of air bubbles.
    2. In the case of more expensive substances, use mineral oil to fill the injectrode and the chemical agent can then be introduced by aspiration. As the density difference between mineral oil and water is relatively high, this substance is a good candidate for the injection of aqueous solutions.
       NOTE: A dye can be added to confirm the separation between both liquids.
3. To fill the injection pipette, fill a 1 ml syringe with the solution by aspiration and then slowly inject the solution into the injection pipette.
4. Pay careful attention for leaks in regions indicated in **Figure 1** by swabbing these areas clean and observing leakages by further injecting the solution slowly with the syringe.
5. Remove the 1 ml syringe. When doing so, be sure to keep light pressure on the plunger so that the vacuum does not remove the solution from the injection pipette.
6. Fill the microsyringe with mineral oil by aspiration and attach it firmly to the filled injection pipette, then carefully wipe away any excess solution from the assembled injectrode with gauze.
7. Verify that the tip is not blocked by injecting a very small volume, enough to see a small drop forming at the tip of the glass pipette.
8. Mount the injectrode on the micropump system and ensure that it is well fixed.
9. Carefully position the injectrode tip at the target coordinates and lower the tip to the surface of the cortex.
10. Slowly lower the injectrode using the stereotaxic apparatus to the target structure (superior colliculus in this case) using the appropriate anatomical coordinates.
11. Cover the exposed cortex with warm agar to prevent tissue desiccation.

4. Injection and Reversible Inactivation

1. Set the microinjection pump to inject 400 to 800 nl at 40 nl/min and press Run to start the injection. Note that spike rate will show reduction during the injection.
   NOTE: In the experimental setup, neural activity recovered within an hour after the end of GABA delivery. Any calibrated mechanical device can be used to apply pressure on the microinjection syringe in order to conduct the injection.
2. After acquisition of the electrophysiological data, euthanize using a method approved by the local Animal Ethics Community.

Representative Results

The construction of the injectrode is illustrated in **Figure 1.** A silver wire (C) is fed into a glass pipette (D) with a portion of the wire bent and protruding out from the opening. A 30 G needle (B) is attached and sealed to the opening of the glass pipette with glue. After the pipette has been filled with the injection substance, a glass micro syringe (A) is attached to the needle. It is important that there is a good seal where the micro syringe connects with the needle (E) and where the silver wire protrudes from the glass pipette (F). **Figure 2** shows a photograph of what the injectrode looks like after completing assembly.

Visually evoked multiunit activity were obtained in the superior colliculus following a 300 msec flash to the contralateral eye as illustrated in **Figure 3**. Upon the injection of GABA, spiking activity in response to a flash stimulus was suppressed. visually evoked multiunit activity typically returned between 45 to 60 min after injection has ceased.

Figure 4 illustrates the setup of the microinjection system. The injection pump controller allows the user to specify the settings for injection. A spring electrical connector connects the silver wire that protrudes from the glass pipette. The connector leads to a head stage with ground and reference electrodes and then plugged into an amplifier. An analog/digital (A/D) interface is used to acquire the electrophysiological data, and a speaker is used for complementary audio monitoring of neuronal activity.

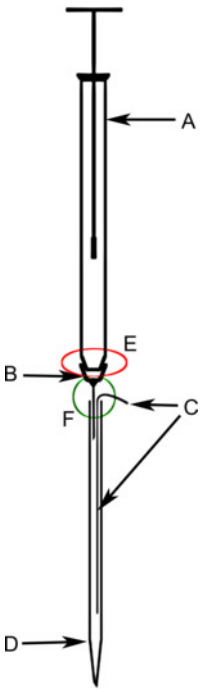

Figure 1: Schematic representation of the injectrode assembly. A micro syringe (**A**) is attached to the recording-injection pipette which consist of a 30 G hypodermic needle (**B**) adhered to a silver wire (**C**) inside a glass pipette (**D**). Regions circled (**E**-**F**) highlight areas that may be susceptible to leaks.

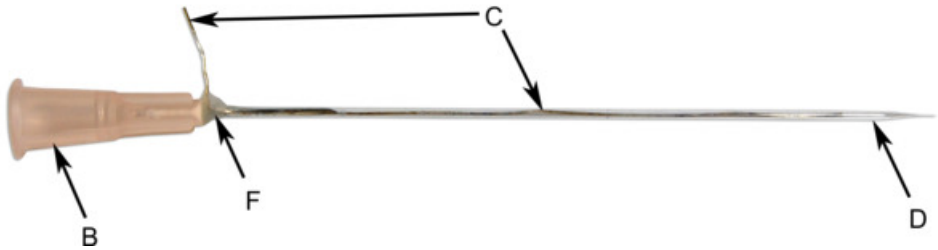

Figure 2: A photo of the constructed pipette using a 30 G needle (**B**), waterproof adhesive glue (**F**), a silver wire (**C**) and a glass pipette (**D**).

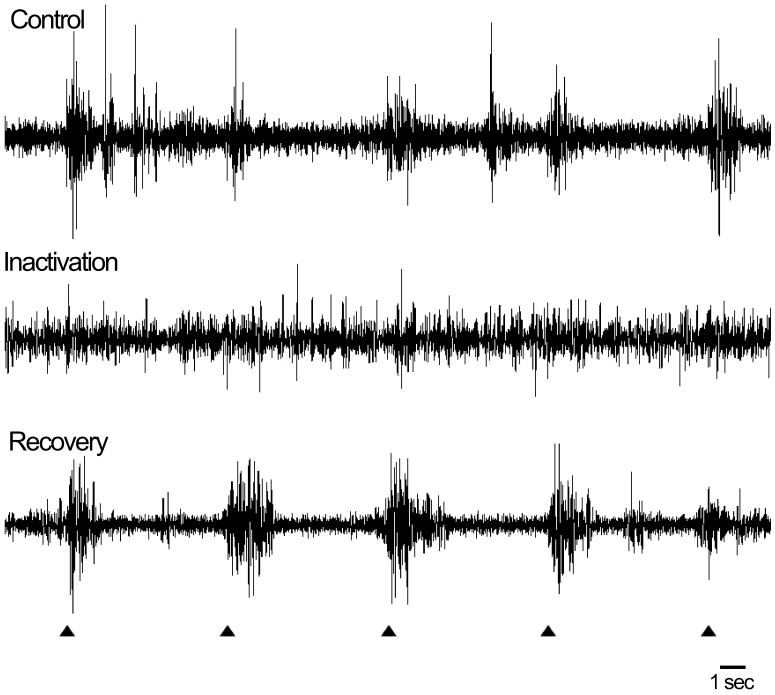

Figure 3: An illustration of the inhibitory effect of the injection of GABA (300 µM) on visually evoked multi-unit activity in the superior colliculus, arrows indicate flash onset. Electrical signals were filtered using a band-pass filter set between 30 and 3,000 Hz.

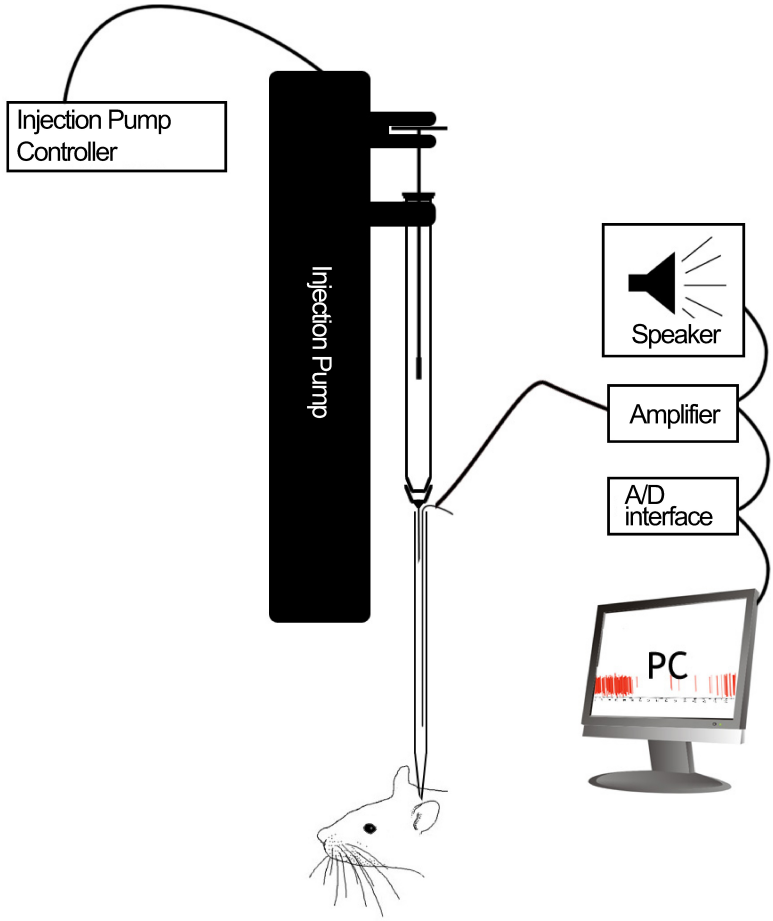

Figure 4: Schematic representation of the complete micro-injection system.

Discussion

The proposed protocol was designed to solve the challenges arising from current reversible inactivation methods. Specifically, this project aimed at refining the methods used for chemical microinjections of substances modulating neural activity, particularly in deep brain structures. A technical challenge emerging from this type of setup is the need for both probes to be colocalized in the same restricted space *in vivo* in order to derive precise recordings at the injection site. This issue can be overcome by using devices, such as the one presented here, which are capable of both injection and recording at the same site. Alternative methods include the use of devices based on gas pressure pulses. Such tools have been available for many years, but the use of a compressible intermediate reduces the control over injection rates and volumes, two parameters that are important to control to insure reversibility. Other methods such as iontophoretic injection systems are also available, but the diffusion dynamics of the liquid are different versus bolus injection, reducing the potential range of inactivation. These methods have the advantage of having a spherical diffusion pattern as opposed to the elliptical pattern observed for micro-injections[7]. Hence, the choice of the inactivation method should be planned according to the target region and the experimental design. Even though commercial alternatives exist, the proposed protocol provides a cost efficient manner of monitoring the pharmaceutical substance delivery as well as allowing for a high degree of customization. Such freedom in the crafting of the injection device favors a large range of experimental flexibility and tuning for specific application contexts.

With regards to the proposed protocol, the critical step is the process of filling the glass pipette. Air bubbles should be avoided, as air compression will render the monitoring of injected volumes intractable. A very minimal resistance should also be felt when manually pushing liquid through the pipette, confirming free flow in the system. An absence of liquid with manual injection may indicate a leak in the system or incorrect pipette preparation resulting in an obstructed tip. The impedance of the pipette should also be measured in order to obtain the desired type electrophysiological recording (LFP, evoked potentials, multi-unit activity, *etc.*), as larger tips sizes will result in lower impedances.

If the injection is successful, a volume of 400 to 800 nl of the 300 µM GABA solution or the 2% lidocaine solution is enough to abolish spiking activity. To have an idea of the injection spread in space and time, agar can be used to simulate nervous tissue. The spread of the injection can then be easily observed with a CSB solution. After simulations, it is essential to characterize injection spread histologically through the use of dyes such as CSB, by autoradiography using radiolabeled drugs or by using metabolic approaches such as glucose autoradiography as indirect proxies to measure activation or inactivation of neural activity[1].

It is also important to note that fast injections (≥100 nl/min) will likely result in lesions making full reversibility unattainable. A major advantage of the proposed protocol is the potential of integrating the injection system with software that would feedback-control the injection rate for a set neuronal activity level. Such an implementation would allow researchers to focus on the inactivation (or activation) parameters rather than on technical parameters such as injection rates or volumes while delivering only the right amount of drug for the considered application. This would minimize probe displacement by optimizing the required drug volume, allow for more time-sensitive control of the drug delivery, favor reproducibility and allow direct-paired comparison of data.

This technique combines a system for substance delivery and recording of electrophysiological signals. We demonstrated its efficacy by using the recording capacity of our pipette to functionally locate the superior colliculus by inducing trains of multi-unit activity using flash stimuli[11]. During inactivation, multi-unit activity diminished and gradually recovered after injection offset. Reversible inactivation techniques, such as the one presented here, provide considerable advantages over mechanical or chemical lesions techniques that provide absent or poor recovery[3]. Reversible inactivation techniques reinforce the statistical significance of experiments since paired comparisons are possible[3], thereby eliminating idiosyncratic differences. We have developed a cost efficient and customizable technique that allows precise control over the duration of the substance delivery and the robust probing of a target cerebral area.

Disclosures

We have nothing to disclose.

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