Title: Single-unit *in vivo* recordings from the optic chiasm of rat

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Short Abstract
Retinal ganglion cells transmit visual information from the eye to the brain with sequences of action potentials. Here, we demonstrate how to record the action potentials of single ganglion cells *in vivo* from anesthetized rats.

Long Abstract
Information about the visual world is transmitted to the brain in sequences of action potentials in retinal ganglion cell axons that make up the optic nerve. *In vivo* recordings of ganglion cell spike trains in several animal models have revealed much of what is known about how the early visual system processes and encodes visual information. However, such recordings have been rare in one of the most common animal models, the rat, possibly owing to difficulty in detecting spikes fired by small diameter axons. The many retinal disease models involving rats motivate a need for characterizing the functional properties of ganglion cells without disturbing the eye, as with intraocular or in vitro recordings. Here, we demonstrate a method for recording ganglion cell spike trains from the optic chiasm of the anesthetized rat. We first show how to fabricate tungsten-in-glass electrodes that can pick up electrical activity from single ganglion cell axons in rat. The electrodes outperform all commerical ones that we have tried. We then illustrate our custom-designed stereotaxic system for i*n vivo* visual neurophysiology experiments and our procedures for animal preparation and reliable and stable electrode placement in the optic nerve.

Protocol:

Part I: Introduction

Part II: Fabrication of Tungsten-in-Glass Electrodes

1.) A graphite ring is placed in a beaker filled with a concentrated solution of sodium nitrate (0.71g/mL) and potassium hydroxide (0.34g/mL) dissolved in water[1], and the beaker is placed under the sewing arm of a modified sewing machine.
2.) Tungsten wire (0.5mm diameter, 6cm length) is taped to the sewing arm so that it is partially immersed (1-2cm) in solution.
3.) 2V are applied across the tungsten wire by attaching a positive lead to the sewing arm and a negative lead to the graphite ring.
4.) The sewing machine is turned on and the tungsten wire is dipped repetitively into solution at a rate of ~4Hz for 2 min, which electrolytically etches the metal to a sharp point.
5.) The etched wire is placed on a manual micromanipulator and the point aligned under microscopic guidance (40x magnification) with the shaft of a glass pipette (0.25mm OD, 0.175mm ID) pulled to a 1μm tip with a glass puller. To prevent movement during wire insertion, the glass is temporarily secured to the microscope stage with putty.
6.) The wire is carefully advanced into the unpulled end of the glass, down the shaft, and through the 1μm opening. A small piece of glass is seen to break off the tip in the video, indicating a tight fit of the tungsten in the glass. The wire should extend around 5-10μm beyond the tip for rat optic fiber recordings.
7.) Cyanoacrylate is applied to the unpulled end of the glass and after hardening the electrical impedance is measured, where 1MΩ electrodes generally work best.

Part III: Stereotaxic Setup and Spike Train Recordings

1.) The stimulus display system is illustrated in the video, along with the stereotaxic apparatus and microdrive for positioning and lowering the electrode into the brain.
2.) To perform retinal spike train recordings the rat is anesthetized and later paralyzed. Standard procedures (not illustrated) are used for surgical insertion of a femoral vein cannula for drug delivery and a tracheal (Y-tube) cannula for mechanical ventilation during paralysis. The experiments are terminal for the animal.
3.) After preparatory procedures, the animal is placed on the heating blanket and the head is fixed with ear and tooth bars into stereotaxic position, as defined by a rat brain atlas[2].
4.) The rectal probe is inserted, which controls the heating blanket via a homeothermic control unit that maintains body temperature at 37°C.
5.) Metal needles are used as leads to measure the electrocardiogram and monitor heart rate.
6.) The skin is opened on top of the skull and the intersection of skull plates (bregma) is exposed. A custom-designed crossbar frame is set in place, a circle is marked on the skull around begma through a hole in the crossbar, and the crossbar is temporarily removed.

7.) The stereotaxic system is calibrated by positioning the guide needle over bregma. A 5-mm hole is drilled into the skull at the marked location and the bone is removed, exposing the brain. A thin ring of putty is placed around the opening in the skull to form a seal around the hole in the crossbar, which is reset into place.
8.) If the animal has been at a stable plane of anesthesia for awhile, it is injected with paralytic in order to prevent eye movements during data collection. After breathing stops the animal is mechanically ventilated. A custom program tracks ventilation and physiological parameters throughout the experiment and informs the experimenter if compensatory action is needed should any parameters go outside normal levels.
9.) The guide needle is backloaded with the tungsten electrode and lowered into the brain. After brain penetration the crossbar hole is filled with agar (not shown), and the microdrive assembly is clamped to the crossbar for mechanical stability. The tungsten electrode is then slowly advanced (1µm/sec) out the guide needle until the tip is about 8-9mm below the skull, where the optic chiasm is located.
10.) Spike trains belonging to retinal ganglion cells are identified by their response to visual input. Upon isolation of a single optic fiber the receptive field of the recorded cell is mapped in visual space and its response properties are characterized with drifting gratings or other visual patterns of interest. The stability of the system allows for single-unit recordings of several hours[3].

Materials:

Adult Brown-Norway rats (250-400g) were purchased from a commercial vendor and housed under a regulated (12/12) light/dark cycle. Anesthesia was induced with an intraperitoneal injection of ketamine hydrochloride and xylazine (70 and 2 mg/kg, respectively, Henry Schein Inc) and maintained for the duration of the experiment with an intravenous infusion of ketamine and xylazine (30 and 1 mg/kg/hr) mixed with dextrose, saline, and gallamine triethiodide (40 mg/kg/hr, Fischer Inc) through a catheter (0.13mm OD, Small Parts Inc) in the right femoral vein. Gallamine is included in the mixture to paralyze eye movements. The infusion rate of the pump (WPI Inc) was adjusted as needed to maintain a stable plane of anesthesia as assessed by heart rate and blood pressure variability. After paralysis, the animal was mechanically ventilated (Harvard Apparatus, Model 683) through a tracheal cannula at 60-80 breaths/min (2cc volume), with the rate adjusted as necessary to maintain end-tidal CO2 measured with an in-line capnometer (Novametrix Inc, Model 710Sp) at 30%. Body temperature was regulated via a homeothermic blanket control system (Harvard Apparatus Inc). Body temperature, end-tidal CO2, heart rate, and blood pressure were continuously monitored throughout the experiment by a LABVIEW program. Visual stimuli were presented on a Sony Multiscan 17e CRT monitor (mean luminance of 30 cd/m^2) running at 100 Hz with a resolution of 800x600 pixels. Data acquisition and monitor output were controlled with custom software written in Matlab and LabView in conjunction with a video image processor (Cambridge Research Systems Inc, Bits++) and the Psychophysics Toolbox[4]. The animal viewed the stimulus display (40.4 x 30.2 cm) at a distance of 16.5cm through contact lenses (Ocular Instruments Inc), and ophthalmic solution was applied periodically during the experiment to keep the eyes moist. The visual field accessible to stimulation was maximized by reverse mounting the animal in a stereotaxic apparatus (Stoelting Co) elevated 14cm above the surface of a floating table (TMC Inc) and setting the monitor on a custom-designed sled that moved on the table along a ±100 deg arc about the nose. This provided a repositionable display field that extended 60-deg above and 35-deg below eye level and ±42-deg laterally from the center of monitor. Tungsten electodes were fabricated with wire obtained from Small Parts Inc and custom borosilicate glass obtained from Friedrich and Dimmock Inc. A standard micropippete puller (Sutter Instruments, Model P-97) was used to shape the glass tip. The electrode impedance tester was purchased from Bak-Electronics. Electrodes were advanced with a motorized

microdrive system (Newport Inc, StepperMike). Electrocardiogram and optic nerve signals were amplified and filtered with a high input impedance multielectrode amplifier (FHC Inc, X-Cell 3x4). Heart beats were detected with a window discriminator (WPI Inc, Model 121). Nerve action potentials were detected and time stamped with 0.1ms resolution by a digital spike discriminator (FHC Inc, APM). The spike discriminator was gated by a trigger signal from the video image processor so that spike times were locked to the stimulus.

Discussion:

Optic fiber recordings are an attractive approach for addressing experimental questions about retinal information encoding and transmission that require an intact eye. Moreover, the signaling properties of both eyes can be studied in virtually the same physiological state if the electrode is positioned in the optic chiasm or tract where the activity of crossed and uncrossed optic nerve fibers can be recorded with a single electrode penetration. Optic fiber recordings are common in cat but not in other popular animals models used in vision research, such as rodents perhaps owing to their small size. We have tried a variety of commercial electrodes of similar impedance and material, none of which were successful at picking up single fiber activity in rat, let alone recording it for several hours like our electrodes can. This implies that the particular geometry of our electrodes, which have a long, sharp tip as opposed to the wide blunt tip of typical commercial ones, is important for reliable and stable isolation of ganglion cell axon spike trains. In addition to showing how to fabricate these electrodes, we illustrate our custom-designed stereotaxic system for *in vivo* visual neurophysiological research. The system is constructed to protect the high impedance microelectrode from environmental vibration and from electromagnetic noise. This is critical for recording the small action potentials produced by axons (as compared to cell bodies) for a prolonged period of time, especially with a computer monitor positioned nearby. It does so with exceptional stability and signal-to-noise ratio, with typical recording times of an hour or more and noise levels on the order of tens of microvolts. These features make the setup particularly useful for vision researchers aiming to record *in vivo* from nerve fibers of the retina or other brain regions.

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