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INTRACELLULAR RECORDING AND STAINING IN THE ACUTE IN VIVO RAT USING SHARP ELECTRODES

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Introduction

For a number of years now we have been studying the discharge properties of cells in the hippocampal formation (HPC) and related structures in relation to the simultaneous occurrence of the HPC field activities of theta and large amplitude irregular activity (LIA) (Bland, 1986). Colom and Bland (1987) developed a system for classifying theta-related cells with the assumption that such cells participate in the mechanisms underlying oscillation and synchrony (theta) in limbic and associated structures. An important aspect of our studies has been the necessity of establishing the morphological identity these theta-related cells. In order to do this, we utilized intracellular recording techniques with sharp electrodes in urethane-anesthetized rats, first classifying the cells during the occurrence of spontaneous field activities and then staining them with neurobiotin following completion of the experimental protocols. In this article we describe our methodology for intracellular recording and staining using sharp electrodes in the acute in vivo rat. At this point we would like to acknowledge the very important advice we received years ago on this topic from our "Spanish-Uruguayan" connection, Washington Buno, Angel Nunez and Elio Garcia-Austt.

Methods

Acute Surgery

We have found that the weight of the rat is critical, the optimal range being 125 grams to 130 grams (100 grams is too small for tracheotomy and recording success diminishes greatly above 130 grams). Animals are first inducted in a halothane anesthesia chamber at 2.5 MAC and the switched to a facemask with the vaporizer set around 2.0 MAC. Under stable anesthetic conditions a tracheal tube is inserted, followed by insertion of an external jugular vein cannula. Wound points are treated with xylocaine and the opening is sutured. While observing the rat, halothane anesthesia is terminated and then carefully the switch is made to intravenous urethane anesthesia. Details of this procedure may be found in Bland and Oddie (1998). Once the rat is stabilized on urethane it is placed in the stereotaxic instrument. The skull is then exposed and leveled. The procedure we describe below is for providing recording access to the dorsal hippocampus. At right angles to the midline make 3 marks, one in the middle for the electrode hole at 3.3 mm, one in front at 2.3 mm and one behind at 4.3 mm. Make another mark parallel to the midline at 2.2 mm from the midline. These serve as reference marks for the bone removal (see Figure 1). At this stage all locations of recording electrodes should also be marked on the skull.

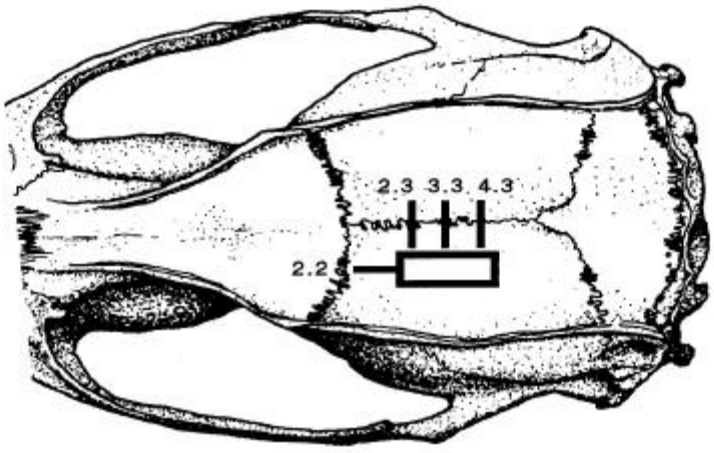

Figure 1. Diagram of the skull illustrating the markings for the removal of the bone.

Once the reference and indifferent electrodes are secured, make a dental acrylic circle on the skull encompassing the whole recording area on the left side of the skull. This will serve to form the pool for holding either glycerine, or later when recording, distilled water (see Figure 2).

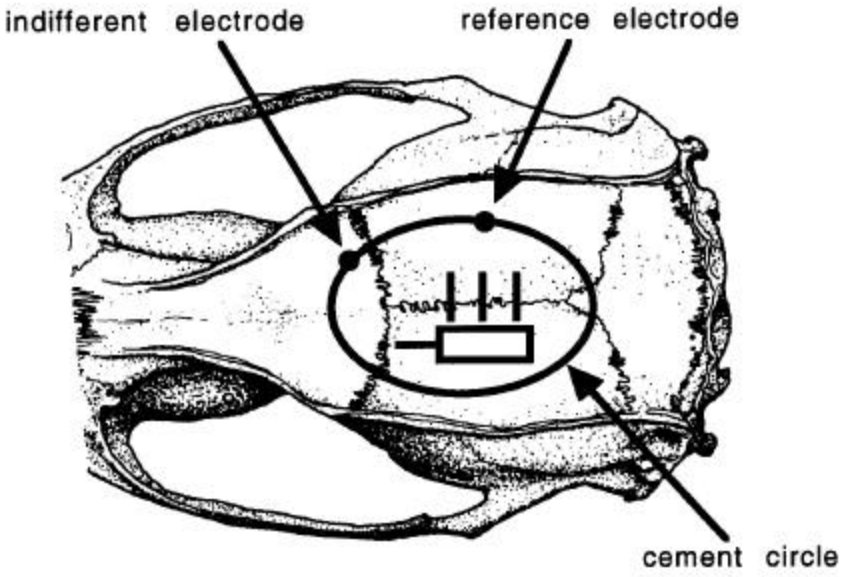

Figure 2. Diagram of the skull illustrating the location of the dental acrylic circle.

At this point the bone window can be drilled out using a ¼ carbide drill bit. The dimensions are approximately 3.0 mm long by 2.0 mm wide. Center this around the mark for the location of the glass recording track. Do not drill out the hole for the glass recording track at this point. After carefully removing the bone, turn it upside down and place it over a convenient place on the skull. Fill the well with glycerine to keep the brain moist. Using the same carbide drill bit and holding the bone fragment down with a pair of forceps, drill a hole in the center of the bone. Remove any rough edges from the bone fragment by polishing with the drill bit. Place the bone fragment aside in a convenient location and proceed in carefully removing the dura using a # 26 syringe with a small hook in the end and a pair of fine forceps. Carefully observe the position of the large blood vessels since this will determine the exact location of the bone fragment (i.e. avoid positioning the bone fragment such that the electrode hole ends up over a large blood vessel). With the layer of glycerine in the pool and using fine forceps, place the bone fragment **upside down** in the hole in the skull with the long side oriented to the midline.Gently push it under the skull at the midline enough to allow it to be pulled back under the lateral edge. Finesse the location with respect to blood vessels, as discussed above. Note that by placing the bone fragment in the skull upside down the slight convexity of the bone fragment makes it an effective pressure plate and it also removes the necessity of using glue.

Leveling procedure

The objective is to elevate the rat's body with respect to the head in order to minimize the pressure differential between the body cavities and the skull cavity. We achieve this by placing the rat on a scissor jack and elevating the body while observing the pulse pressure in the electrode hole as shown by the movement of the glycerine in the hole. When the optimal elevation is achieved, tie some silastic tubing around the back legs and after firmly stretching the body; anchor the silastic tubing to the end of the stereotaxic frame.

Electrode preparation

Glass micropipettes are pulled and backfilled with a solution 1 molar potassium acetate and 2% Neurobiotin (Vector Laboratories). When the tips are filled, pressure fill with Neurobiotin to the level of the shank, using a Hamilton 150 microliter gas tight syringe and then pressure fill the remaining shaft of the electrode with 1 molar potassium acetate. The optimal resistance of the microelectrode is between 75 and 80 megohms (see Figure 3).

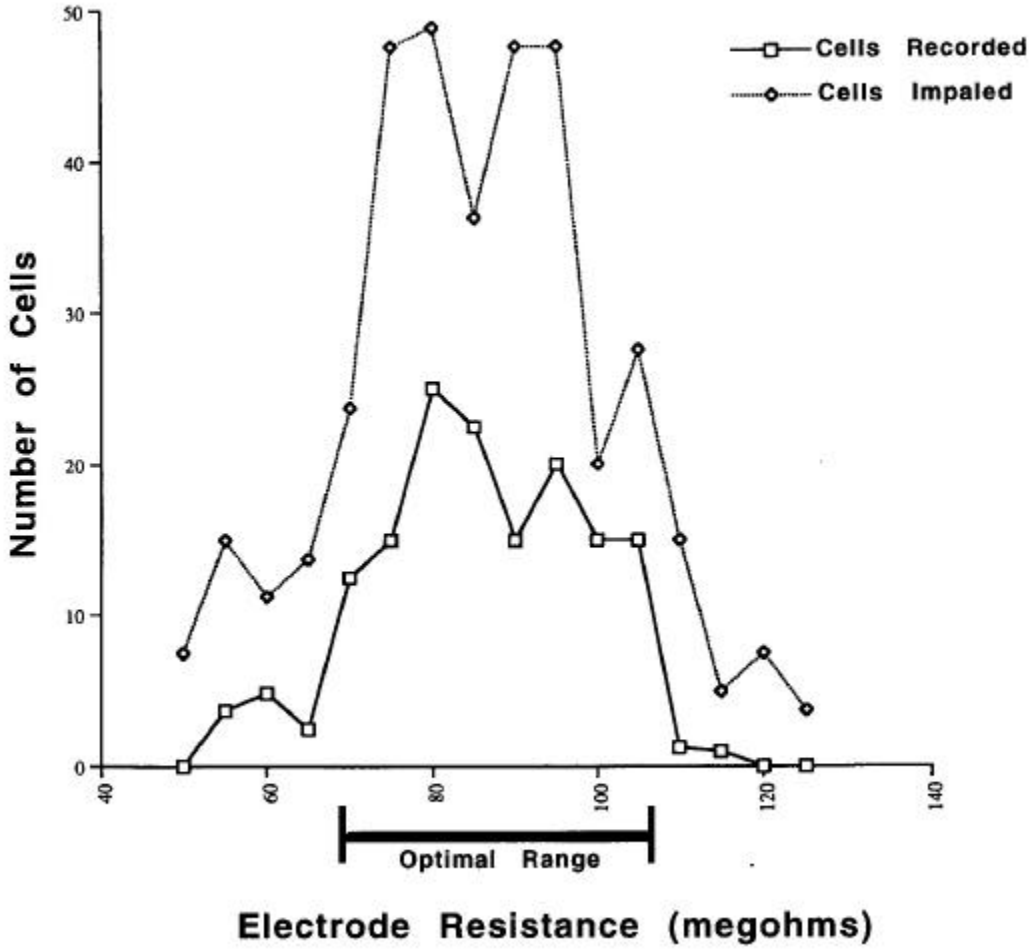

Figure 3. Graph relating the number of cells impaled and number of cells successfully recorded, in relation to the resistance of the electrodes.

Electrode lowering procedures

After trying a number of micropositioners we determined that our best success came with the use of the Kopf 660 micropositioner. The total electrode travel distance in our experiments is 4.5 mm. First, gently suck away the glycerine from the pool and manually lower the electrode in close proximity to the hole, but not on the brain surface. Switch to **Run:** speed – 4mm/low range and the green button – 4 microns/sec. After hearing the microelectrode contact the brain surface, reset the counter button to zero and continue with the same settings until the electrode is at the level you wish to begin recording. As the electrode is advancing, add **distilled** water to the pool to keep the brain moist. Switch to **bursting mode at a setting of 2 micron steps**, keeping the same speed and range the same. Begin current pulses, balance the bridge and record the electrode resistance from the bridge balance control. Adjust the capacitance until it "rings" and then back off slightly until ringing stops. We use the "buzz" technique for cell penetration, using the shortest buzz duration and only buzzing when the electrode is not moving. Upon cell penetration, hyperpolarize until the cell is stable. We generally use an electrode for a maximum of three tracks. Figure 4 shows an example of a layer 5 neocortical pyramidal cell recording.

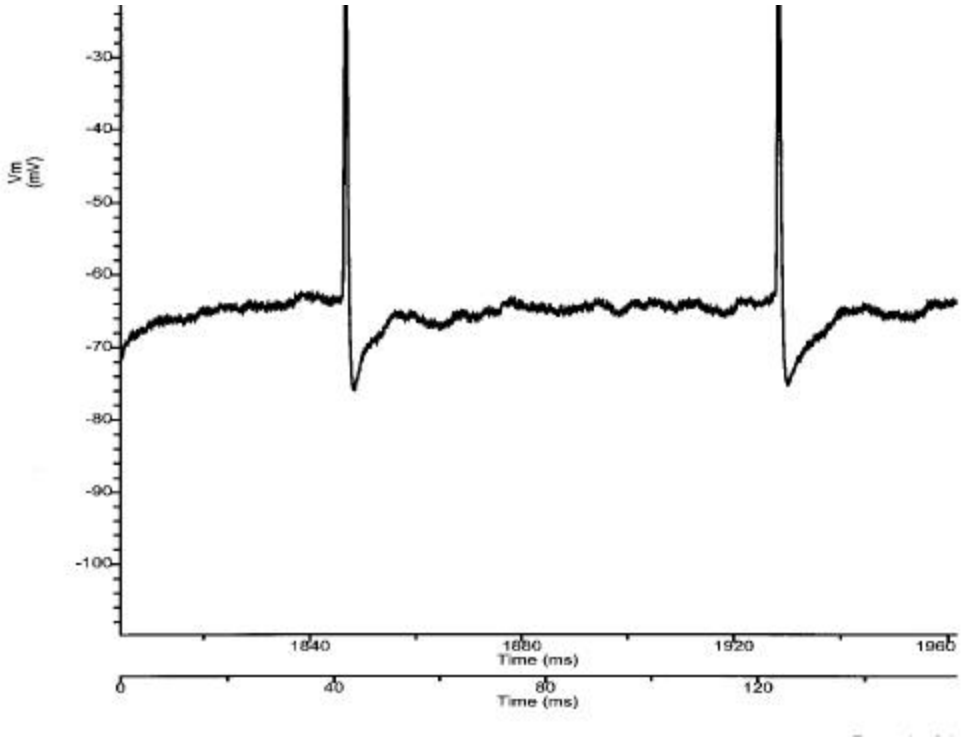

Figure 4. An example of an intracellular recording of a layer 5 neocortical pyramidal cell.

Staining protocol

Upon completion of our experimental protocol the staining procedures are initiated. These are modified only slightly from the procedures previously described (Kita and Armstrong, 1991). Apply 5 nA, 100 msec positive pulses for 1 to 10 minutes (we have successfully stained after 1 minute but longer times are preferable). Following completion of the staining protocol leave the electrode inside the cell for 2 to 3 minutes. Withdraw the electrode on the **RUN** setting, low range, speed 4 mm/sec. Figure 5 shows the neocortical cell from which the recordings shown in figure 4 were made.

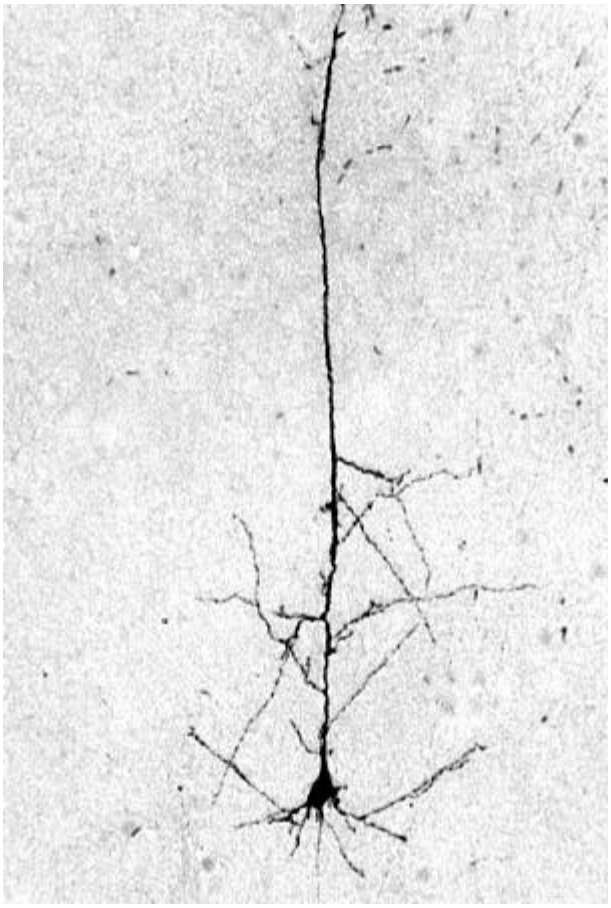

Figure 5. The layer 5 neocortical pyramidal cell, stained with Neurobiotin, from which the recording shown in Figure 4 were made.

Intracellular recording carried out in anesthetized animals using sharp electrode technology is typically a very difficult and often frustrating task. Using the procedures described above we have achieved stable recordings in over 200 cells and we hope that others may benefit from our experience.

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