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Learning To Do in Vivo Neural Responses in Mice

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Abstract

PURPOSE: To learn how to perform in vivo neuron stimulation in mice and how to remove the brain after perfusion. Also, to investigate the relationship between neuronal responses in the locus coeruleus and blood corticosterone levels.

METHODS: Mice are anesthetized and placed in a stereotaxic surgical apparatus. Next, electrodes are placed in the brain to stimulate and record neuronal activity. When stimulation is complete, a blood sample is collected and the mice are perfused to preserve their brains. After perfusion, the brain is surgically removed to be sliced for subsequent electrode placement verification. The collected blood will be used with an ELISA kit to measure corticosterone at a later date.

ANTICIPATED RESULTS: A strong relationship between the neuronal responses of the locus coeruleus and the levels of blood corticosterone is anticipated.

Introduction

This research is a stereotaxic electrophysiology study. Using electrodes, small areas of the brain can be stimulated. The locus coeruleus is a nucleus that is known to be involved with stress. Corticosterone is a glucocorticoid that is released by the adrenal cortex into the blood as part of the stress response. This study aims to identify the role that locus coeruleus stimulation plays in the physiological stress response of the body.

Purpose of the Study

The main purpose of this study is to investigate the relationship between neuronal responses in the locus coeruleus and the levels of corticosterone present in the blood after stimulation. Another purpose of this study is to refine the methods of using the locus coeruleus that are key for investigating the inflammatory processes that contribute to auditory dysfunction in the cochlea of the inner ear.

Figure 1: Stereotaxic Apparatus

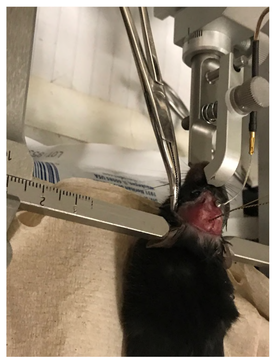

Figure 2: Mouse with electrodes

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Methods and Procedures

HABITUATION: Prior to any work being done with laboratory animals, those planning to handle the mice must be trained in the proper and ethical use of laboratory animals. Thus far, there are 29 mice (n=29) for the study transferred from other protocols, which allows us to reduce the total number of animals necessary to gather this important data. The mice must be habituated to handling and to the lab where surgery will take place primarily by taking them to the lab individually for a few hours. After habituation, the mouse are weighed to determine the dose of the anesthetic.

ANESTHESIA: A 0.04 mg/kg dose of atropine sulfate is given via intraperitoneal (IP) injection to help dry secretions. After 10 minutes, a half dose of the dissociative anesthetic urethane at 1.0 g/kg is administered IP. After 20 more minutes, the second half of the urethane is administered. After a further 15 minutes, the mouse is tested for the tail pinch, foot pinch and corneal reflex. If the mouse exhibits any of those reflexes, another 10 minutes will be given for the urethane to take effect.

Surgical POSITIONING: Once the reflexes are absent, the mouse is inserted into the stereotaxic apparatus. Ear bars are positioned into the external auditory canal and the top incisors are positioned into the mouth bar to hold the head completely steady. When the positioning is correct, the researcher will not be able to move the skull. Once the skull is secure, gel pads heated to 37℃ are placed underneath the mouse to maintain body temperature.

SURGERY: Next, the head of the mouse is shaved. Using a small scalpel, a midsagittal incision running from the mouth bar to the base of the skull is made. Using hemostats, the skin is pulled to the sides to expose the skull. A cotton swab and saline can be used to clean the skull so that bregma and lambda, which are skull sutures, are visible. The skull is leveled in the apparatus and a stereotaxic drill arm is used in conjunction with a mouse stereotaxic brain atlas that uses the bregma skull suture as the reference point. Holes are drilled directly above the locus coeruleus so that electrodes may be sunk into the nucleus. Once the electrodes are sunk to the appropriate depth, stimulation can occur.

STIMULATION: A combination of DataWave software, Neuralynx hardware and a Getting brand isolated stimulator are used to find an appropriate stimulation voltage. Once the voltage is identified, a stimulation pattern that has been shown to elicit a strong stress response from the locus coeruleus is applied for 20 minutes. Immediately after that, an electrolytic burn is used to scar the brain location where the electrodes are placed.

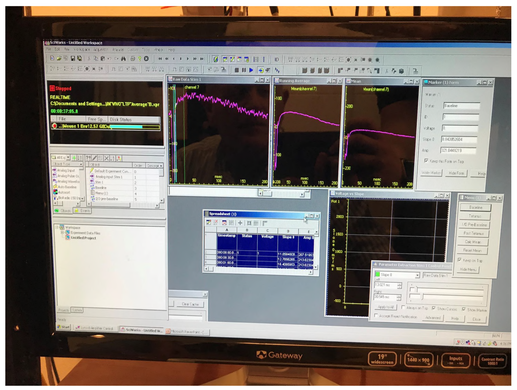

Figure 3: Neuronal Response in DataWave

PERFUSION: Upon completion of stimulation, the electrodes are removed from the skull and the mouse removed from the apparatus. The anesthetized mouse is secured in the supine position . Ventral skin is reflected, the thoracic cavity is opened and the right atrium nicked so that the escaping blood can be collected with a pipettor. Next, a peristaltic pump flushes out the remaining blood with saline, followed by 10% formalin to fix all the tissues but especially the brain. During this time, the collected blood is centrifuged to separate the plasma from the formed elements. The plasma is collected and frozen to determine corticosterone levels later.

BRAIN REMOVAL: After perfusion is complete the brain is accessed from the dorsal position. A special instrument called a bone rongeur is used to carefully remove the top of the very thin skull, exposing the fixed brain underneath. A small spatula is then used to cut the cranial nerves and to gently loosen the brain and remove it from the base of the skull. It is paced in 10% formalin for subsequent histologic placement of electrodes.

Anticipated Results

It is anticipated that locus coeruleus electrophysiologic stimulation will robustly increase blood corticosterone compared to sham surgery controls that are treated identically but do not receive any stimulation. Blood corticosterone levels will be assayed using Enzyme Linked Immunosorbent Assay (ELISA) plates to compare controls and experimental subjects.