

LAB #5: PERFUSIONS & HISTOLOGY

Background:

Recently, we radio frequency-lesioned the hippocampus of one group of rats and sham lesioned the other. Lesioned, sham-lesioned and unoperated rats were then compared on the elevated plus maze, runway maze, and BG levels. We looked for any differences which could reliably predict which rat was which.

At this point, any group differences are irrelevant, as is a finding of “no difference”. This is because we have no way of knowing if a) the lesion generator worked, i.e. is there in fact *any* lesion at all, b) did we “hit” the hippocampus and if so, what percentage of it was destroyed, and c) was any other structure damaged? These questions must be answered in order to properly interpret the data.

For any given rat, the following scenarios lead to different interpretations:

- a) no lesion: interpretation?
- b) incomplete hippocampal lesion: interpretation?
- c) hippocampus + other structure(s): interpretation?
- d) no hippocampus, but other structure(s) damaged: interpretation?

Often, valuable information can be obtained by comparing the behavioral differences of rats which fall into each of these categories. Of course, to be able to make such comparisons, you need several rats in each category.

The next step... to analyze coronal sections of brains from hippocampal lesioned rats to determine the extent and placement of each lesion.

PERFUSIONS

This technique is similar to the embalming process in humans. First, a saline solution is pumped into the rat's circulatory system to replace the blood. Why? A section of brain that has traces of blood in and around it will obscure the lesion (i.e. the lesion will be poorly visualized). Second, a solution of 10% buffered neutral formalin is pumped into the circulatory system, replacing the saline. This solution hardens and preserves the brain, which is then stored for a minimum of 48 hours in the same formalin solution to await the next step.

Read pages 76-78 of your lab manual to learn “how to perfuse a rat”, and take notes during the lab of anything not addressed in the manual.

CAUTION: We will be using some very toxic chemicals... lab rules are strictly enforced!

NOTE: Witnessing a perfusion for the first (or even the 100th time) can be disturbing for some people. If it's a simple matter of being squeemish, watch from a distance and let me

know if you are “not feeling well”. If it’s a matter of concern for the welfare of the rat, it might help to keep several points in mind.

First, perfusions are not done to conscious (or anything close to conscious rats). We will wait until respiration has ceased before we begin the procedure. Even so, it is possible (and even desirable) that the rat’s heart will still be beating. This does not mean that the rat has any chance of regaining consciousness. Many physiological processes take a while to stop, even after the animal is “technically dead”.

Second, keep in mind that scientists who perform the kinds of experiments that require these procedures do so with a firm belief that they will increase our knowledge of the brain, which in turn will benefit both humans and animals. Of course, you may agree with this statement and know that this kind of career is not for you... and there’s nothing wrong with that. You are also free to believe that no animal research should ever be performed. Although I would hope to convince you otherwise, I respect this opinion and hope that you do the same. These issues aside, the only pain felt by a rat during a perfusion is the initial prick of the needle from the injection.

HISTOLOGY

This refers to a set of procedures; using a microtome to slice the brain into thin (usually coronal) sections, mounting brain section onto slides, staining these sections to view cell bodies and/or fibers, and finally analyzing each section to determine the extent and placement of the lesion.

Please read page 79-80, 87-89 of you lab manual to learn more about histology. Take notes during the lab of anything not addressed in the manual.