# STEREOTAXIC SURGERY: HIPPOCAMPAL LESIONS

In this lab, you will learn and do the following:

Calculate the correct dose of drug for a given rat's weight
Know what drugs are used, when, and why – and where to inject them (e.g. im vs ip)
Locate bregma and lamda and know what they are used for
Know how to read a vernier scale and what directions DV, AP, and ML refer to
Know how to use a stereotaxic atlas and its limitations
Know why we make sure that DV for bregma = DV for lamda
How to determine and monitor depth of an aesthesia
Know what problematic signs are during recovery (failure to gain weight, lack of grooming, puss/swelling around surgical site, any other unusual rat behavior which might suggest pain or an inability to eat or drink)

## Injections

**Determining the dose -** the dose is dependent on the rat's weight. If the concentration on the bottle is what you need, then take the rat's weight (e.g. 300g) and move the decimal over 3 places left (e.g. dose = .300 ml). In other words, use .1 ml for every 100 g of weight.

**Preparing the needle -** prepare the needle carefully. Draw out a little more than needed. With the needle pointing up and away from people, gently tap the top of the tube to get the air bubbles out. Then eject some of the drug to obtain the desired amount.

**Restraining and injecting the rat** – the method of restraint depends on the injection type. We will be doing intramuscular injections. Roll the rat up firmly in a towel. Reach in and pull out one of the hind legs (note which one). Inject xylazine into the muscle located on the back of the upper leg. Approximately 5-10 minutes later, inject ½ the dose of ketamine into the same place but in the leg not previously injected. Wait approximately 5 minutes and inject the remaining dose into the other leg (the one you injected with xylazine). To minimize discomfort, insert the needle point first, not flat side first.

First aid for you- **seek immediate medical assistance if you are inadvertently injected.**

## Anaesthesia Procedure

**Xylazine (sedative & analgesic)** **10 mg/kg i.m**.

Our concentration is double what we need, so cut the volume of the injection in half (i.e. use 0.05 mL per 100 grams of weight). This drug takes about 5-10 minutes to sedate the rat. It also makes the ketamine more effective.

**Ketamine (dissociative anaesthetic)** **100 mg/kg i.m.**

0.1 mL of drug is injected for every 100 grams of weight.
Ketamine is has cataleptic, amnestic, profound analgesic, anti-inflammatory, and dose dependent anesthetic actions

**signs of respiratory distress**- Gurgling, wheezing, breathing too shallow
**signs of deep anesthesia**- slow, steady breathing, tail pinch and no response, lack of corneal flinch when a q-tip is touched to the eye, testicular pinch
**signs of light anesthesia –** urination, flinching

## Preparing the rat for surgery

**Shaving** be careful to not shave the whiskers, ears, or eyes. Also, don't push down hard on the head - you might kill the rat by closing off the airway.

**Temperature** taking and monitoring the rat's temperature is only critical for long surgeries (2+ hours)

**Stereotaxic** when the rat is placed in the stereotaxic, make sure that the readings on the ear bars match so that the rat is centered. When placed correctly, the head should easily move up and down, but not side to side. The incisors go over the incisors bar. Make sure that the top teeth are in front of the lower teeth. The rat should have "elephant ears", and when the ear bars are inserted, the rat's eyes will blink (note: rats do not close their eyes when anesthetized).

**parts & pieces**
Ear bars, nose clamp, electrode arm & holder, three Vernier scales (DV, ML, and AP)

**stereotaxic maps** the map we use was developed from neuroanatomical studies of male rats weighing 250g. Our co-ordinates could be slightly off if using a different sex or if rats weigh more/less than 250g

the lines on the skull - bregma (front) and lambda (back) – are used to ensure the skull is flat (dv bregma = dv lambda). Bregma coordinates (ml, dv, ap) are then used as the starting (reference) point for locating the lesion site.

stereotaxic co-ordinates are determined by using the lab atlas (see my movie)

**Vernier scales:** allow us to make very precise AP, ML, and DV measurements (down to .1 mm).

***** see diagram at the end of the handout *****

## Surgical Equipment & Supplies

| | |
|---|---|
| Gauze & Q-tips | peroxide & alcohol |
| Mosquito forceps | autoclip applier & 9 mm wound clips |
| Bone file | 1.7 mm diameter drill bit |
| Pin vice | |
| 200 µ electrode | |

## Radio Frequency Lesions: Procedure

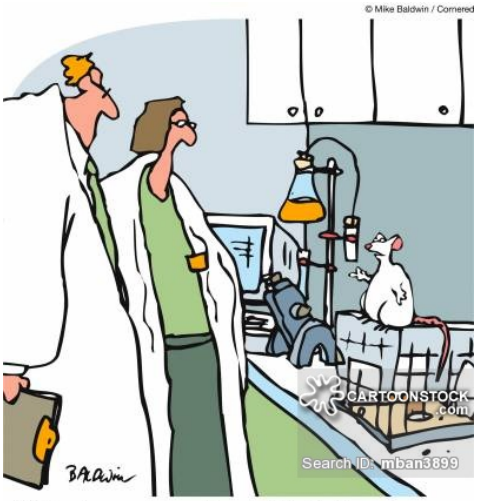
1) clean shaved head with alcohol (watch the eyes)
2) make 1.5 cm incision (beginning from just behind the eyes) using cauterizer
3) retract skin with forceps
4) scrape surface with bone file to expose skull sutures (lambda & bregma)
5) determine DV co-ordinates for lambda and bregma and adjust til equal
6) determine AP, ML, DV coordinates of bregma
7) move electrode to hippocampal lesion co-ordinates relative to bregma

AP =-3.8 ML =±2

8) lower electrode barely to skull surface and mark point of contact with needle tip
9) raise electrode, swing stereotaxic arm out of the way, and drill hole over the mark with a pin vice
10) reposition electrode and lower to appropriate depth, DV = 3.2
11) pass radio frequency current (fine setting 2,5 mA for 30 sec)
12) wait 1 minute, then raise electrode
13) repeat steps 7 - 12 for other hemisphere
14) swing stereotaxic arm out of the way
15) remove forceps
16) clean wound with peroxide
17) apply 3 - 4 wound clips
18) remove rat from stereotaxic
19) apply antibiotic (nitrofurazone .2%)
**you can't double dip, so take plenty the first time**
20) place in recovery cage: remove when awake or has urinated

## Sham Surgery

identical to above except current is not passed through the electrode
rationale? –**this surgery is done to see how they respond to the trauma of the surgery itself.**



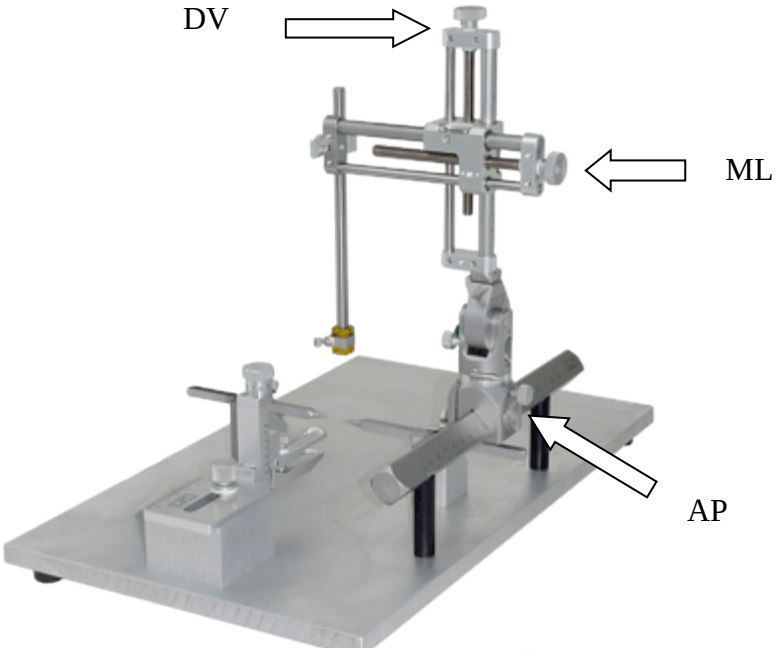
"I feel much better. I think the cheese treatments must be working."

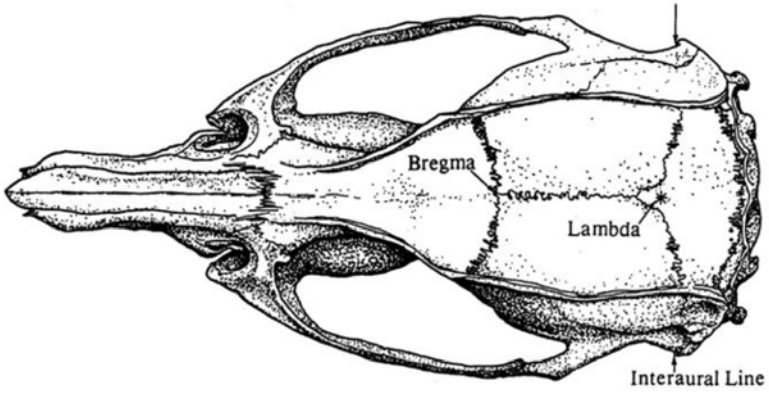
## Important!

During any and all surgical procedures, monitor the rat for clear, deep and even breathing. Periodically check for responses to pain. Re-anaesthetize at first sign of light anesthesia. **ALWAYS CHECK FOR A RESPONSE! If rat urinates after being under for a while (about 30 min)- this is one of the first signs that he/she is coming out of the anesthesia**

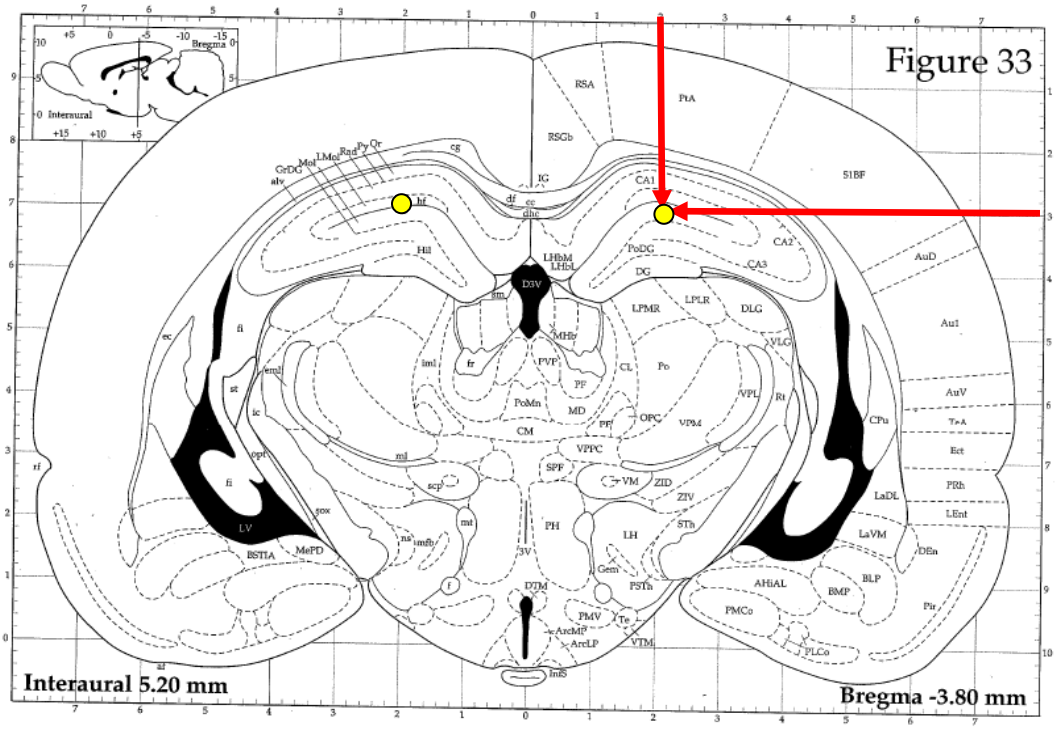
## Post-operative Issues

infection**: swelling, puss, lack of weight gain and grooming**
antibiotic treatment & analgesic administration **if rat shows signs of infection and/or pain**
veterinary consultation
caution when handling - **lesioned rats can be unpredictable**



# Figure 33




V 3rd ventricle
f amygdaloid fissure
AHiAL amyghip area, antlat
AHiPM amyghip a, postmed
lv alveus of the hippocamp
APir amygdalopir trans area
APTD ant pretectal nu, dors
ArcLP arc hyp nu, lateropost
ArcMP arc hyp nu, med post

Au1 primary aud cx
AuD 2ary aud cx, dors area
AuV 2ary aud cx, vent area
BLP basolat amyg nu, post
BMP basomed amyg nu, post
bsc brachium sup collic
BSTIA BST, intraamyg div
CA1-3 fields of hippoc
cc corpus callosum

cg cingulum
CL centrolateral thal nu
CM central medial thal nu
cp cerebral peduncle, basal
CPu caudate putamen
D3V dorsal 3rd ventricle
DEn dorsal endopiriform nu
df dorsal fornix
DG dentate gyras

dhc dorsal hip commissure
DLG dorsal lat geniculate nu
DTM dorsal tuberomammill
ec ext capsule
Ect ectorhinal cx
eml ext medullary lamina
F nu of the fields of Forel
f fornix
fi fimbcia of the hip

fr fasciculus retroflexus
Gem gemini hypothal nu
GrDG granular layer dentate
hbc habenular commissure
hf hippocampal fissure
Hil hilus dentate gyras
IBl inner blade dentate gyras
ic internal capsule
IG indusium griseum

IGL intergeniculate leaf
IMA intramedullary th area
iml internal medull lamina
InfS infundibular stem
LaDL lat amyg nu, dorsolat
LaVM lat amyg, ventromed
LEnt lateral entothinal cx
LH lateral hypothalamic area
LHbL lateral habenular , lat

LHbM lat habenular nu, med
LMol lacunosum moleculare
LPLR lat post nu, laterorostr
LPMR lat post nu, mediorost
LV lateral ventricle
MD mediodorsal thal nu
MePD med amyg, postdors
mfb med forebrain bundle
MHb med habenular nu