Protocol for electrolytic lesion of brain in tetrode-implanted animals

Required material:
- Direct current generator
- 2 cables with connectors adapted to your drive pins
- Anaesthetic system, unless you want to try this out on a freely-moving animal, which should be possible (?)
- Schematic of the drive with ground position, select 1 functional electrode per tetrode
- (for testing: ammeter & 2 testing cables)

Note: to be done just before perfusion (but some people do it a few days before to wait for glial reaction)

The current generator I'm using:

Parameters testing:
Connect the ammeter to the circuit: red cable -> current measuring input; black cable -> ammeter ground (usually black). Set the ammeter on an adapted current measure and switch on the current on the generator.

Check the current on the ammeter, the pulse duration and the generator lights:
- **Green "event" light**: closed circuit, OK
- **Red "event" light**: open circuit, NOT OK (bad cables, wrong ammeter connectivity…)
- **Red "timing overlap" light**: inter pulse period is smaller than pulse duration, NOT OK.

Actual lesion protocol:
- anaesthetise the rat (5% isoflurane in 3 L/min O_2) in the chamber
- transfer the rat to the nosecone or equivalent (allowing access to drive while anaesthetised)
- insert black cable (connected to negative outlet) to drive's ground pin.
- for each channel/pin to be lesioned:
  - plug the red cable (connected to positive outlet) in the chosen pin
  - turn on the stimulation, make sure the **green light** stays on long enough.

POSSIBLE ERROR CAUSES (**red light**):

- bad ground connection: try another ground pin if possible, otherwise connect the black cable to a part of the rat's body (usually, the foot) with a wet cloth in between.
- bad channel: try another channel of the same tetrode.
- bad connectivity of one of the cable: insert the cables within the drive pin better.

Lesion parameters tested so far:

NOTE that the current parameters were optimized for either ventral or dorsal hippocampus and do not allow to discriminate each tetrode location, rather they create a large lesion, larger if the tetrodes are shorter (see details in table below). The results will depend on tetrode impedance.
Optimal parameters so far for dorsal structures are shown in green.
Let me know if you try different parameters and what was the outcome!

	Trig Slope	Pre-train delay	Burst width	Pulse duration	Interpulse period*	Pulse sign	Baseline amplitude	Output (current intensity)	Lesion diameter for 1 lesion/tetrode
17 µm Pl-Ir, MEC, DV 1.5 mm	N.A.	0	0	**10s**	15s	**+**	0	**10µA**	Better-sized lesions Note, ground on rat's paw
17 µm Pl-Ir, dorsal HPC, DV 1.5 mm	N.A.	0	0	**15s**	20s	**+**	0	**15 µA**	Lesions still big, same as below, even for only 4 lesions
17 µm Pl-Ir, dorsal HPC, DV 1.5 mm	N.A.	0	0	**20s**	30s	**+**	0	**15 µA**	~ 0.3 mm ∅ (8 lesions, see example)
17 µm Pl-Ir, dorsal HPC, DV 1.5 mm	N.A.	0	0	**30s**	35s	**+**	0	**15 µA**	~ 0.4mm ∅
17 µm Pl-Ir, dorsal HPC, DV 1.2 mm	N.A.	0	0	**30s**	35s	**+**	0	**20 µA**	~ 0.4 mm ∅
17 µm Pl-Ir, dorsal HPC, DV 1.2	N.A.	0	0	**50s**	60s	**+**	0	**45 µA**	~ 0.5-0.7 mm ∅
17 µm Pl-Ir, ventral HPC, DV 7 mm	N.A.	0	0	**35s**	40s	**+**	0	**35 µA**	~ 0.2-0.4mm∅ (4 Tetrodes) ~ 0.6 mm∅ (8 Tetrodes)
17 µm Pl-Ir, ventral HPC, DV 6.2 mm	N.A.	0	0	**50s** (10*5)	60s (10*6)	**+**	0	**45 µA** (100 µA * 0.1 * 4.5)	~ 0.35 mm ∅

* *interpulse period = Pulse duration (where current is active) + refractory period (where no current is delivered). It just has to be longer than the pulse duration, or you'll get a "timing overlap" error.*