Stereotaxic Surgery Preparation

- The mouse was anesethetized by 7 A.M. when corticosterone levels are the lowest in nocturnal animals such as mice.
- The mouse was placed in the stereotaxic device and burr holes drilled for recording and stimulating electrode placement.
- The surgery process is identical for the experimental (stimulation) and control group with the exception of the stimulation itself. It is exclusive to the experimental group.

Brainstem Stimulation

- The placement of the recording and stimulating electrodes was designed to target the locus coeruleus and was determined through a stereotaxic mouse atlas.
- Brainstem stimulation data was gathered by connecting the reference electrode to a digital stimulus isolation amplifier (10 V) which sent a signal to the stimulating electrode to depolarize the neurons in the brain.
- A pattern of six .2 millisecond biphasic pulses in a 50 milliseconds interval was applied. This pattern repeated every 2 seconds at 10 volts for 20 minutes.

CSF and serum collection

- Incision made to expose the cisterna magna.
- A capillary pipette with an average tip diameter of 126 µm was used to extract cerebrospinal fluid from the cisterna magna.
- A slight vacuum was created with the syringe and 5-10 microliters were slowly collected.
- CSF was transferred into a tared microcentrifuge tube where a 1:100 ratio of acetic acid was added in accordance with 50% of the CSF volume to prevent any enzymatic degradation.

Trans-cardial perfusion and brain removal

- The ventral surface of the mouse was exposed using