Standard Operating Procedures

#AU 00026 Electro-olfactogram (EOG) Experiments

An electro-olfactogram (EOG) directly measures the response of the olfactory tissue to specific olfactory cues. The procedure is as follows:

- 1) A fish is anesthetized with MS-222 (150-200 mg/L depending on the species, buffered to pH 7.0 with sodium bicarbonate)
- Flaxedil (3 mg/kg) is injected intramuscularly to prevent opercular movement as this interferes with measurement of EOG
 - a. Once anaesthesia has taken effect, the fish is weighed and the weight of the fish is used to determine the volume of flaxedil to inject into the fish. The concentration of the flaxedil solution is such that only a small amount of flaxedil (0.1 0.3 mL) will need to be injected into the animal
 - b. Once the volume required is determined, it is drawn into an insulin syringe, and ensuring no bubbles are present in the syringe the flaxedil is injected into the muscle on the side of the fish posterior to the pectoral fin. This placement is used to ensure opercular movement does not occur
- 3) Fish are placed into a holding chamber and a tube delivering aerated water containing MS-222 is placed into the mouth to allow water flow over the gills for respiration and to keep the fish under anesthetic for the duration of the experiment
- 4) A microscope is placed over top of the olfactory chamber, and a flap of tissue the covers the olfactory epithelium is carefully excised
- 5) A probe is placed between the flaps of tissue in the olfactory epithelium, with a second reference probe placed on the edge of the olfactory chamber
- 6) A tube is placed above the olfactory chamber that will deliver olfactory cues into the chamber
- 7) The response of the tissue to various odours is monitored via a differential amplifier and an integration unit (e.g., a PowerLab). When the experiment is completed, fish are euthanized by an anesthetic overdose followed by a sharp blow to the head prior to being placed into a freezer. Fish must be euthanised after this procedure due to the removal of a flap of tissue covering the olfactory chamber

Based on previous work a minimum of 10 fish per cue is required for sufficient statistical power to compare responses between different concentrations of cues or exposed and control animals

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