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Short Report

A procedure for making precision stereotaxic surgery in the goldfish[1]

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Abstract A new device and a surgical procedure for placing electrodes in goldfish are described. They are used in stereotaxic surgery that is performed in lesion and electrophysiology studies. We modified DKI's stereotaxic apparatus in which the subject is fixed on a mouthpiece and two rubber plates in order to produce highly localized lesions in fish. A body holder and a new method of administering anesthesia were also developed. By stabilizing the water flow into the subject's gill using a three-pronged pump, we were able to maintain the anesthetic effect.

Key words : Stereotaxic surgery, Goldfish, Maintenance of anesthesia with a three-pronged tube.

Introduction

Fish have been used extensively as experimental animals for numerous applications. Precision surgical techniques are necessary to understand the functions of the brains of goldfish. Many techniques and surgical practices used in goldfish have been developed for original studies: measurement of eye movement (Fetcho & Faber, 1988), electrophysiology (Lee, Eaton, & Zottoli, 1993; Pastor, Torres, Delgado-Garcia, & Baker, 1991; Paul & Roberts, 1981), and brain lesions and behavior (Kyle & Peter, 1982; Rodriguez, Lopez, Vargas, Gomez, Broglio, & Salas, 2002). In general, these fish workers have described home-made systems to hold fish and to perfuse their gills, but there is no standard procedure for goldfish. The existing stereotaxic technique for fish depends on a strong heart, cutaneous respiration, and effective local anesthetic agents, such as MS 222 (tricaine methane sulphonate), to prevent the generation and conduction of nerve impulses. This method cannot preserve the anesthetic effect and, thus, subjects experience more pain than necessary, which raises some ethical questions.

We have developed a high-precision surgical approach for stereotaxic surgery in goldfish. In the present article, we describe a new apparatus for pain management that alternates 0.5 % solutions of urethane and fresh water, maintaining an anesthetic effect to lessen the subject's pain, and anesthesia and postoperative care that are consistent with new standards required by many ethics committees and national bodies regulating animal research. This technique allows the development of lesions of the partial telencephalon in goldfish. This will permit future behavioral comparisons between the telencephalons of goldfish and other vertebrates, such as mammals and birds.

Assembly

The apparatus consisted of a flame (DKI, FLAME ASSEMBLY 1530), ear bars for a pigeon (DKI, EAR BAR 856), an electrode holder (DKI, ELECTRODE HOLDER 1779), an electrode carrier (DKI, ELECTRODE CARRIER 1460), and an adapter for a small bird (DKI, 1515). The mouthpiece had a tip with a diameter

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of 5 millimeters and it was substituted for the tip of a Micro Pipette (NICHIRYO, JUSTOR 1100DG), which was connected with a flexible tube to a 0.5 % solution of urethane or distilled water and inserted into the mouth of the fish so that it could breathe through the liquid flow from the mouthpiece (Figure 1). The subject's body was wrapped with gauze to avoid damage to the skin and muscles. Two rubber plates held the abdomen of the subject (the front, Figure 2 and the photo obliquely from above, Figure 3). The rubber plates were attached to the ear bars of the stereotaxic apparatus (DKI model). One side of the rubber plate had a drain that held the abdomen of the subject and the other side had a patch attached to the ear bar. The rubber plate was not attached to the gills of the subject so that it could move freely. The mouthpiece was connected to the pump (EYELA, Micro Tube Pump MP3-B) with a three-pronged tube, which was connected to distilled water and an anesthetic solution. A switch was used to control the flow of the two solutions. The subject was put on an instrument tray. The tray was filled with water up to the subject's forehead, which enabled it to breathe. The water level was kept by the other pump. The amount of flowing liquid was from 25 to 30 ml.

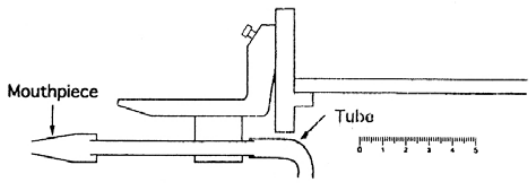

Figure 1. Mouthpiece and tube inserted into the mouth of the fish.

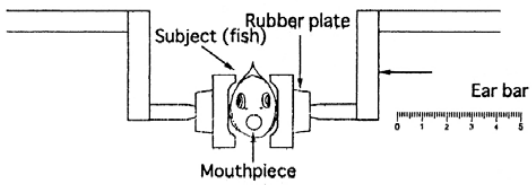

Figure 2. A diagram illustrating the fixed goldfish.

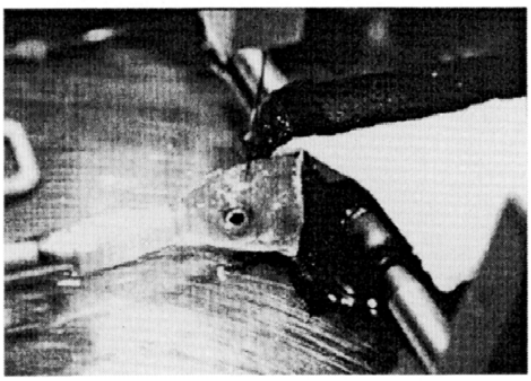

Figure 3. A photo of the stereotaxic surgery from front.

An electrode (RADIONICS, TC) with high frequency was used to create lesions. The electrode tip was 0.25 mm, with a length of 0.25 mm and a shaft of 100 mm. Power was supplied to the electrode by a lesion generator (RADIONICS, RFG-4A). The electrode was held by an electrode holder on an electrode carrier. The present apparatus could be adjusted to less than 10 cm of the subject's body length.

Surgical procedure

The subject's weight was recorded. Prior to fixing in the stereotaxic apparatus, the animals were forced to swim in a 1.0 % solution of urethane (Aldrich) for about 10 min to induce anesthesia. The subject was fixed on a mouthpiece and two rubber plates. We controlled the subject's level of consciousness by switching the liquid flow between solutions of urethane and distilled water. The skin was carefully retracted with a surgical knife to expose the skull, and the skull bone was drilled under a microscope (OLYMPUS, OME-NB).

We used the position of the subject's eyes as a landmark for drilling the skull and detecting the telencephalon because the goldfish telencephalon is near the eyes. However, because of individual differences in the position of the telencephalon in relation to the skull bone, the position of the eyes and the coordinates of the skull bone cannot be used as extracranial landmarks.

The lipid between the skull bone and the surface of the telencephalon was removed with a cotton-tipped swab to dry the brain surface.

The coordinates of telencephalon size (distance: anterior-posterior and depth: dorsal-ventral), the position of the sulcus, and the position of the joint between the telencephalon and the midbrain were measured and recorded. The electrode holder was verified and the lesion site was determined in combination with the position of the joint.

The temperature of the electrode was set to 60-70 ℃ for 1 min. The hole in the skull was filled with dental cement after retraction of the electrode.

After the dental cement dried, the goldfish was removed from the stereotaxic instrument and placed in its aquarium. The subjects were kept at about 20 ℃.

In our routine procedure, we use a 0.5 % solution of urethane for 10 min when the subject is fixed in the stereotaxic apparatus after being forced to swim in a 1.0 % solution of urethane. Thereafter, we switch to distilled water for 15 min. The 10-min period with urethane corresponds to the time needed to drill the skull bone and damage a target brain area. The 15-min period with distilled water corresponds to the time needed for the dental cement to dry. In fact, the subject wakes up after 15 min in flowing distilled water. When the surgical procedure is delayed, we prolong the time to flow 0.5 % solution of urethane or repeated switching 0.5 % solution of urethane for 10 min for distilled water for 15 min. I switch back and forth between urethane for 10 min and distilled water for 15 min until the surgical procedure is completed.

Histological data

A photo of an example of a typical lesion shows the electrode lesions centered on the dorsal telencephalon (Figure 4). The subject was sacrificed seven days after the surgery. The lesion site was located in +1.0 mm of the atlas made by Peter & Gill (1975). The depth of the lesion of the right hemisphere was 0.2 mm and 1.5 mm lateral from the body center. The depth of the left hemisphere was 0.1 mm and 1.5 mm lateral from the body center. We evaluated the lesion area using three morphological conditions: 1) disappearance of cells: 2) cell pruning or loss of processes, but cell nucleus remains present: 3) glial cell activation. Figure 4, arrow 3, shows that glial cells accumulated in the lesion site.

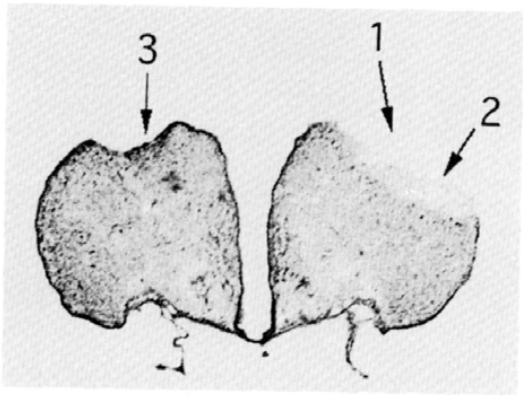

Figure 4. An example of a typical lesion: 1) disappearence of cells; 2) cell pruning or loss of processes, but cell nucleus remains prestent; 3) glial cell activation.

Discussion

Figure 4 shows that the target area was precisely damaged. The lesion area spread from the injection site of the electrode. The parameters that determine lesion area and volume are electrode placement, duration of the electricity flow, and temperature of electrode. It is evident that the present stereotaxic apparatus has high reliability. We had the best results with goldfish that weighed 9 g or less.

In surgery, the experimenter often inflicts more pain than is necessary because the subject's level of consciousness cannot be controlled. By alternating between 0.5 % solutions of urethane and fresh water, the anesthetic effect is maintained.

These techniques and practices have allowed us to make important gains in productivity, as well as improve the overall care and health of goldfish. Since developing this technique, we have performed this surgery on 42 goldfish that were used for behavioral testing. We have seen excellent recovery from surgery in all subjects and no problems with long-term survival. We have achieved a postoperative mortality of 0 % with the 42 subjects.

References

Fetcho, J. R., & Faber, D. S. 1988 Identification of motoneurons and interneurons in the spinal network for escapes initiated by the mauthner cell in goldfish. *Journal of Neuroscience*, **8**, 4192-4213.

Kyle, A. L., & Peter, R. E. 1982 Effects of forebrain lesions on spawning behaviour in the male goldfish. *Physiology & Behavior*, **28**, 1103-1109.

Lee, R. K. K., Eaton, R. C., & Zottoli, S. J. 1993 Segmental arrangement of reticulospinal neurons in the goldfish hindbrain. *Journal of Comparative Neurology*, **329**, 539-556.

Pastor, A. M., Torres, B., Delgado-Garcia, J. M., & Baker, R. 1991 Discharge characteristics of medial rectus and abducens motoneurons in the goldfish. *Journal of Neurophysiology*, **66**, 2125-2140.

Paul, D. H., & Roberts, B. L. 1981 The activity of cerebellar neurones of an elasmobranch fish (Scyliorhinus canicula) during a reflex movement of a fin. *Journal of Physiology*, **321**, 369-383.

Peter, R. E., & Gill, V. E. 1975 A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carassius auratus*. *Journal of Comparative Neurology*, **159**, 103-128.

Rodriguez, F., Lopez, J. C., Vargas, J. P., Gomez, Y., Broglio, C., & Salas, C. 2002 Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *Journal of Neuroscience*, **22**, 2894-903.

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