

## A METHOD FOR THE NEUROPHYSIOLOGICAL STUDY OF REFLEXES ELICITED BY NATURAL STATOCYST STIMULATION IN LOBSTERS

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In the neurophysiological investigation of crustacean statocyst function, the requirement to administer a physiological stimulus is not easy to reconcile with the need to maintain a stable preparation for microelectrode recordings. In studies of sensory activity this dilemma has been overcome by using an isolated statocyst preparation with its associated sensory nerve (Cohen, 1955). In many cases where such preparations have been used, stimulation has nevertheless been by artificial means: irrigation of the statocyst sac (Sandeman & Okajima, 1972; Silvey & Sandeman, 1976), magnetic attraction of an artificial iron lith (Ozeki, Takahata & Hisada, 1978) or displacement of individual hairs with a needle (Cohen, 1960; Takahata & Hisada, 1979; Takahata, 1981). For studying reflex activity, it has proved possible to record with needle electrodes from large statocyst interneurons in dissected crabs mounted on pivoted platforms (Fraser, 1974, 1977; Fraser & Sandeman, 1975).

This paper describes the first published method for recording simultaneously from statocyst interneurons and other elements in the reflex pathway during application of a tilting stimulus, in the decapod, *Nephrops*.

A saline was developed using a simple formula given by Burton (1975) in conjunction with a plasma analysis given by Robertson (1949) for this species. Compared with values for the plasma (Table 1), the magnesium concentration in the saline is decreased to less than half, sulphate is retained at an isotonic level while sodium and chloride are adjusted to compensate. Whereas sulphate has been omitted from previous salines, for example Evans, Kravitz, Talamo & Wallace (1976), it is incorporated in the new saline since Cole (1941) demonstrated its importance for normal heart function. Tris (Sigma T-1378) was used as the buffering agent but without maleic acid as Burton (1975) had observed that the acid made little contribution to the overall buffering capacity above a pH of 7.2. This saline gave good support of physiological function. Activity recorded from swimmeret muscles in intact animals was lost within 2-3 h of carapace puncture using previous salines (e.g. that of Evans *et al.* 1976). In contrast, with the new saline, activity in the isolated preparation was recorded for many hours, in particular reflex activity to head tilts continued for up to 10 h.

Adults of both sexes of *Nephrops* were used with body lengths, measured from rostrum to telson tips, of 10-25 cm. Each animal was immersed in oxygenated,

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Table 1. Ionic concentrations (mM)

	Cl <sup>-</sup>	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	SO <sub>4</sub> <sup>2-</sup>	Tris
<i>Nephrops</i> plasma (Robertson, 1949)	518.9	517.4	7.57	13.8	8.88	18.5	0.00
<i>Nephrops</i> saline	515.6	507.1	7.70	14.9	4.00	18.5	20.0

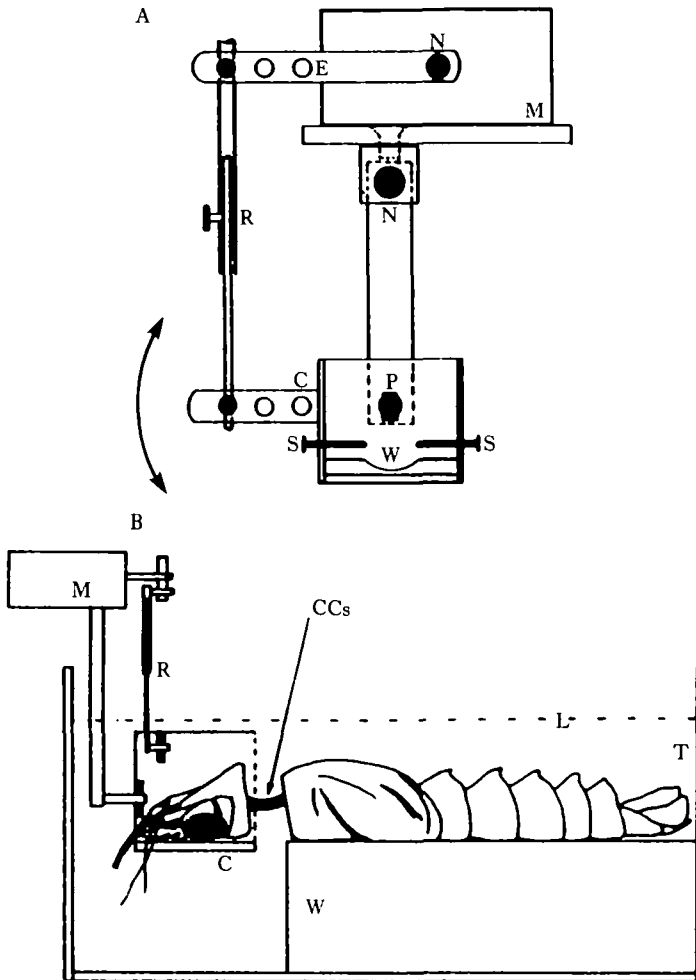


Fig. 1. (A). Frontal view of tilt apparatus. M is the scanner drive motor, connected *via* an eccentric arm (E) to an adjustable bar (R), delivers an imposed movement (arrows) to the cradle (C) which rotates about the axis P. N are locknuts, S are screws used to clamp the 'head' in place on a wax bed (W). (B) Side view of a typical experimental arrangement. The main body of the animal is clamped, dorsal side down onto a wax bed (W) in the experimental tank (T). The 'head' is held in the cradle (C) of the tilt apparatus. The whole preparation is immersed in circulating, oxygenated saline (circulation system not shown) up to the level indicated (L). Following the procedure outlined in the text, the only connections between the body and 'head' of the animal are the paired circumoesophageal connectives (CCs).

circulating saline throughout the experiment. The preparation was initially dissected in a bath of saline and washed thoroughly in fresh saline until the blood seen pumping from wounds was clear and mixed into the surrounding saline without clouding. The whole tank was then flushed with fresh saline before continuing the experiment. The 'head' of the animal was severed from the rest of the cephalothorax, by cutting the cuticle all the way around the cephalothorax just behind the eyes; subsequently the stomach was drained and removed along with the mouthparts, antennal glands and fat deposits. The brain was retained in the anterior section leaving the paired circum-oesophageal connectives as the only connection between the 'head' and the rest of the body. These connectives are sufficiently long to allow a large degree of head tilt without damage to them.

The 'head' was then placed in a wax-lined cradle and clamped in position with screws (Fig. 1A). The cradle was positioned on an axis with the animal's body and rotated about this axis. Adequate clearance between 'head' and thorax was obtained by cutting thin strips of cuticle from their edges. The cradle was connected *via* an adjustable bar to the eccentric arm of a scanner drive motor (General Scanning Inc. G-300) which moved through a set angle driven by a ramp/signal generator. Using this drive control, the amplitude, frequency and stimulus waveform could be

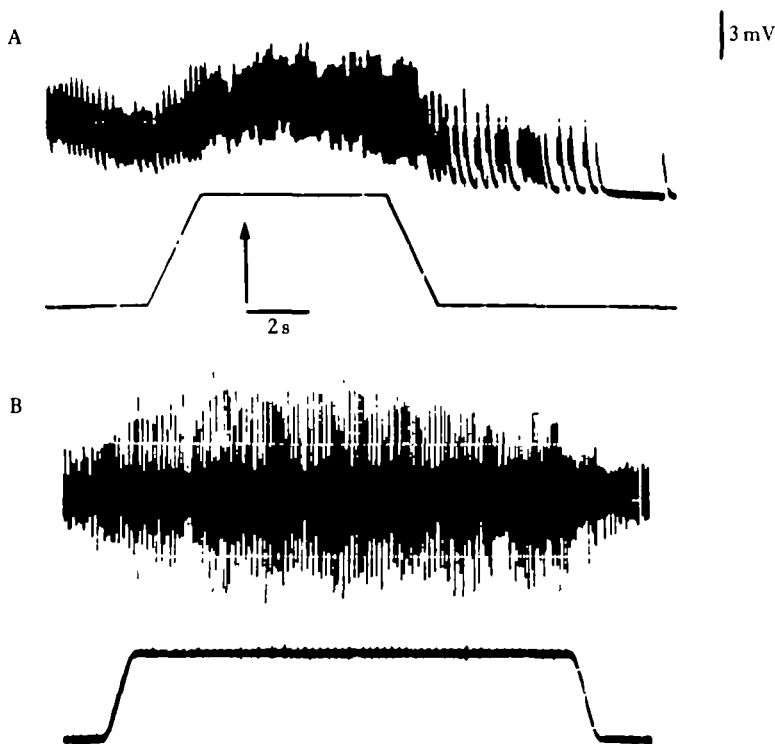


Fig. 2. Two recordings from swimmeret twister muscles demonstrating that intracellular micro-electrode recordings (A) obtained using 'head' tilt stimuli show similar responses to 'natural' statocyst stimulation as those seen in myographic records from intact preparations (B). Note the slow wave generator potential seen in the intracellular record (A) that is absent from the myogram (B). The time mark applies to both records. Arrow indicates upward movement of the ipsilateral statocyst to the recorded swimmeret.

precisely set, while the stimulus range could be changed using the adjustable drive bar.

Thus, the 'head' containing the paired statocyst organs, could be rotated to give adequate stimulation to the sensory systems within them independently of the rest of the animal, which was held rigid enough for intracellular penetrations at any point along the pathway from the connectives to the last abdominal ganglion (Fig. 1B).

Intracellular microelectrode recordings from fibres of the twisting muscles of the swimmeret motor system demonstrate that the responses to tilts of the isolated 'head' (Fig. 2A) compare closely with the responses obtained to tilts of the whole animal (Fig. 2B). They illustrate, in addition, the slow wave generator potential that is not observed in myograms recorded from whole animal preparations. The preparation has been used successfully in detailed intracellular studies of this system (Miyán, 1982; J. A. Miyán & D. M. Neil, in preparation).

*Nephrops* is an ideal subject for studying statocyst mediated reflexes because it is effectively blinded by normal laboratory light intensities (Loew, 1976) eliminating any complicating visual inputs to the general equilibrium system. It would be of value to compare this lobster with *Homarus* which relies much more heavily on its visual system and is not blind under normal laboratory conditions.

With the present technique, microelectrode penetrations can be held both during the administration of tilt stimuli, and for the subsequent long periods necessary for dye-injection of, for example, horseradish peroxidase (see Mesulum, 1982), Lucifer Yellow (Stewart, 1978) or cobalt chloride (see Strausfeld & Miller, 1980). Therefore, this technique should be invaluable for future work on the central mechanisms and neuroanatomy of this, and other equilibrium sensitive systems.

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