Reaction time of the topminnow *Aplocheilus lineatus* to surface waves determined by video- and electromyogram recordings

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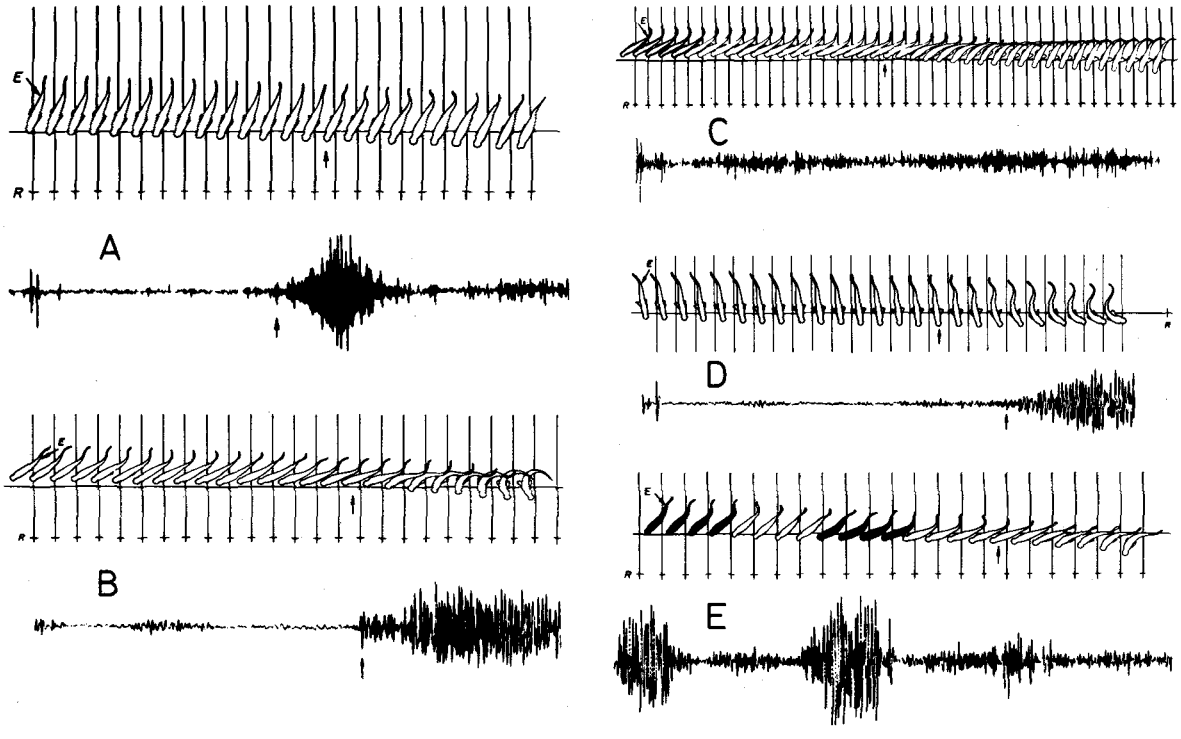
Summary. Evoked muscle potentials during a localizing response for a wave center occur in *Aplocheilus lineatus* 30 ± 1.5 msec ($\bar{X} \pm SE$) earlier than the simultaneously monitored body movements. Considering this time, only the first 8–10 wave cycles of a total wave signal are utilized to identify and localize a wave source.

Like clawed toads[2,3] and surface dwelling insects[4] the surface feeding fish *Aplocheilus lineatus* responds to concentric surface waves with a movement towards the wave center[5]. The reaction times of such localizing responses give information about that portion of a wave signal which is essential for identification, as well as localization, of a wave source. EMG-recordings in fishes have been done so far to differentiate functionally between white, pink and red swimming musculature[6–8] or to study nervous activation and control especially of respiratory or swimming movements[9–12]. We used this technique to define the behaviorally determined reaction times of *A. lineatus*[13] in order to get a better idea of the time needed for neuronal processing. Furthermore we studied the practicability of EMG-recordings analyzing the oriented responses of these topminnows.

The potentials were recorded in blinded unrestrained animals with a copper wire (diameter 0.05 mm) insulated except at the tip. The wire was firmly attached with histoacryl to the red body musculature of the fish just ventral to the horizontal myoseptum. EMG-activity and the behavioral responses of the fishes were registrated simultaneously and in every single case we compared the onset of the evoked EMG and the onset of the orienting response. Both were determined by means of a signal averager (Nicolet 1070) respectively an electronically inserted clock into the video monitor (time resolution 10 msec).

The figure, A, shows a sequence of pictures taken during slow swimming and the initial phase of an evoked response. The corresponding muscle activity (lower trace) begins 53 msec earlier. This time is not expected to be a constant value throughout. Since the stimulus-evoked responses are directed ones, attention was directed to the target angle prior to perception of the stimulus. For example, at a target angle section of ±40° (0° medially in front of the fish) primarily, only some caudally located body segments show strong deflections. The best experimental results for the very first change of electrical muscle activity have indeed been achieved with such caudal and contralateral implanted electrodes.

For target angles between 40° and 90° the responses are normally characterized by a more rostrally located initial deflection of the body and a flip of the tail fin directed away from the wave source (figure, B and D). Therefore



Video-recorded single consecutive sequences at 20-msec intervals of the orientation response towards the wave source R (upper traces) and the simultaneously registered local muscle activities in E (lower traces). The vertical arrows mark the onset of the behavioral or the muscle response. In order to obtain the real reaction times the period of time needed for wave propagation (reference point middle of the fish's head) was subtracted from the time interval indicated by the arrows. The vertical and horizontal lines are fixed reference lines. Swimming speed of the fishes prior to stimulation and target angle at the arrival of the wave front are in *A* 4.1 cm/sec, −25°; *B* 6.5 cm/sec, −80°; *C* 3.5 cm/sec, −80°; *D* 3.8 cm/sec, −85°; *E* 12.5 cm/sec, −90°.

one might expect distinct muscle contractions of ipsilateral parts of segments in the middle of the trunk as well as contralateral parts of more caudally situated segments.
In some cases, however, where the momentary position of the tail fin was most unfavourable with respect to target angle and electrode location, it was impossible to determine the reaction time by EMG-recordings. In the figure, C and D for example, the body of the fish was going to bend itself just to the contralateral side of the electrode at the onset of the response. Therefore the recorded activity was too small (figure, C) or occurred only with the return of the tail flip and hence appeared to be delayed (figure, D).
If the fishes swam faster than 8–10 cm/sec the recorded muscle activity level was too high most of the time to be used to determine the reaction time. The periods of high muscle activity correspond to certain phases of fast swimming movements (black fishes in figure, E).
For presenting stimuli to the left side of the fishes, the most favourable electrode location was therefore 1. on the right side of the body (contralateral) 5–7 scales away from the tail fin for target angles between 40° right to 90° left and 2. on the left side of the body (ipsilateral) 15 scales away from the tail fin for target angles between about 40° and 90° on the left side of the fishes. Insertion of 2 or more electrode wires proved to be unsatisfactory, because their increased weight and water drag altered the natural position of the small fishes (6–8 cm) at the water surface and hence their responsiveness as well as normal swimming movements.
In 169 out of 399 experiments the onset of the behavioral response by means of concave deflection of the body segment we recorded from, and the beginning of an increased muscle activity, could unequivocally be determined. Based on these 169 trials the activation of red body muscles occurs 30 ± 1.5 msec ($\bar{X} \pm SE$) earlier than the behavioral response itself. The concrete measured times ($\bar{X} \pm SD$) using wave stimuli with pp-amplitudes between 9 and 3 µm are 144 ± 33 msec (video) and 114 ± 31 msec (EMG). The value of 30 msec, which is independent of swimming speed and target angle, agrees well with contraction times of white muscles of several other fishes of similar body length[14] and with the time delay between muscle activation and visible leg movements during prey localization in *Gerris remigis*[15].
Considering the nature of wave signals[13] we can state that the signal analyzing processes of *A. lineatus* last just as long as the first 8–10 wave cycles of a stimulus. Thus the results imply that mainly the initial part of a wave train lasting several hundred msec is utilized by the fish and that neuronal processing is highly sensitive to the frequency spectrum of a wave signal, as has been reported also for prey identification on the water surface in the back swimmer *Notonecta glauca*[16–18].

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Endogenous gibberellins and amylase activity in tall and dwarf strains of rice *(Oryza sativa)*

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Summary. 2 tall and 7 dwarf strains of rice were sprayed with 0 or 40 µg ml^{-1} GA_3. 4 dwarf strains responded to exogenous GA_3, and showed a markedly lower endogenous gibberellin content than the tall strains, while 2 dwarf strains did not respond to GA_3 application and had considerably higher endogenous gibberellin levels than the tall ones. Amylase activity in germinating seeds showed a significant negative correlation with the endogenous gibberellin content.

The phenomenal agronomic success of dwarf cereals, particularly of wheat and rice, has triggered detailed biochemical investigations of generic dwarfness. Gibberellins (GA) have been implicated in genetic dwarfness in wheat (*Triticum aestivum* L. em. Thell); some dwarf strains show limited GA utilization[1,2], while others exhibit reduced GA biosynthesis[2]. Some dwarf mutants of rice (*Oryza sativa* L.) also show limited GA biosynthesis[3,4] but there is no clear-cut evidence for restricted GA utilization in rice[5,6]. Here we describe 2 rice mutants that show restricted GA utilization.
2 tall and 7 dwarf strains of rice were planted in a field in a split plot design with 3 replications, and were sprayed 5 times with 0 or 40 µg ml^{-1} GA_3 (gibberellic acid) at 15-day intervals. Plant height at maturity was recorded on 10 random plants from each subplot. Endogenous gibberellins were extracted from 80-day-old plants grown for this purpose[1], and assayed by endosperm bioassay[7] using half seeds of the wheat cultivar 'K 68'. For investigating amylase activity and isozymes, hand dehulled seeds were germinated in petri dishes at 30 °C for 5 days and homogenized in 0.05 M phosphate buffer (pH 7.0). The homogenate was stored in a refrigerator for 30 min and centrifuged at 4 °C. The amylase activity in the clear supernatant was assayed according to Bernfeld[8]; protein content was estimated following Lowry et al.[9]. Amylase isozymes were separated by polyacrylamide gel electrophoresis[10]; the gels were prepared by adding 2 ml of 4% starch solution to 100 ml of 8% solution of polyacrylamide gel. The gels were stained with iodine reagent after they had been incubated for 30 min in 1% starch solution at room temperature and washed with distilled water. The α-amylase bands were clear, while the β-amylase bands had a reddish tinge.
The 2 tall strains and 5 of the dwarf strains showed a significant increase in plant height in response to exogenous GA_3. 2 dwarf strains, Shyama and Cigar Mutant, did not respond to GA_3 application. The 5 dwarf mutants that