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Behavioral evidence for post-pause reduced responsiveness in the electrosensory system of *Gymnotus carapo*

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Summary

Gymnotiform weakly electric fish find their way in the dark using a continuously operating active sensory system. An electric organ generates a continuous train of discharges (electric organ discharges, EODs), and tuberous high-frequency electroreceptors monitor the pattern of transcutaneous current flow associated with each EOD. Here, I report that a prior interruption to the continuous train of EODs dramatically affects a response shown by many pulse-type gymnotids. In this so-called novelty response, fish normally raise their electrosensory sampling rate in response to novel sensory stimuli. The gymnotid *Gymnotus carapo* was induced to pause its EODs briefly, and the novelty response to sensory stimuli given post-pause was analyzed. Mechanosensory stimuli given as early as 20 EODs after a pause elicited clear novelty responses, but strong high-frequency electrical stimuli were ineffective at this time. Moreover, high-frequency electrical stimuli remained less efficient in eliciting normal-sized responses until approximately 2000 EODs, or 40 s, after a pause. The post-pause inefficiency of high-frequency stimuli was not due to an inappropriate choice of intensity or their temporal patterning and did not result from the stimulation that caused the pausing. Low-frequency stimuli that also recruited ampullary electroreceptors were more efficient than high-frequency stimuli in eliciting post-pause responses. These findings show that continuous activity is required either to maintain sensitivity to high-frequency electrical stimuli or to ensure that such stimuli are able to modulate efficiently the pacemaker that sets the discharge frequency.

Key words: active sensory system, pacemaker, novelty response, cessation, electrosensory, mechanosensory, electric organ discharge, weakly electric fish, *Gymnotus carapo*.

Introduction

Weakly electric fish find their way in the dark by using an active sensory system. They continuously send a train of current pulses through their skin and measure, using tuberous electroreceptors, the associated pattern of transcutaneous current flow, from which they are able to derive a picture of their surroundings (e.g. Heiligenberg, 1977; Moller, 1995; von der Emde, 1999). In contrast to other active sensory systems such as those used by echolocating bats, cetaceans or oil birds, the electrosense is continuously active during the entire life of a fish.

In some of these fish, it is possible to disrupt experimentally the otherwise continuous train of electric organ discharges (EODs). This should enable an assessment of how critical the continuous operation of this sensory system is for its performance. I attempted here to explore the effects of a pause in EOD activity on the subsequent performance of the so-called novelty response shown by many pulse-type weakly electric fish. In this response, a fish briefly raises its discharge frequency after it has detected a novelty in its environment. Thereby, it increases its electrosensory sampling rate whenever something novel transpires. The response can generally be elicited not only by changes in the EOD feedback but also by a variety of other stimuli (Lissmann, 1958; Bennett and Grundfest, 1959; Westby, 1975; Heiligenberg, 1980; Kramer et al., 1981; Meyer, 1982; Barrio et al., 1991; Falconi et al., 1995; Ciali et al., 1997; Corrêa and Hoffmann, 1998). Thus, it is possible to compare post-pause novelty responses driven by different sensory stimuli. This comparison, in turn, may provide hints as to whether an EOD pause affects stimulus detection or the execution of the response. The present study demonstrates a dramatic failure of electrosensory, but not mechanosensory, stimuli to elicit novelty responses after pausing of EODs in the gymnotid fish *Gymnotus carapo*. The findings point to two new mechanisms in which continuous activity is required to ensure either maintained sensitivity to high-frequency electric stimuli or a high efficacy of tuberous receptor-driven synaptic input to its target cells in the pacemaking structures.

Materials and methods

Experimental animals

Ten *Gymnotus carapo* L. (length 14–24 cm), obtained

from Aquarium Glaser, Rodgau, Germany, were kept individually in tanks of various sizes. All experiments were performed in a 70 cm×40 cm×30 cm (length × depth × height) tank. Water conductivity was approximately 360 μS cm^{-1}, pH approximately 7, and temperature 26 °C in all tanks. During the experiments, a fish rested almost motionless in a porous shelter placed centrally on the bottom of the tank and oriented parallel to the long axis of the experimental tank.

Experimental paradigm

The derivation of 'post-pause efficiency', $\eta(n)$, of a stimulus in eliciting normal-sized novelty responses when given n EODs after a pause is illustrated in Fig. 1. The stimulus was given twice: first at the nth post-pause EOD and then a second time 10 000 EODs, or approximately 200 s, later during steady firing. The second stimulation served to determine the 'normal' response size, with which the first post-pause response was then compared. The interval of approximately 200 s between the first and second stimulus was selected to ensure independence of the second response from the occurrence of the first.

Monitoring interpulse interval

To monitor the interpulse interval continuously, the EODs of the experimental fish were recorded using two silver wires fixed on the front and the back of the tank. The voltage across these wires was amplified (EG&G 5113) and fed into a computer using a data-processing card (DAP 3200a/415, Microstar Labs; software written in DAPL and Borland Turbo-Pascal 7.0).

Pausing the EODs

Pausing was usually elicited by electrical signals (1 kHz square waves of up to 1 s in duration, field strength in the fish's shelter of up to approximately 300 mV cm^{-1} peak-to-peak) generated by a pulse generator (Master-8, AMPI) and delivered by a T-shaped dipole electrode (Westby, 1974, 1975), whose distance to and orientation with respect to the fish was varied. Pauses could also be elicited by optical and mechanical stimuli. However, these stimuli were less efficient in eliciting pauses than the electrical stimuli. The efficiency of non-electrical stimuli in eliciting pausing was therefore increased in three fish. In these, a pause-eliciting electrical stimulus was preceded by a mechanical stimulus (tapping the tank's wall). After a few trials, the mechanical stimulus itself was sufficient to elicit pausing. Approximately 10–20 pauses were elicited each day. At least 10 000 EODs were required between the second stimulus of a previous experiment and a subsequent attempt to elicit a pause. In some fish, it was not possible to elicit more than one pause a day. In experiments that required a large number of post-pause responses to be evaluated, three fish (gc1–gc3) were generally used since pausing could most readily be elicited in these.

Electrical stimuli

The isolated output of a generator (DS 345, Stanford Research) was automatically triggered at the rising phase of the nth (and n+10 000th) post-pause EOD and delivered *via* carbon electrodes that straddled the fish. Stimulus intensity was determined at the fish's usual position using two silver wires (1 cm apart, insulated except at their tips).

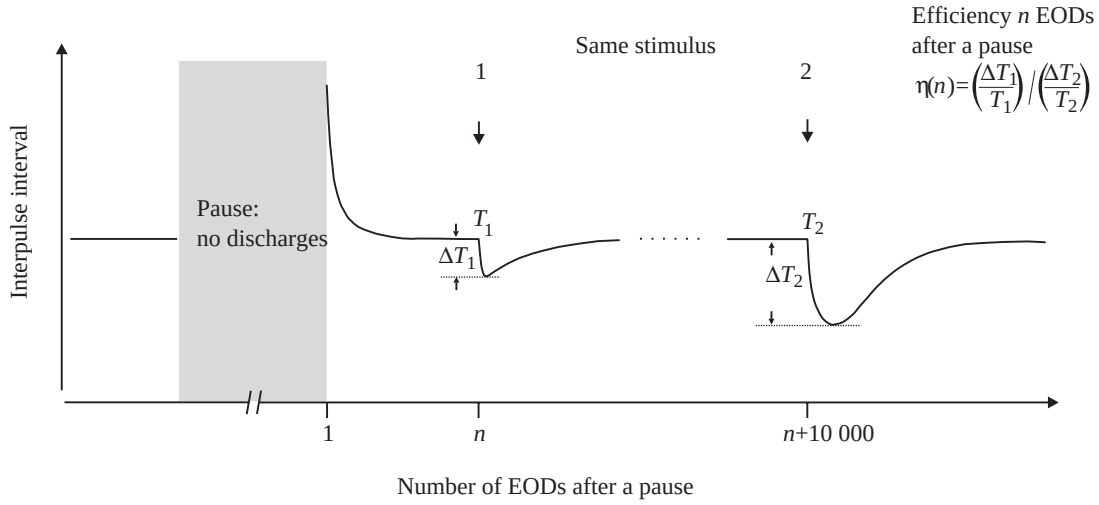

Fig. 1. Measurement of the efficiency with which a given stimulus elicited a novelty response after a pause in the ongoing electric organ discharges (EODs). The stimulus was given twice (vertical arrows): (1) n EODs after the pause and (2) 10 000 EODs, or approximately 200 s, later when the fish had returned to steady firing. The response is a transient shortening in the interpulse interval between successive EODs (ordinate). In the schematic trace shown, a *Gymnotus carapo* fired with a stable interpulse interval of approximately 20 ms. The fish was then induced to pause its EODs for the period indicated in grey. For each of the two subsequently elicited responses, the maximum reduction ΔT in interpulse interval from the pre-stimulus interval T was determined, and ΔT was normalized to T. The efficiency of the stimulus when given n EODs after the pause, $\eta(n)$, was taken as the ratio of the two normalized interval modulations, as indicated.

Mechanical stimuli

A standardized strong mechanical stimulus that could be triggered *n* EODs after a pause was generated by mounting a large contactor (AEG Elfa VI4040M5) on the desk on which the experimental tank stood. The pressure wave produced by the knock that resulted from activation of the contactor was reproducible both in details of its time course and in peak-to-peak amplitude and remained approximately constant within the porous shelter of the fish (deviations less than 1 %). This was verified by monitoring the waveform and amplitude of the pressure wave using a miniature hydrophone (Bruel & Kjaer, 8113) and a charge-conditioning amplifier (Bruel & Kjaer, Nexus 2692). The peak-to-peak amplitude corresponded to 167 dB re 1 μPa.

Quantifying the stimulus efficiency

In responses elicited during normal firing, the stimulus-induced maximal excursions ΔT from the pre-stimulus interpulse interval T_0 will be larger for larger values of T_0 (S. Schuster, unpublished observation). To make response strengths obtained at different values of T_0 comparable, the strength (R) of a novelty response was assayed using the ratio $R=\Delta T/T_0$. The efficiency, $\eta(n)$, of the stimulus in eliciting a response n EODs after a pause was defined as the ratio of the post-pause response strength R_1 to the subsequently determined 'normal' response strength R_2, i.e. $\eta=R_1/R_2$.

Unless stated otherwise, Student's *t*-tests were used to determine, for each individual, whether the mean efficiencies η obtained for that individual under different stimulus regimes differed significantly.

Results

Failure of strong electrosensory stimuli to elicit post-pause responses

Even electrical pulses (1 cycle of a 1 kHz sine, 235 mV cm^{-1} peak-to-peak) that were far above threshold and elicited strong novelty responses when given during steady firing failed to elicit novelty responses immediately after a pause. Pulses delivered early, i.e. 10–30 EODs after a fish resumed its discharges following a pause, never elicited a response (80 tests with 10 fish). When given 100 EODs after a pause, the stimulus pulses still yielded only 'small-sized' novelty responses. Only long after a pause, after more than approximately 2000 post-pause EODs had occurred, were the stimuli able to elicit 'normal-sized' novelty responses. Fig. 2 illustrates these findings with typical responses obtained when a stimulus pulse was given *n* EODs after a pause.

To obtain a quantitative measure of the reduction in response after a pause, I determined the post-pause efficiency, $\eta(100)$, i.e. the efficiency with which the stimulus elicited a novelty response when given 100 EODs after a pause (see Fig. 1 for an illustration of how efficiencies were measured). Several measurements were made of $\eta(100)$ in each of 10 fish, yielding a set of $\eta(100)$ values obtained after a total of 118 pauses that ranged from 1.1 to 76 s in duration (17.5±14.1 s, mean ± S.D.). Within this data set, no correlation existed between the efficiency $\eta(100)$ and the duration of the prior pause. The mean efficiencies did not differ between fish that could be induced to pause several times a day and those in which only a few pauses could be elicited each day. Hence, all post-pause efficiencies of the data set were pooled to obtain an average efficiency: $\eta(100)=0.43\pm0.03$.

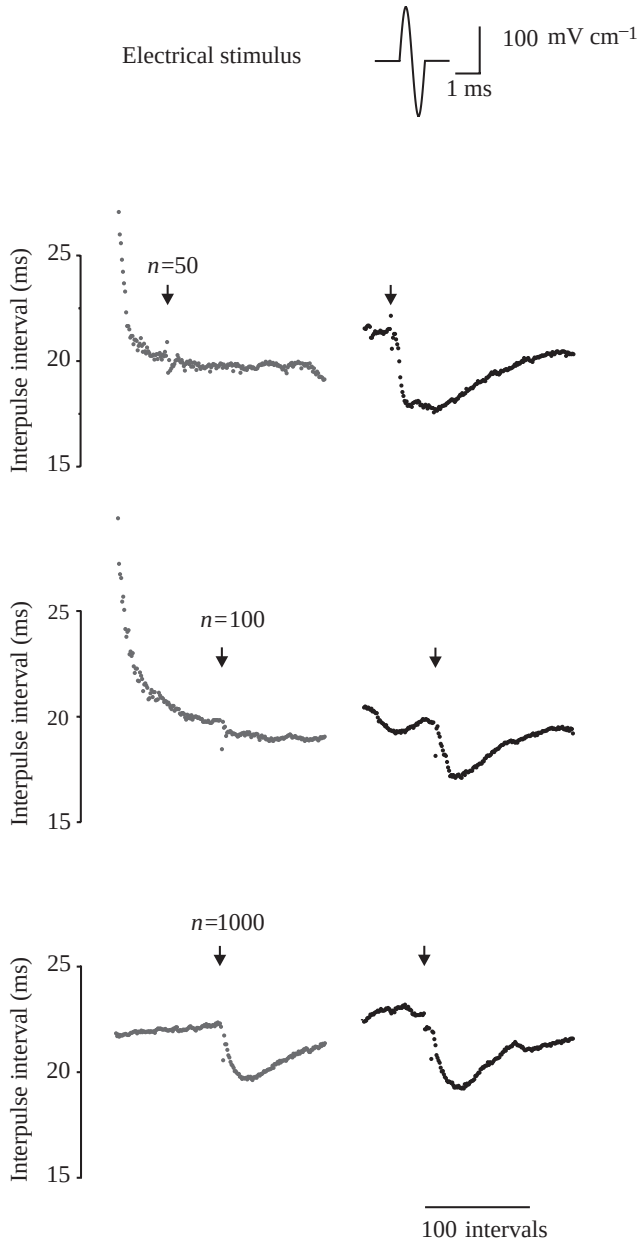

Fig. 2. Even strong electrosensory stimuli failed to elicit novelty responses when given directly after a pause in the electric organ discharge (EOD). The stimulus was a single cycle of a 1 kHz sine wave delivered far above threshold at a peak-to-peak field strength of 235 mV cm^{-1}. Each row illustrates the two responses obtained, first when the stimulus was given at the *n*th post-pause EOD (left traces; *n*=50, 100, 1000, top to bottom row) and, second, 10 000 EODs later during steady firing (right traces). Stimulus timing is indicated by arrows. Pause durations were 3.9, 16 and 17 s (top to bottom).

Post-pause responses to a mechanical stimulus

The failure of electrical stimuli to elicit responses immediately after a pause could mean that novelty responses simply cannot occur after a prior pause. However, using a mechanosensory stimulus, responses could readily be elicited, even immediately after a pause (Fig. 3). In these experiments, the mechanical stimulus was chosen so that it elicited, during normal firing, responses of strength comparable with that of responses elicited by the electrical stimulus used previously.

The mechanosensory stimulus elicited clear novelty responses immediately after a pause. However, the post-pause responses were smaller than those obtained during steady firing (i.e. $\eta<1$). This was analyzed using a set of 82 experiments in which the mechanical stimulus was given 100 EODs after a pause. This set involved data from all 10 fish, obtained after pauses ranging in duration from 1 to 75 s (19.6±18.9 s, mean ± S.D.). No correlation existed between pause duration and post-pause efficiency, and mean efficiencies did not differ between fish that could be induced to pause several times or only a few times per day. The efficiencies were therefore pooled, and an average efficiency, $\eta(100)=0.71\pm0.02$, was obtained. The 71 % average efficiency of the mechanical stimulus when given 100 EODs after a pause was significantly greater than the average efficiency (43 %) of the electrical stimulus (difference between the two averages $P<0.001$; *t*-test).

The efficiency is independent of how pausing is induced

The most efficient stimulus to make a *Gymnotus carapo* pause its ongoing train of EODs is a strong electrical 'shock'. Such shocks have also been used to elicit pausing in the previous datasets. Hence, the reduced efficiency, or even failure, of electrical stimuli in eliciting a novelty response immediately after a pause could result from an aftereffect of the electrical shock. This was tested by training three fish so that they reproducibly paused their EODs in response to mechanical stimuli. In a series of subsequent experiments, these trained fish were induced to pause by either an electrical shock or a mechanical stimulus. For each fish, the mean efficiency of the electrical stimulus (1 cycle of a 1 kHz sinewave, 235 mV cm^{-1} peak-to-peak) 100 EODs after the pause was determined after these two differently elicited pauses. In all three fish, the mean efficiencies were independent of whether the prior pause had been elicited by an electrical or a mechanical shock (Fig. 4). This demonstrates that the reduced responsiveness to electrical stimuli is not caused by an aftereffect of a pause-inducing electrical shock.

Would other electrical stimuli be more efficient in eliciting post-pause responses?

Both the mechanical and electrical stimuli elicited strong novelty responses of comparable size during steady firing. Hence, insufficient 'strength' of the electrical stimuli was not a likely cause for their reduced efficiency in eliciting post-pause novelty responses. However, this conclusion would not hold if the efficiency of the electrical stimuli was very sensitive to stimulus intensity. Therefore, a series of experiments with three fish explored how the efficiency, $\eta(100)$, of the electrical sine-wave pulses varied as their intensity was varied over a range of almost three orders of magnitude so as to elicit smaller (field strength 0.9 mV cm^{-1}) or slightly stronger (field strength

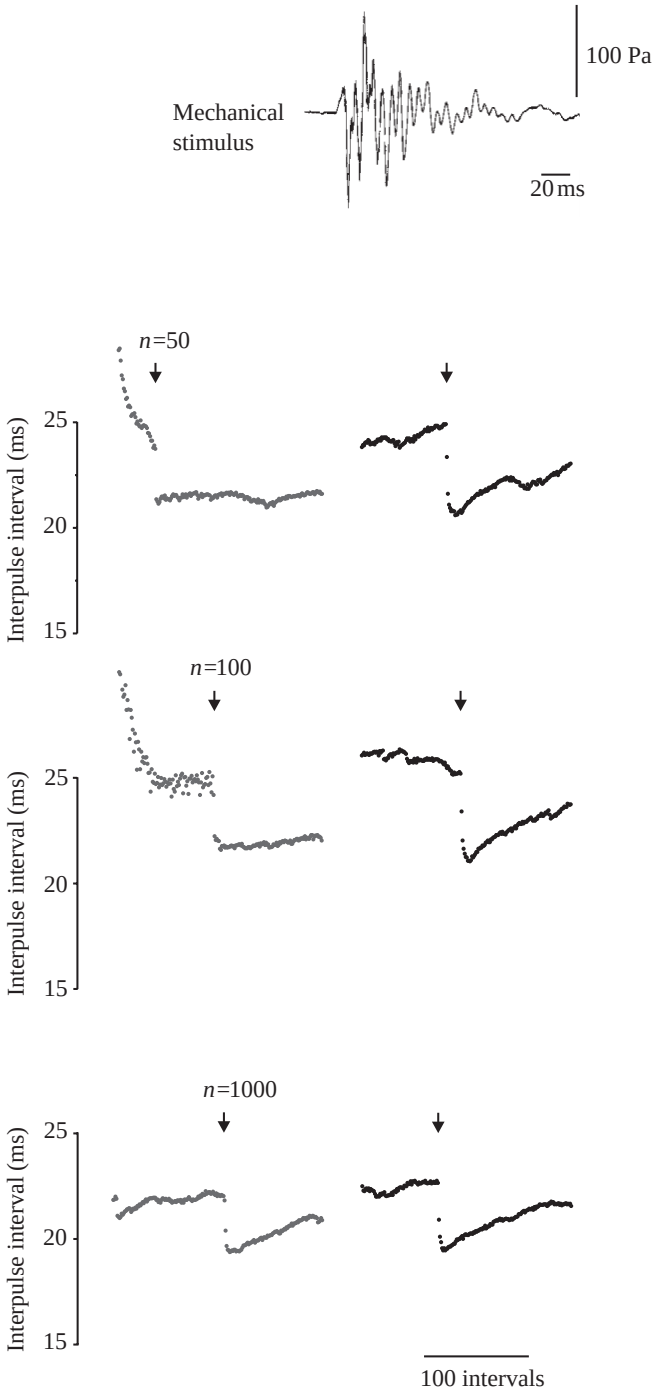

Fig. 3. Mechanosensory stimuli were able to elicit novelty responses immediately after a prior discharge interruption. The three rows illustrate pairs of responses obtained when the stimulus (top trace) was given *n* electric organ discharges (EODs) after a pause (left traces; *n*=50, 100, 1000, top to bottom row) and 10 000 EODs later during steady firing (right traces). Stimulus timing is indicated by arrows. Pause durations were 7, 75 and 20 s (top to bottom).

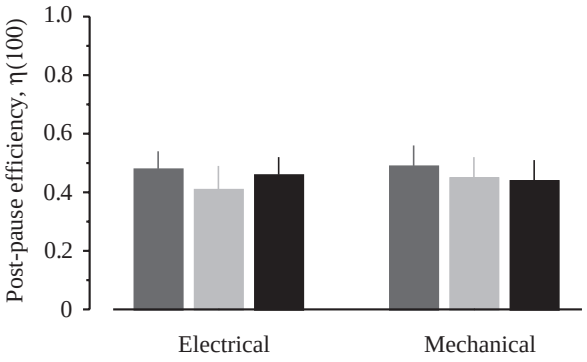

Fig. 4. The dramatic post-pause reduction in the size of electrically elicited novelty responses is not an aftereffect of a pause-eliciting electrical 'shock'. Post-pause efficiency, η(100), was determined 100 electric organ discharges (EODs) after a pause. The stimulus used was an electrical pulse (one cycle of a 1 kHz sinewave, peak-to-peak field strength 235 mV cm^{-1}). Pausing was elicited either by electrical shocks ('Electrical'; fish gc1, dark grey columns, 18 pauses; fish gc2, light grey columns, 10 pauses; fish gc3, black columns, 15 pauses) or, after appropriate training of the fish, by gently tapping on the wall of the tank ('Mechanical'; 12, 10 and 15 pauses, respectively). Values are means + S.E.M.

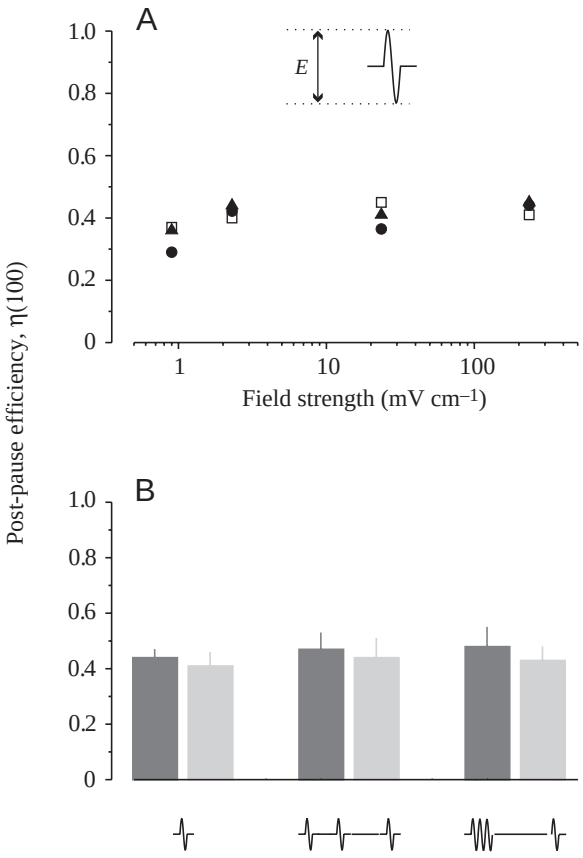

Fig. 5. The reduced efficiency of electrical pulses in eliciting a normal-sized post-pause novelty response was not due to insufficient intensity (A) or temporal patterning (B). (A) One cycle of a 1 kHz sinewave was delivered at one of the set field strengths indicated on the abscissa (0.9–235 mV cm^{-1} peak-to-peak). Different symbols relate to three fish, gc1 (circles), gc2 (squares) and gc3 (triangles), and show η(100), average efficiencies 100 electric organ discharges (EODs) after a pause. Measurements were obtained after 220 pauses. The number of pauses for each fish, given in order from low to high intensity, was: gc1, 20, 20, 7, 53; gc2, 10, 30, 10, 30; gc3, 10 each). The steady-state response strengths increased with increasing intensity but so did the post-pause strengths, leaving their ratio η constant. (B) The post-pause efficiency η(100) did not differ when either a single pulse or an extended series of pulses was given as stimulus. Means + S.E.M. are shown, obtained in experiments with two fish, gc1 (dark grey columns) and gc2 (light grey columns). The different time courses of the stimuli are illustrated schematically below the columns. Stimuli were a single cycle of a 1-kHz sine wave, a group of six such pulses with 20 ms silent intervals separating successive pulses or a continuous wave of 100 cycles. All stimuli had the same intensity (235 mV cm^{-1} peak-to-peak).

235 mV cm^{-1}) responses during steady firing than elicited by the mechanosensory stimulus. Within this range, the post-pause efficiency was independent of stimulus intensity (Fig. 5A).

The mechanical stimulus had a longer duration than the electrical stimulus and a different time course. Could the low post-pause efficiency of the electrical stimulus be due to its shorter duration? To analyze this possibility, two stimulus patterns of longer duration were tested: (i) a continuous sine burst, 100 ms in duration, and (ii) a series of six sine pulses (period 1 ms) with 20 ms intervals of silence between them (Fig. 5B). Both stimuli had an intensity of 235 mV cm^{-1} (peak-to-peak). The average post-pause efficiency obtained with these stimuli was not statistically different from the average efficiency of a single pulse of the same intensity. Furthermore, for each fish and for each stimulus condition, the average efficiencies differed significantly from the average 71 % post-pause efficiency obtained from the pooled data set described above using the mechanical stimulus (difference between each of the averages shown in Fig. 5B from the 71 % average P<0.001; t-tests).

The efficiency of low-frequency electrical stimuli

Besides their tuberous high-frequency electroreceptors, which monitor the ongoing EODs, all weakly electric fish possess ampullary receptors that detect low-frequency stimuli (direct current to approximately 100 Hz; for a review, see Zakon, 1986). As these receptors are not tuned to the EODs, they should be little affected by EOD pausing. It was therefore interesting to determine the post-pause efficiency of low-frequency stimuli that recruit ampullary receptors. Unfortunately, in *Gymnotus carapo*, there is no skin region where only ampullary or only tuberous receptors occur, so the two types of receptor cannot be activated selectively by localized stimulation (unlike in *Apteronotus leptorhynchus*; e.g. Zakon et al., 1998). Moreover, many of the tuberous receptors of *Gymnotus carapo* are very broadly tuned, with their sensitivity extending far into the low-frequency region (Watson and Bastian, 1979), so that separating ampullary and

tuberous receptors by varying the spectral energy of the electrical stimulus will be only partially successful.

While it is thus not possible to stimulate selectively either tuberous or ampullary receptors in *Gymnotus carapo*, it is still possible to investigate whether a greater involvement of ampullary and lesser involvement of tuberous receptors caused by a transition from a high-frequency to a low-frequency electrical stimulus might affect post-pause efficiency. To do this, a single sine-wave cycle of either 2 or 10 Hz, presented at a smaller than previously used stimulus intensity of 2.35 mV cm^{-1}, was chosen as the low-frequency stimulus that activates ampullary receptors. The two frequencies were chosen to assess the possible effects of the absolute pulse duration. A low-frequency sinusoid of 2 or 10 Hz might still be above the threshold of some tuberous receptors at these frequencies and, therefore, might not be expected to be sensed exclusively by ampullary receptors. However, from the tuning curves reported by Watson and Bastian (1979), it is clear that, at the chosen intensity, the high-frequency stimulus will recruit more tuberous receptors than the low-frequency stimulus. Thus, the relative contribution of ampullary receptors to the response will be higher for the low-frequency stimuli.

In the corresponding experiments, the average efficiency 100 EODs after a pause, $\eta(100)$, was determined for the low-frequency stimulus and compared with that obtained with a high-frequency stimulus of the same intensity. The post-pause efficiency of the low-frequency stimuli was higher than that obtained for high-frequency stimuli (Fig. 6). Because the average efficiencies of the 2 Hz and 10 Hz stimuli did not differ significantly, both stimuli were included in Fig. 6 in a single low-frequency efficiency for each fish that lay between the high-frequency and mechanical efficiency. In both fish, the observed average low-frequency post-pause efficiency differed significantly from the averages obtained with both the high-frequency and mechanical stimuli (all averages differed by at least $P<0.05$, *t*-tests). While the present experiments cannot exclude the possibility that a complete elimination of tuberous receptor activity would have yielded a still higher efficiency, they do show that an increase in the proportion of ampullary receptors recruited by an electrical stimulus increases its post-pause efficiency.

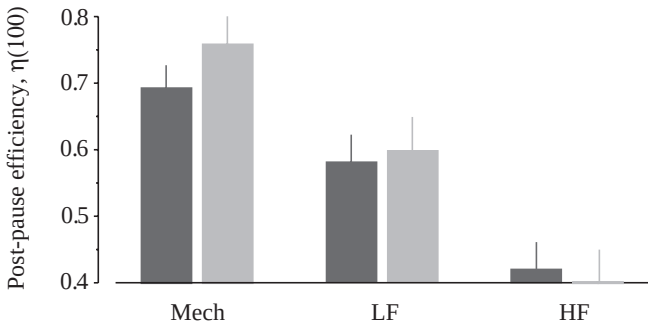

Fig. 6. Low-frequency (LF) electrical stimuli that recruit the ampullary electroreceptors more and the tuberous electroreceptors less than high-frequency stimuli (HF) are more efficient in eliciting post-pause responses. Means + S.E.M. of efficiency, $\eta(100)$, 100 electric organ discharges (EODs) after a pause were determined for three different types of stimulus: the mechanical stimulus ('Mech'; shown in Fig. 3), a low-frequency stimulus (a single cycle of a 2 Hz or a 10 Hz sine wave, selected with equal likelihood) and a high-frequency electrical stimulus (one cycle of a 1 kHz sine wave). LF and HF electrical stimuli had the same intensity (2.35 mV cm^{-1} peak-to-peak). Data were obtained from 171 pauses in two fish (fish gc1, dark grey columns, 26, 35 and 20 measurements for mechanosensory, LF and HF stimuli, respectively; fish gc2, light grey columns, 30 measurements for each stimulus type).

The course of post-pause efficiency

For both the standard electrical (see Fig. 2) and mechanical (see Fig. 3) stimuli, an attempt was made to determine the time course of efficiency from immediately after the pause until normal response levels were reached. To this end, a series of experiments was conducted with two fish, in which 486 pauses were elicited and average post-pause efficiencies $\eta(n)$ were determined for various set values of n (Fig. 7).

The mechanical stimulus elicited responses of approximately 70 % of the normal response strength as early as 20 EODs after a pause. Interestingly, its efficiency was constant at this level until approximately 1000 EODs after the pause, and only then approached the steady-state efficiency (i.e. $\eta=1$). In contrast, the efficiency of the electrical stimulus started at zero for $n=20$ and appeared to increase continuously with n, attaining the same efficiency as the mechanical stimulus approximately 1000 EODs or approximately 20 s after a pause. At 2000 EODs, or 40 s, after the pause, both the electrical and the mechanical stimulus elicited 'normal-sized' responses.

Discussion

By interrupting the otherwise continuous train of electric organ discharges (EODs), the results of this study demonstrate the importance of continuous EOD activity for the ability of a gymnotid weakly electric fish to respond to novel electrosensory stimuli. While such stimuli failed to elicit novelty responses immediately after an EOD pause, responses could readily be elicited by mechanosensory stimuli. The failure of electrosensory stimuli to elicit a response after a discharge cessation was not due to their insufficient intensity or their temporal patterning or to the way in which pausing had been elicited. The findings show that continuous activity is required, either to maintain the sensitivity to high-frequency electrical stimuli or to ensure that such stimuli are able to modulate efficiently the pacemaker that sets the discharge frequency. In the following, a brief overview is given of the structures that might be affected.

Pacemaker and motor output

In gymnotiform fish, each EOD is commanded by a discharge of the medullary pacemaker nucleus (PMn) (for reviews, see Bennett, 1971; Dye and Meyer, 1986), which

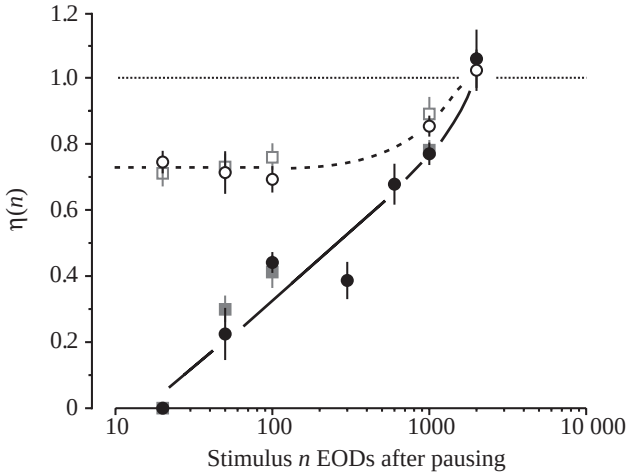

Fig. 7. The post-pause recovery of efficiency η in eliciting a novelty response. Means ± S.E.M. of η are shown for the electrical stimulus (filled symbols) and for the mechanical stimulus (open symbols). The diagram comprises 972 responses of two fish (fish gc1, circles; fish gc2, squares) after a total of 486 pauses. The electrical stimulus (see Fig. 2) was given at various preselected values of *n* after 153 (gc1) and 120 (gc2) pauses; the mechanical stimulus (see Fig. 3) was given after 93 (gc1) and 120 (gc2) pauses. When given 20 electric organ discharges (EODs) after a pause, the electrical stimulus failed to elicit a response in either fish. Tentative courses of post-pause efficiency towards the 'normal' efficiency (η=1; indicated by the dotted horizontal line) are fitted by eye for the electrical stimulus (continuous line) and the mechanical stimulus (broken line).

contains a network of pacemaking (PM) and relay (R) cells. The R cells transmit the command pulse to the spinal electromotor neurons that innervate the electric organ. This basic organization seems also to hold in *Gymnotus carapo* (Bennett et al., 1967; Bennett, 1971; Trujillo-Cénoz et al., 1993). At present, we are ignorant of the processes that occur within the PM and R cells of *Gymnotus carapo* during a pause in the EOD. Likely possibilities would be that the PM cells cease to fire during a pause or that the R cells are blocked. The latter possibility is realized, for instance, in the pulse-type fish *Hypopomus*, in which sudden interruptions are mediated by *N*-methyl-D-aspartate (NMDA)-receptor-activated depolarization of the relay cells (Kawasaki and Heiligenberg, 1989, 1990; Spiro, 1997) so that a spike in the PM cells is unable to elicit an R spike. Such a mechanism seems unlikely for the interruptions of *Gymnotus carapo* given the course of post-pause interval changes (Schuster, 2000; also see left-hand traces in Figs 2, 3). In its so-called sudden interruptions, *Hypopomus* fires at a constant frequency and then suddenly stops firing. If the fish then resumes its discharges after the pause, it fires immediately at the pre-pause frequency (Kawasaki and Heiligenberg, 1989). The situation is quite different in *Gymnotus carapo*. This fish was never observed to restart its firing at the pre-pause frequency but usually at a greatly reduced frequency. This might indicate that *Gymnotus carapo* PM cells stop their spontaneous activity during a pause. However, a direct demonstration of this is currently not available. In the present interpretation of the changes in post-pause responsiveness, it is important to consider potential post-pause changes in the PM and/or R cells, although their nature is unknown. Such changes could be important in determining the efficiency of a given stimulus-driven synaptic input to these cells in eliciting postsynaptic potentials.

Novelty-related input to the pacemaker

Tuberous (high-frequency) and ampullary (low-frequency) electroreceptors send their afferents to the electrosensory lateral line lobe (ELL), which projects to the torus semicircularis of the midbrain. Recordings from the torus semicircularis in *Gymnotus carapo* and *Hypopomus* (Grau and Bastian, 1986) suggest that this structure is involved in detecting the novelty of electrosensory stimuli. Unfortunately, we are ignorant of the sites of novelty detection for non-electrosensory stimuli. However, all such sites would issue a 'novelty command', probably *via* pre-pacemaker structures to the PMn, thereby causing a brief increase in its firing rate. It is likely that the sites of novelty detection and the paths over which a novelty command is sent to the PMn differ for different sensory modalities. Different paths, affecting different target cells in the PMn and possibly using different types of transmitter, seem likely and may explain possible differences in the courses of novelty responses elicited by stimuli that are sensed by different modalities (e.g. compare the steady-state responses in Figs 2 and 3). Moreover, a command to the PMn, elicited by input from one sense, may affect the PMn through more than one path to either the PM or R cells. Even co-activated multiple input to the same cell is possible, as has recently been demonstrated in *Gymnotus carapo* (Curti et al., 1999): in Mauthner-cell-induced pacemaker accelerations (Falconi et al., 1995), both NMDA and metabotropic glutamate receptor subtypes appear to be co-activated on a single PM cell.

Prior pausing affects the tuberous-driven input to the PMn

The failure of the high-frequency electrical but not of the mechanosensory stimuli to elicit a novelty response after a discharge cessation could be explained by two, not necessarily exclusive, mechanisms. (i) Both the tuberous electroreceptors, which sense the ongoing EODs, and their afferents are continuously (from EOD to EOD) active and could be less sensitive after an EOD pause. Also, the pathway from the ELL to the site where the novelty is detected is probably also continuously active (to account for the rapid detection of the novelty). Interrupting the ongoing activity of the receptors and their afferent pathway may well lower their sensitivity in an unknown way. It is not implausible that such effects could occur: preliminary data indicate post-pause changes in the alternating-current-resistance of the skin of *Gymnotus carapo* (S. Schuster, in preparation). It is not yet clear whether these changes occur as a result of activity-dependent resistance changes in the receptors. However, even if the receptors are not directly affected, such changes in skin impedance would

be likely to affect the current flow sensed by the receptors. (ii) The effect could also be caused by the interaction between synaptic input and the post-pause state of cells in either the PMn or perhaps also in pre-pacemaker structures. The efficiency of input to these cells might change as a result of postsynaptic mechanisms: as these cells are likely to undergo changes at the onset of firing after a pause, even presynaptic input of fixed size might lead to postsynaptic potentials of less than normal size, thus causing smaller rate modulations. Such a mechanism could also explain the 30 % reduction in efficiency observed for the mechanical stimulus, but would probably be most relevant for the novelty command input driven by the tuberous electrosensory input and less for mechanosensory- and ampullary-driven inputs. Mechanosensory- and ampullary-driven novelty commands presumably activate other paths, possibly using different transmitters and receptors, in which the efficacy of synaptic transmission could be less affected by the state of their target cells.

Relevance for studies on the consequences of post-pause changes in EOD waveform

The novelty response appears to be a powerful tool for studying how changes in the waveform and amplitude of its EOD (Franchina and Stoddard, 1998; Zakon et al., 1999; Schuster, 2000) might affect the ability of a weakly electric fish to electrolocate. The fastest EOD changes occur when a *Gymnotus carapo* resumes its EODs after a preceding pause (Schuster, 2000). To address the implications of such EOD changes, it should be possible to design experiments in which the fish signals with its novelty response whether it has detected a novelty in its EOD feedback. Placing such a novelty at various stages of the post-pause recovery in which the successive EODs either vary in known ways or remain constant could determine whether EOD changes are detrimental to the fish's ability to electrolocate. However, in such experiments, I have never observed novelty responses to changes in EOD feedback during the post-pause period in which the dramatic EOD changes occur. The present investigation was started as a result of this failure and has shown that high-frequency electrical stimuli are simply unable to drive a novelty response after a preceding pause. This finding might also be important to bear in mind in comparable research on species that show EOD changes without prior pausing. If EOD changes occurred in correlation with changes in discharge rate, then the efficiency of a given change in EOD feedback in eliciting a novelty response could well be reduced as a result of the rate changes rather than the EOD changes.

Is there an advantage of a lower post-pause responsiveness?

At present, it is only possible to speculate whether the reduced responsivensess to high-frequency electrical stimuli might be more than a mere by-product of disrupting an otherwise continuously active system. In a natural situation, *Gymnotus carapo* would switch off its electric organ both as a strong submissive signal to conspecifics (Black-Cleworth, 1970; Westby, 1974) and during an encounter with one of its predators, the electric eel (Westby, 1988). In both types of encounter, it is not implausible that increases in discharge frequency, such as those occurring during a novelty response, could be detrimental. Increases in discharge frequency can be interpreted as aggressive signals by conspecifics (Black-Cleworth, 1970; Westby, 1974), and electric eels are most attracted by high discharge rates of approximately 100 Hz (Bullock, 1969), which is above the resting frequency of a *Gymnotus carapo*, so it might be a good strategy to suppress rate increases in response to the electrical stimuli emitted by a superior conspecific or an electric eel if one is still around after a pause.

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