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Plasticity in a cerebellar-like structure: suppressing reafference during episodic behaviors

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SUMMARY

Detection of relevant sensory signals requires the filtering out of irrelevant noise, including noise created by the animal's own movements (reafference). This is accomplished in the electrosense of little skates (*Raja erinacea*) by an adaptive filter in the cerebellar-like electrosensory nucleus (dorsal nucleus) in the medulla. We have shown that electrosensory inputs reliably coupled to the regularly recurring movements of breathing over time are eliminated selectively in the principal neurons (ascending efferent neurons, AENs) by a cancellation signal that is a negative of the reafference and is supplied by a parallel fiber system. Similarly, electrosensory inputs repeatedly linked to passive fin movements are eliminated suggesting that the filter also functions in relation to other behaviors besides breathing. To determine whether this adaptive filter can eliminate reafference created by brief and infrequent episodic behaviors like swimming in skates, we initiated a series of coupling tests in which an external electrosensory stimulus was coupled to short bouts of either parallel fiber stimulation or passive fin movements, and then measured the ability of AENs to generate a cancellation signal. Following five brief coupling periods (30–60 s) separated by long rest periods (1–9 min), 38.5% of the AENs developed a cancellation signal when the coupling was to parallel fiber stimulation, and 73% when the coupling was to passive fin movement. We demonstrate that the cancellation signals can be developed incrementally, persist for at least a 3 h rest period without reinforcement, and are extinguished within minutes when the association of sensory stimulus and fin movement or parallel fiber stimulation no longer exists. The results indicate that the adaptive filter has the properties necessary to cancel reafference associated with even brief and infrequent behaviors.

Key words: electrosensory, parallel fiber, sensory reafference, synaptic plasticity.

INTRODUCTION

One of the essential functions of information processing in most sensory systems is to remove expected or predictable sensory inputs which convey no new information. Perhaps the best understood examples of this come from studies of the electrosense in elasmobranch fish, and mormyrid and gymnotid electric fish in which adaptive filters work to subtract predictable self-stimulation (Bell, 1982; Bell et al., 1993; Bell et al., 1999; Montgomery and Bodznick, 1994; Bodznick et al., 1999; Bastian, 1995; Bastian, 1999).

Like most aquatic animals (Kalmijn, 1974), skates inadvertently generate weakly electric fields around themselves in part as a result of osmoregulatory ion pumping. Skates are electroreceptive and possess dermal sensory organs that are extremely sensitive to the electric fields generated by other animals and by themselves, making it important to separate new information from self-stimulation. With our co-workers we have previously shown that in skates the dorsal octavolateral nucleus (dorsal nucleus), which is the primary electrosensory nucleus in the medulla, plays a critical role in the filtering of electrosensory signals *via* modulation of the responses of the dorsal nucleus projection neurons, the so-called ascending efferent neurons (AENs). In particular, the AENs learn to suppress unwanted responses to the electrosensory self-stimulation (reafference) that accompanies the fish's own behaviors. Consequently, in skates and rays, although primary afferents respond vigorously to electrosensory stimuli created by the fish's breathing, the AENs are much less activated by the same reafference, so the signal-to-noise ratio of the output from the AENs is significantly increased over that in the afferents (Montgomery, 1984; Bodznick and Montgomery, 1992; Bodznick et al., 1992).

The underlying anatomical organization for this reafference suppression is as follows. The basilar dendrites of the AENs are monosynaptically activated by the primary electrosensory afferents. In addition, thousands of parallel fibers and also inhibitory interneurons synapse on the spiny apical dendrites of the same AENs in an overlying molecular layer of the nucleus, and these inputs carry motor corollary discharge, propriosensory and descending electrosensory information. The molecular layer inputs can modify the AEN response to the electroreceptor inputs based on previous and ongoing experience. When an external excitatory electrosensory stimulus is reliably coupled to the animal's ventilation for 5–10 min or longer, the AEN response to the external stimulus decreases. When the stimulus is removed, the mechanism for the reduction in the AEN response is evidenced by a cancellation signal (or negative image of the original response to the stimulus) in the AEN, which is phase locked to the ventilation (Montgomery and Bodznick, 1994; Bodznick et al., 1999). The cancellation signal can be altered and continually updated as a result of an active re-matching process associated with an absence of or a change in the reafference.

According to the adaptive filter model (Montgomery and Bodznick, 1994), the cancellation signals are constantly contained in the parallel fiber matrix and the differential weighting of its synapses with the AENs. Furthermore, the cancellation signals are modified through the adjustment of the strength of these synapses.

The AENs eliminate the electrosensory reafference by extracting a cancellation input from the parallel fiber matrix that is equal to the negative of the reafference. This model is directly supported by recent *in vivo* patch clamp studies showing the predicted changes in parallel fiber excitatory synaptic potentials (and probably also interneuron inhibitory synapses) in AENs during the development of new cancellation signals (Bertetto, 2007). Similar findings were previously reported in the independently evolved electrosensory systems of weakly electric fish (Bell et al., 1993; Bastian, 1996).

The adaptive filter in the skate dorsal nucleus, and the filters in mormyrids and gymnotids, have been tested in almost all cases for the elimination of reafference caused by nearly continuous behaviors, such as ventilation and electric organ discharges. In skates, direct parallel fiber stimulation coupled to an electrosensory stimulus in a similar continuously recurring manner gives the same result (Bodznick et al., 1999). In each case a sensory stimulus repeatedly coupled to movements or parallel fiber stimulation for a sufficient duration will result in the development of a cancellation signal. However, episodic behaviors, such as swimming in skates, often occur in only short bouts that are each seemingly much too brief to generate a cancellation signal *de novo*. To create a cancellation signal that works for episodic behaviors, the development of the signal must happen incrementally and persist during inter-episodic periods. Each newly developed contribution to the cancellation signal must add to the previous one that is preserved during inter-episodic intervals of varying duration. In this study, we mimicked such behaviors by episodically coupling an external electrosensory stimulus to either passive fin movement or direct parallel fiber stimulation and show that the cancellation signal is incrementally developed during repeated short co-activations. We further show that, after the cancellation signal is fully constructed, it can last for at least 3 h in the absence of further parallel fiber stimulation or fin movement. These results demonstrate that the adaptive filter mechanism in the dorsal nucleus has the properties necessary to eliminate self-stimulation generated by even rare and episodic behaviors.

MATERIALS AND METHODS

Animals and surgery

Little skates (*Raja erinacea* Mitchill 1825) were anesthetized by immersion in 0.04% benzocaine and surgical procedures were performed as previously described (Duman and Bodznick, 1996). The brains were exposed by the removal of the overlying cartilage, and decerebrated by diencephalic section. In most cases, the skates were then injected with tubocurare (0.1 mg kg^{-1}, i.v., Sigma) to eliminate all movements. In a few cases where noted, skates were only partially paralyzed by destroying the spinal cord to eliminate trunk and tail movements but leave normal breathing movements intact. After surgery, the fish were transferred to a Plexiglas experimental tank of cold seawater, and positioned with a Plexiglas head holder so that the cranial opening was just above the water surface. A gentle flow of seawater (0.1–0.4 l min^{-1}, 9°C) was directed into the mouth as an extra support to ventilation.

All procedures followed NIH guidelines for the care and use of experimental animals and were approved by the Animal Care and Use Committees of Wesleyan University and the Marine Biological Laboratory.

Electrophysiological methods

Unit activity was recorded extracellularly using Pt–black-tipped indium microelectrodes (2–7 MΩ, 1–2 μm tip). The AENs were identified by their antidromic response to electrical stimulation of the contralateral lateral mesencephalic nucleus. All neural responses were filtered, amplified and then acquired and analyzed using Spike 2 software (CED, Cambridge, UK). In experiments with only partially paralyzed fish, the fish's breathing activity was continuously monitored with a force transducer placed against the skin over the branchial chamber.

The parallel fibers that originate in the dorsal granular ridge (DGR) and synapse on the spiny apical dendrites of the AENs were electrically stimulated using a tungsten microelectrode in the DGR and a return electrode in the seawater. The location of the DGR stimulating electrode was chosen based on the known topography of the DGR to dorsal nucleus projection (Conley and Bodznick, 1994) and optimized to elicit the largest evoked potential response from the dorsal nucleus recording site. The parallel fiber stimuli were delivered as 250 ms, 25 Hz trains of pulses; each pulse was of 0.2 ms duration, 2–5 V amplitude. Trains were repeated every 2 s (Fig. 1B). Passive fin movement was generated by attaching plastic clips to the ipsilateral pectoral fin and using strings to connect these clips to an arm extending from a servo motor controlled by a function generator. The movement had the form of a single cycle sinusoid of 1 Hz repeated every 2 s. Excitatory electrosensory receptive fields of AENs were localized and stimulated by a 2–10 μV, DC step, dipole electric field (dipole electrodes were 2 mm glass tubes filled with 1.5% agar in seawater and poles were separated by 0.5 cm).

Our experimental protocol had three phases (Fig. 1): (1) a precoupling phase in which the parallel fiber stimulus trains or fin movements were given alone in order to measure AEN responsiveness before coupling; (2) a coupling phase during which an electrosensory stimulus (250 ms duration) was presented simultaneously with each parallel fiber stimulus train or fin movement either continuously for 5 min or episodically, with each episode of coupling lasting 30 s to 2 min separated by rest periods of 1–9 min without any stimulation (parallel fiber or electric field) or fin movements [one coupling period plus the following rest period was defined as a coupling cycle; in general, five coupling cycles were given (Fig. 1A)]; and finally (3) a postcoupling period during which the AEN responsiveness to parallel fiber stimulation or fin movement alone was again measured.

From our past studies we know that under the conditions of our experiments generally only 55–65% of AENs in the dorsal nucleus appear to exhibit the adaptive filter capability. Therefore, in many cases, before testing an AEN with episodic stimulus coupling, we first demonstrated that the AEN was able to develop a clear cancellation signal with the continuous coupling protocol. After a sufficient recovery period (usually at least 30 min) during which parallel fiber stimulation or fin lift continued without an accompanying sensory stimulus until all traces of the previous cancellation signal were gone, the neuron was tested again with the episodic coupling regime.

Data analysis

After an excitatory electrosensory stimulus was coupled to either direct parallel fiber stimulation or fin movements, the AEN firing rate was measured during parallel fiber stimulation or fin movement alone, and the results were compared with those obtained before the coupling. A cancellation signal was shown as a significant reduction in the AEN firing rate specific to the period of the previously coupled excitatory electrosensory stimulus. The relative spike rates were generated by subtracting the background firing rate during a control interval outside the stimulation period from the firing rate during the coupling period as:

$$S_s = (S_t/T_t) - (S_o/T_o)\,, \quad (1)$$

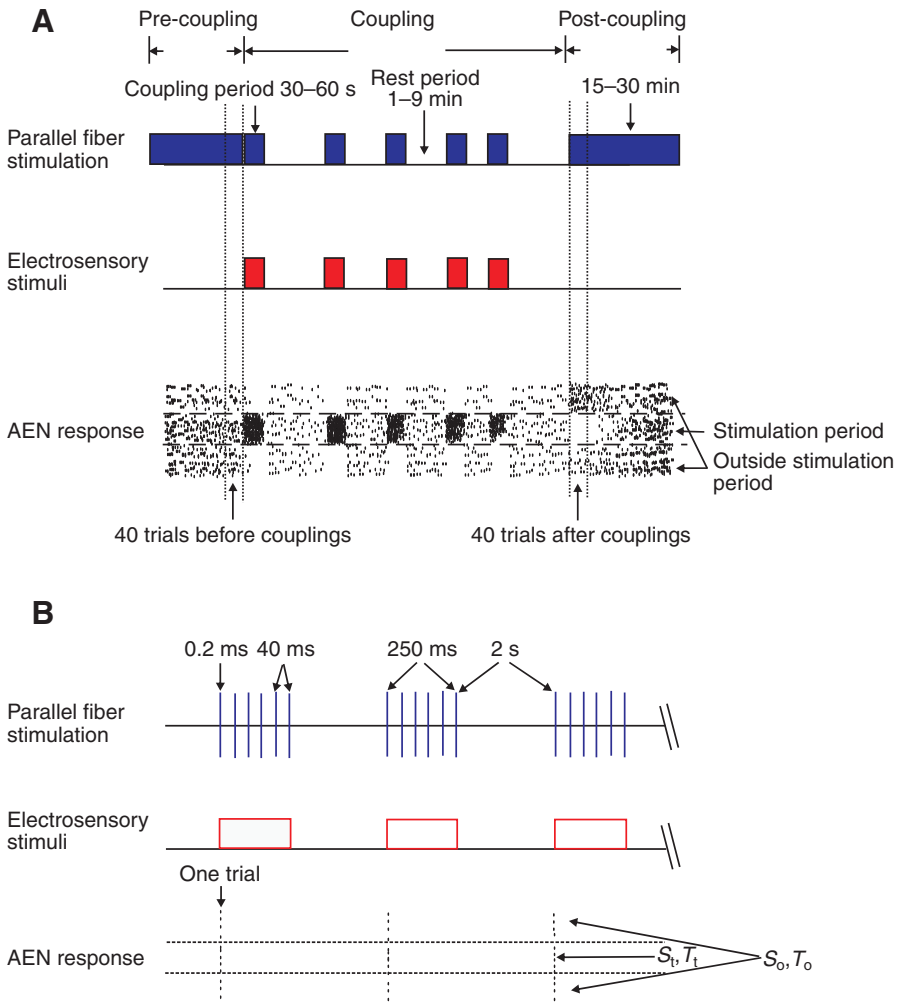

Fig. 1. Coupling test protocol. (A) During the pre-coupling period, only parallel fiber stimulus trains (see B) are given to test the AEN responsiveness before coupling. During the coupling period, an excitatory electrosensory stimulus is delivered simultaneously with each parallel fiber train either continuously for 5 min (continuous coupling) or, as illustrated here, in five short bouts of coupling separated by rest periods during which neither parallel fiber stimuli nor electrosensory stimuli are presented (episodic coupling). One coupling cycle is composed of one coupling period (30–60 s) and one resting period (1–9 min). At the bottom, the AEN activity throughout the experiment is illustrated as a raster plot. During the postcoupling period, parallel fiber stimulus trains are again given alone. (B) The duration of a single parallel fiber stimulus train is 250 ms and the inter-train interval is 2 s. Each train has six pulses, each 0.2 ms in duration (25 Hz intra-train frequency). The dipole electric field (2–10 μV, 250 ms) is presented simultaneously with each parallel fiber train. The AEN response to each parallel fiber train is defined as one trial. In tests with fin movement, a single cycle sinusoidal (1 Hz) passive fin movement replaces the parallel fiber stimulus train. S_t, number of spikes counted during the stimulation period; T_t, duration of the stimulation period in seconds; S_o, number of spikes counted during the arbitrary control interval outside the stimulation period; T_o, duration of the arbitrary control interval outside the stimulation period in seconds; see Eqn 1.

where S_s is the subtracted spike rate (spike s^{-1}), S_t is the number of spikes counted during the stimulation period, T_t is the duration of the stimulation period in seconds, S_o is the number of spikes counted during the arbitrary control interval outside the stimulation period and T_o is the duration of the arbitrary control interval outside the stimulation period in seconds. We refer to this statistic (S_s) as the subtracted spike rate, and use it to detect spike rate changes that are specific to the stimulation period compared with background firing. The AEN response induced by one stimulation period is defined as one trial. There was one S_s for each trial (Fig. 1B). A Wilcoxon–Mann–Whitney test was used to compare the 40 trials (80 s time window) right before and 40 trials right after the coupling (Fig. 1A). Each AEN was analyzed separately. The AEN firing rate after the coupling was continuously monitored to verify the recovery of the AEN activity from the coupling.

RESULTS

The adaptive filter suppresses responses to stimuli repeatedly coupled to ventilation, passive fin movement or direct parallel fiber stimulation

As shown previously (Montgomery and Bodznick, 1994), in more than half of AENs tested the response to an external electrosensory stimulus reliably linked to the recurring movements of breathing is reduced by the action of a cancellation signal that develops during the coupling. This is also true when an electrosensory stimulus is coupled to either passive fin movements or direct parallel fiber stimulation delivered in a similarly repeating pattern for 5 or 10 min (Bodznick et al., 1999). Though the response to the external stimulus markedly declines in many of these cells, the most robust indicator of the plasticity is the cancellation signal associated with breathing movements, fin movements or parallel fiber stimulation right after the electrosensory stimulus is removed. We believe that the lack of a significant reduction in the response to the external stimulus in some cases probably reflects limitations of the adaptive filter under our specific test conditions, including the use of relatively short coupling times and strong sensory stimuli.

The cancellation signal following coupling with an excitatory sensory stimulus is defined as a statistically significant decrease in AEN firing rate after the coupling that is specific to the period during the movement or parallel fiber stimulation at which the excitatory sensory stimulus had been presented. The subtracted spike rate (S_s), as defined in Materials and methods, was the statistic we used to measure this firing rate during the stimulus period relative to background firing, and cancellation signals were evident as a significant decline in this statistic. In the current tests, external stimulus coupling to ventilation resulted in the formation of a cancellation signal in 86% (21 out of 28) of the AENs tested. Coupling an electrosensory stimulus to passive fin movements or parallel fiber stimulation for a period of 5 min resulted in 80% (12 out of 15) and 46% (23 out of 50), respectively ($P<0.05$), of AENs exhibiting a cancellation signal. Representative examples

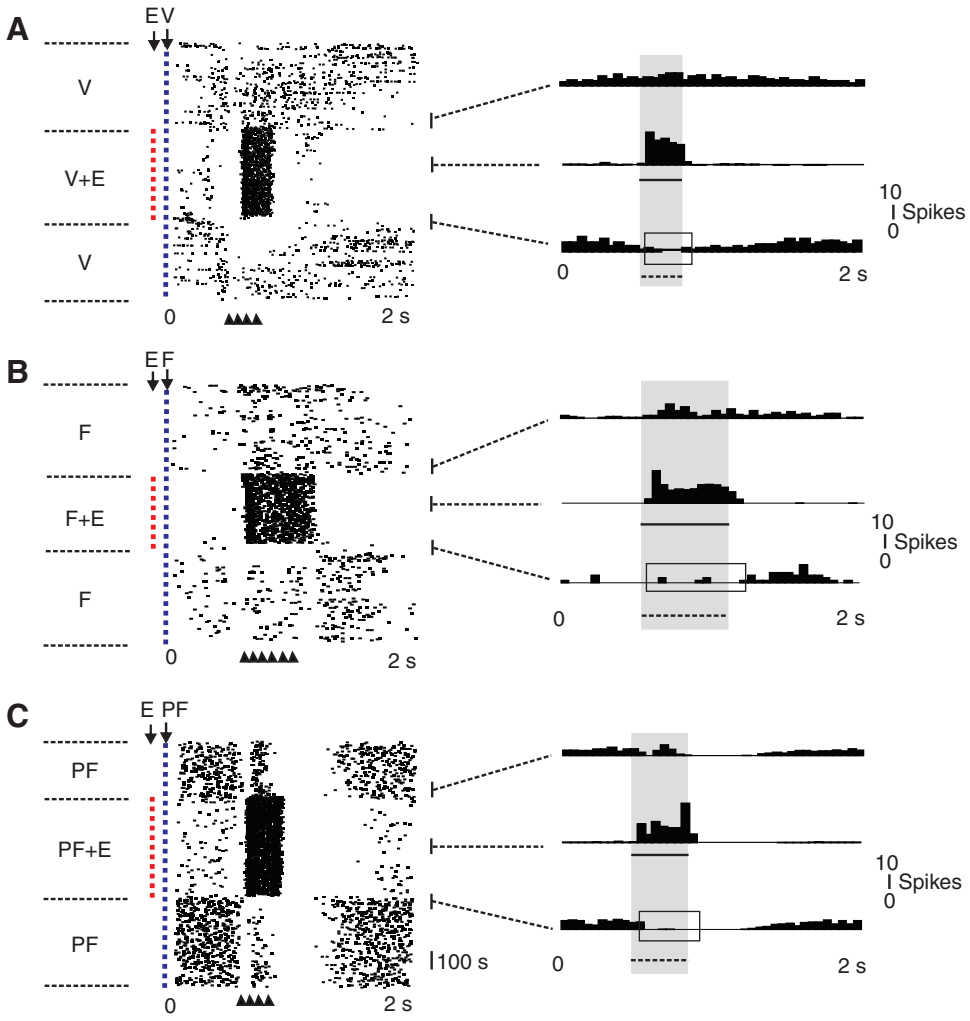

Fig. 2. Cancellation signals are developed after 5 min of continuous coupling of an electrosensory stimulus to ventilatory movements (A), passive fin movements (B) or direct parallel fiber stimulation (C). A raster plot in each case shows the activity of an AEN before, during and after the 5 or 10 min coupling. Note that the red dots on the left indicate trials when the electrosensory stimulus is presented coupled to ventilation, fin movement or parallel fiber stimulation (blue dots). On the right are spike histograms of 30 trials from the times indicated. Boxes on the bottom traces indicate cancellation signals. In each case there is a significant decrease in the AEN subtracted spike rate after the coupling (P<0.01) compared with that before. V, ventilation; V+E, 2 μV local dipole stimulus coupled to ventilatory movements. F, passive fin movement; F+E, 2 μV dipole stimulus coupled to fin movement. PF, parallel fiber electrical stimulation alone; PF+E, 2 μV dipole coupled with parallel fiber stimulus trains. The arrowheads beneath the raster plots indicate the periods of ventilatory movement, fin movement or parallel fiber stimulation. During coupling the E field stimulus is also presented throughout this same stimulus period.

for ventilation, passive fin movement and direct parallel fiber stimulation are shown in Fig. 2. The temporal link is required. When an external stimulus was given randomly at about the same rate but without coupling to ventilation, fin lift or parallel fiber activation, cancellation signals were not observed in any case (N=12).

Episodic co-activation of AENs and parallel fibers results in incremental cancellation signal generation

The demonstration that the adaptive filter mechanism can be studied using an electrosensory stimulus linked to passive fin movement and even direct parallel fiber stimulation enabled us to investigate whether the mechanism has the properties necessary for it to function in the subtraction of electrosensory stimuli linked to episodic behaviors.

For the episodic protocol, following determination of baseline AEN activity, we first initiated a 1 min coupling stimulation and compared the subtracted firing rates of the AEN during the 80 s just before and just after the coupling to determine whether a single short coupling period was sufficient to induce a cancellation signal. No significant difference was found after 1 min of coupling in any of the cases, whether coupling with parallel fiber stimulation or with fin movements (representative example shown in Fig. 3A,B). Thus, a single 1 min coupling is not enough to induce any measurable cancellation signal.

In order to mimic episodic behavior, we divided the 5 min continuous coupling duration into five cycles, with each cycle composed of a 1 min coupling period followed by a 2 min resting period without either external sensory stimulation or parallel fiber stimulation (Fig. 1A). Thus, the total coupling duration remained at 5 min, as above, but was evenly distributed in 1 min episodes over a total period of 15 min. When the AEN subtracted firing rate immediately after the five cycles was compared with that from the period just before coupling, there was a significant decrease in the AEN firing rate specific to the coupling phase (Fig. 3A,C). Under these conditions, coupling an external stimulus (2 μV) to direct parallel fiber stimulation (0.5 Hz, 2–5 V) resulted in the development of a significant cancellation signal in 38% (41/109) of the AENs tested (representative example shown in Fig. 3).

To determine whether the multiple coupling cycles or just additional time are required for the development of cancellation signals, we performed a single 1 min coupling cycle and measured AEN activity immediately after and 14 min after coupling. In this case, the total experimental time was equal to that used for the five 1 min coupling tests. For these experiments, we chose AENs that had successfully developed cancellation signals after five 1 min couplings, but were allowed to recover fully to baseline levels of activity. Under these conditions, cancellation signals as measured by the subtracted spike rates were never observed (P>0.05, N=7,

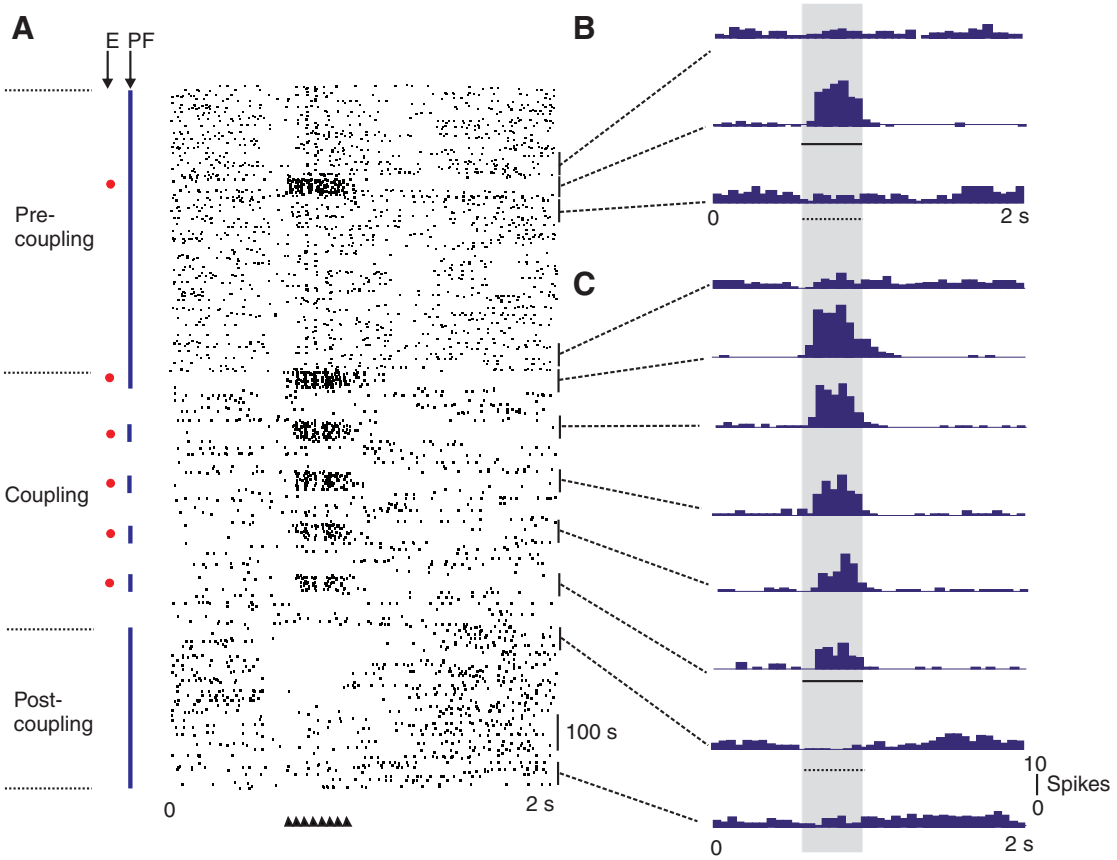

Fig. 3. A cancellation signal appeared in an AEN after five 1 min periods of coupling parallel fiber stimulation to an electrosensory stimulus. (A) Raster plot shows activity of an AEN before, during and after the five 1 min couplings of a 2 μV dipole stimulus to direct parallel fiber stimulation. No measurable change is found after a single 1 min coupling test (P>0.05) during the precoupling period. However, the cancellation signal (or negative image) following five 1 min coupling cycles appears as soon as parallel fiber stimulation is resumed in the post-coupling period (P<0.01), and then fades within minutes as parallel fiber stimulation is given alone. Note that since the parallel fiber inputs are the source of the cancellation signal in the AEN there is no cancellation signal present during the rest periods when the parallel fiber stimulation is stopped along with the electrosensory stimulus. (B) Histograms (30 trials each) from the same data as in the raster plots in A, representing the AEN activity before, during and after a single 1 min coupling test. (C) Histograms before, during and after the five 1 min episodes of coupling. Note the presence of the cancellation signal (seen as a negative image of the initial response of the AEN) when parallel fiber stimulation is resumed after the five 1 min couplings (P<0.01). Also note that during the five 1 min couplings, the response of the AEN to the dipole stimulus gradually declines with each additional coupling cycle. Rasters, histograms and labels as in Fig. 2.

data not shown). Therefore, multiple cycles are required and the cancellation signal is developed incrementally.

The nature of episodic learning is such that if the adaptive filter mechanism is to function to remove reafference, the changes in synaptic strength between the parallel fibers/interneurons and AEN must be preserved during the resting period. In skates the swimming bouts are often very short and separated by quite lengthy periods spent resting quietly on the bottom. To test further the limits of the adaptive filter for such episodic behaviors, we reduced the duration of each individual coupling cycle to 30 s and increased the resting periods to 5 min. Therefore, the total coupling period was shortened to 2.5 min and the total resting period was extended to 25 min. Reduction in the individual coupling periods to 30 s, for a total coupling duration of 2.5 min over a 27.5 min period still resulted in the formation of cancellation signals in 39% of the AENs (15 out of 38) when coupling the electrosensory stimulus to parallel fiber stimulation (representative AEN shown in Fig. 4A). Similar experiments in which five cycles of a 30 s coupling of external stimulation to passive fin movement each followed by a 5 min resting period resulted in 73% (11/15 AENs) developing a cancellation signal (representative AEN shown in Fig. 4B). These results show that incremental changes in synaptic strength occur in just 30 s of coupling (15 trials) and can persist for a minimum of 5 min between couplings of external stimulation with either direct parallel fiber stimulation or passive fin movement.

As can be seen in Figs 3 and 4, the responses of many of the AENs to the external stimulus gradually decreased following each additional coupling cycle, regardless of whether the coupling was to parallel fiber stimulation or fin movements. Quantification of all neurons that developed cancellation signals, regardless of stimulation protocol, revealed that 43% of these AENs (29/67) displayed an incremental decrease with each additional bout of coupling. In addition, similar to that observed for continuous stimulation, an increasing delay between the onset of external stimuli and AEN response was also observed in many of the cells. These results also indicate that, as stated previously, the cancellation signal is the most robust indicator of the plasticity of the adaptive filter.

The cancellation signal can still be developed after varying the resting periods

Natural episodic behavior is irregular. To better mimic the natural behaviors, the interval between two coupling periods was varied

from a minimum of 1 min to a maximum of 9 min. In all instances, the total resting period was maintained at 25 min. AENs that had successfully developed cancellation signals with continuous coupling were chosen, and irregular episodic stimulation was initiated after the AEN activity had returned to baseline levels (a minimum of 30 min). The AEN subtracted spike rate after the five couplings with irregular intervals was compared with that before the couplings, and there was a significant decrease in the AEN firing rate ($P<0.05$; Fig. 5). These results show that the adaptive filter in the dorsal nucleus is capable of canceling noise resulting from irregular episodic stimulation, and that development of the cancellation signal can accommodate at least a single resting period of up to 9 min.

Cancellation signals are long lasting but reversible

To characterize the cancellation signal, and apparent underlying synaptic changes following episodic coupling, as a short-term *versus* long-term phenomenon, AENs that fully developed a cancellation signal with coupling were examined for the persistence of the cancellation signals in the absence of further parallel fiber stimulation and external stimuli. For these experiments, once a cancellation signal was fully developed, another five 30 s coupling/5 min resting cycles were given to reinforce the signal, then both parallel fiber and external electrosensory stimulation were terminated. The maintenance of a negative image was examined after either 2 or 3 h of AEN stimulus deprivation. Due to physical limitations, we could not extend our experiments beyond 3 h. Under these conditions, a cancellation signal was preserved in a total of 60% (6/10) of the AENs tested. A representative example is shown in Fig. 6, where the cancellation signal was preserved for 2 h ($P<0.01$). Of the six AENs that retained the cancellation signal, four were from the 2 h deprivation experiment, and two were from the 3 h deprivation experiment. In each case the cancellation signals then gradually disappeared after parallel fiber stimulation was resumed without the external electrosensory stimulus. Therefore, cancellation signals following episodic coupling, just as those after continuous coupling (Bodznick et al., 1999), are very long lasting but quickly reversible when the association between parallel fiber activity and sensory stimulation changes.

DISCUSSION

The incremental development and storage of cancellation signals in the dorsal nucleus

The detection of behaviorally relevant stimuli involves selection and amplification of only the relevant signals from the full array of sensory inputs reaching the brain. We have used subtraction of electrosensory self-stimulation in skates to study mechanisms by which suppression of non-novel sensory information can occur in the dorsal nucleus, a cerebellar-like structure of the medulla. The key feature of the dorsal nucleus and other cerebellar-like structures is a parallel fiber system which provides the principal neurons (AENs) with a complex array of motor commands and sensory

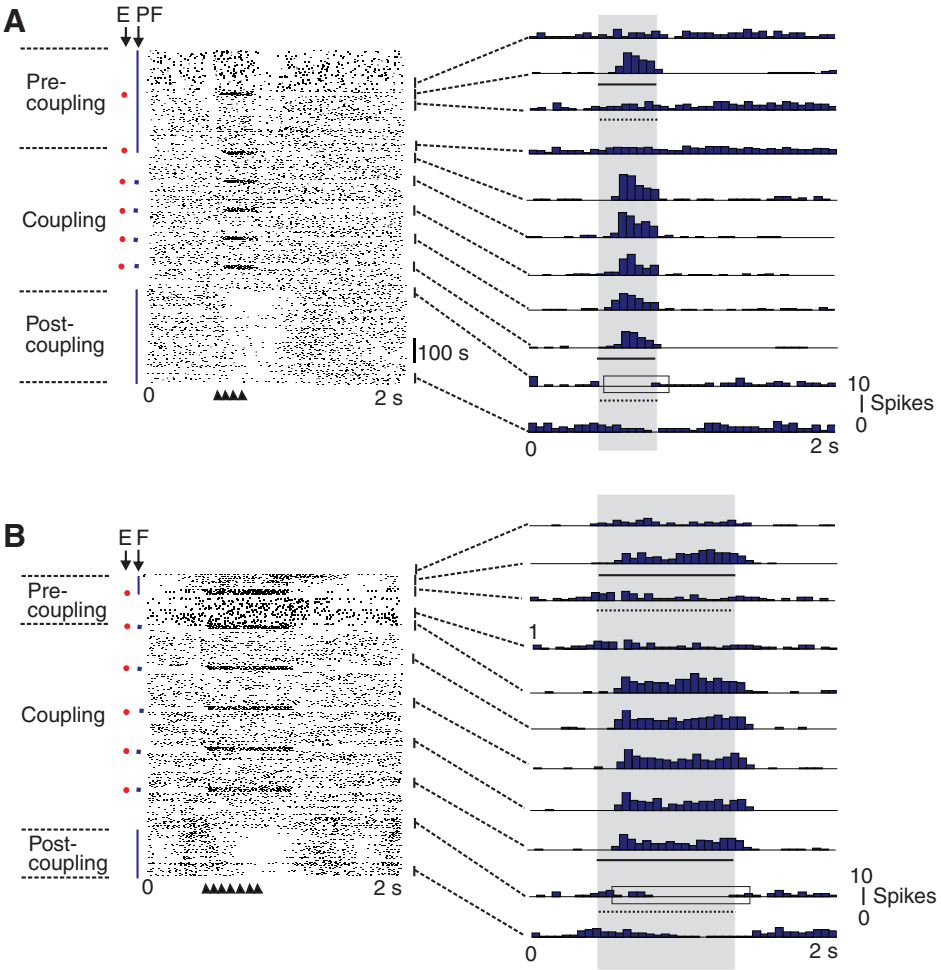

Fig. 4. Cancellation signals are absent after one 30 s coupling period but develop during five 30 s coupling periods separated by 5 min rest periods. An excitatory electrosensory stimulus (2 μV) was coupled with parallel fiber stimulus trains (A) or passive fin movements (B). Note the presence of the cancellation signal in the AEN activity when the parallel fiber trains or fin movements are resumed during the post-coupling period. Rasters, histograms and labels as in previous figures.

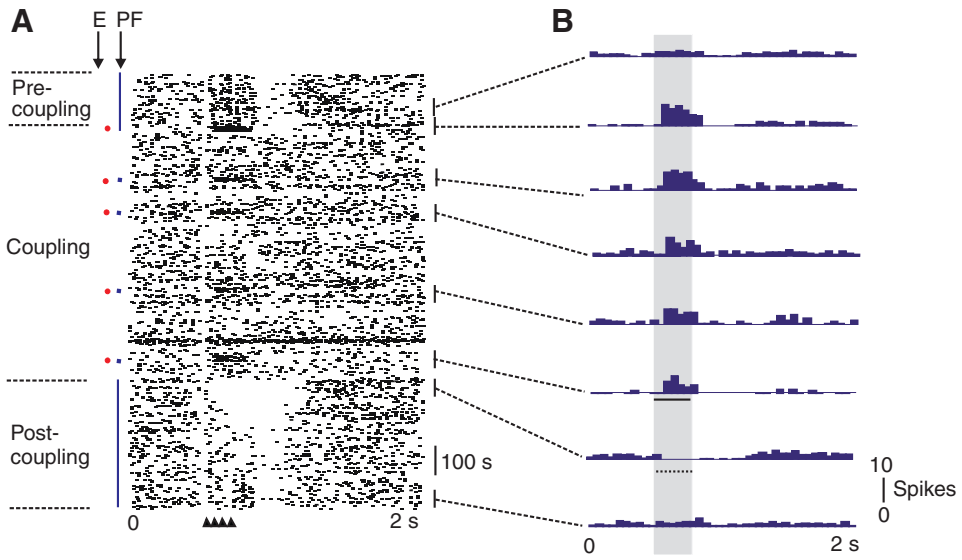

Fig. 5. A cancellation signal is developed after five 30 s couplings separated by irregular rest intervals. (A) A raster plot showing activity of an AEN before, during and after the five 30 s couplings separated by variable rest intervals. The cancellation signal is apparent when parallel fiber stimulation resumes after the last rest period. (B) Histograms of AEN activity from the periods indicated. Note the AEN response to the dipole stimulus gradually declines with each additional coupling cycle. Labels as in previous figures.

feedback related to the animal's own behaviors. From these parallel fiber inputs a cancellation signal is constructed by each AEN that takes the form of the negative of the predictable features of the reafference during each particular behavior. Plasticity of the parallel fiber synapses with each AEN allows the cancellation signal to be updated within several minutes to accommodate changes in the reafference.

The adjustments in parallel fiber synaptic weightings are directed by the output of each AEN. The posited learning rules are that when a given parallel fiber is active and the AEN is active, the gain of the synapse is reduced and conversely when a parallel fiber is active and the AEN is not active, those synapses are strengthened (Montgomery and Bodznick, 1994). This removes excitation from the parallel fiber inputs to the AEN at times when the AEN is consistently excited by the reafference and *vice versa*. The resulting cancellation signal thus counters all AEN excitation or inhibition that is predicted by activity in the parallel fiber system. Note that by these learning rules the synapses of only active parallel fibers are altered. Inactive parallel fibers hold their current synaptic weightings during periods of inactivity.

For behaviors like breathing and even swimming in those fish that swim more or less continuously, the synaptic weightings and thus the form of the cancellation signals associated with the particular behavior are being continually updated with each cycle of the behavior, every 2 s or so in the case of the ventilatory movements of skates. In this study we have attempted to extend the adaptive filter model further by asking whether the same learning rules can also create cancellation signals to eliminate reafference

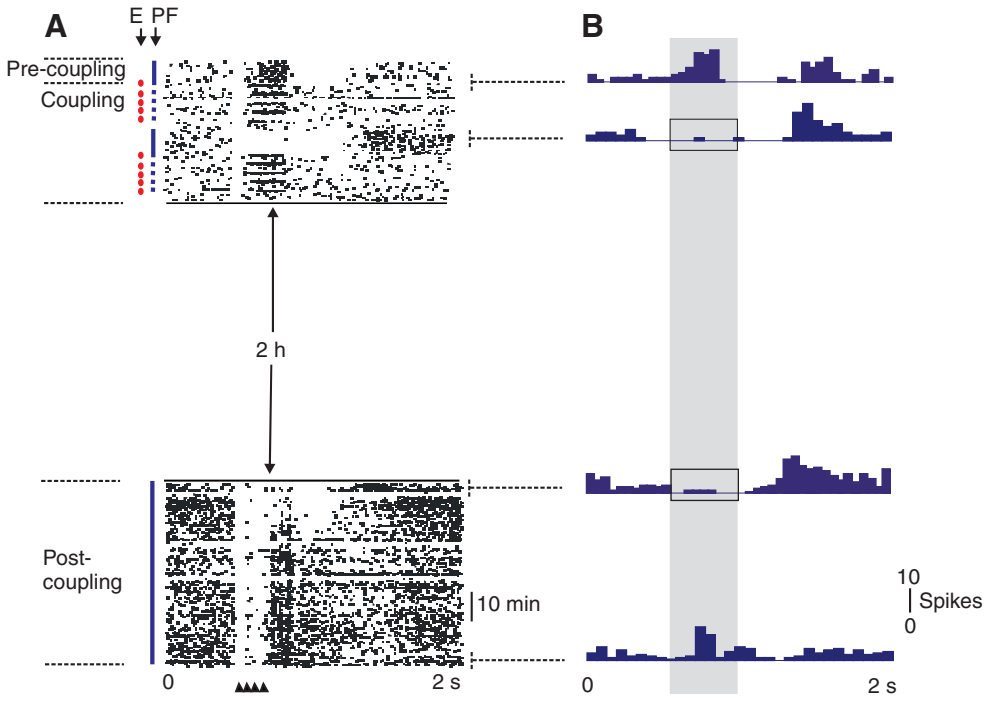

Fig. 6. Once the negative image is fully developed, it can be long lasting. (A) The negative image appears after five 30 s coupling tests, and then another five 30 s couplings are given to reinforce the negative image. The negative image is still apparent after a 2 h rest period without parallel fiber or electrosensory stimulation, but then fades over 20 min after parallel fiber stimulus trains alone are resumed. (B) Histograms as in previous figures.

caused by episodic behaviors, i.e. like most behaviors which occur in only brief episodes separated by much longer and variable rest periods. Swimming in the little skate, *R. erinacea*, is such a behavior. Not only does swimming occur sporadically but also the individual bouts of swimming are usually quite short, with a duration significantly less than the minutes of time usually required in our experimental coupling protocol for the appearance of new cancellation signals. For these behaviors, how do effective cancellation signals ever have time to develop and must they be developed *de novo* each time the behavior recurs?

Here we have shown that, in the dorsal nucleus, episodic AEN/parallel fiber co-activation results in the incremental development of a cancellation signal in the individual AENs. While a single short co-activation of parallel fibers and AENs did not induce the formation of a cancellation signal, a cancellation signal was generated following five such short episodes (30 or 60 s) of co-activation, with inter-episodic intervals ranging from 1 to 9 min (Figs 3, 4 and 5). Furthermore, once developed in this way, the cancellation signal was preserved intact for periods of at least 2 or 3 h when the parallel fibers were not being stimulated. This, we believe, would correspond to the rest periods during which the skates are inactive on the seafloor. Therefore, it appears that the adaptive filter mechanism of the dorsal nucleus does have the properties necessary to efficiently filter out predictable electrosensory inputs associated with a fish's episodic behaviors.

The behavioral relevance and survival importance of this capability of the adaptive filter seems very clear. Following a quiet period, when the skate again becomes active the adaptive filter functions immediately to reduce the associated reafference without the need for a delay of many minutes while an effective cancellation signal is created anew. The cancellation signal associated with the particular movements from their last occurrence would still be intact and would then be subject to the same updating as would be needed to adjust for any changes there might be in the reafference. One further requirement of the adaptive filter mechanism for it to work in this way is that the cancellation signals associated with swimming and other behaviors like breathing must be independent so that, for example, the cancellation signals for swimming are not inappropriately updated or lost during the period when the animal is resting quietly but breathing. This will be the case as long as the parallel fibers active during swimming are not the same as those active during breathing, and as long as the synapses of only active parallel fibers are subject to change. We are currently testing these tenets.

Although the development of cancellation signals is incremental, it is unclear whether the strength of individual synapses among the cluster of synapses of the active parallel fibers changes incrementally or in an 'all-or-none' way. 'All-or-none' would mean that the synaptic strength of an individual synapse changes from a starting level to a minimum or maximum level in one step. Because the development of the cancellation signal is incremental, under this circumstance the change in the total synaptic strength would reflect the increase in the number of synapses that have changed. Alternatively it is possible that the strength of each individual synapse changes gradually and incrementally so that the incremental formation of the cancellation signal might reflect the incremental change in the strength of each synapse of the active array of parallel fibers.

In this study, the external electrosensory stimuli were coupled to both passive fin movements (such as would normally occur during swimming behavior) and direct parallel fiber stimulation, and cancellation signals were developed in both cases (Fig. 4). This provides further support for our model that it is the molecular layer inputs that supply the cancellation signal during normal behaviors. As we noted, in this study as in our previous studies of the adaptive filter, not all of the AENs develop a cancellation signal after the co-activation of the parallel fibers and primary afferents, or after coupling of an electrosensory stimulus to ventilation or fin movements. Because we have found this in all of our studies and even in the same fish in which we have found AENs with strong cancellation signals, we believe it is not due to inadequate stimuli or testing protocols but instead represents real heterogeneity among AENs in the adaptive filter property. A similar finding has been made in the principal neurons of the cerebellar-like electrosensory nucleus of gymnotid fishes by Bastian and coworkers (Bastian et al., 2004). This indicates that there are different types of AEN in the dorsal nucleus of the skates, and that the reafference may be useful for some purposes. In part we believe the reafference relayed as descending feedback through the parallel fiber inputs may contribute to the ability to effectively predict and suppress reafference in other AENs (Bodznick et al., 1999). Also, more AENs developed a cancellation signal when the electrosensory stimuli were coupled to ventilation or passive fin movement than when they were coupled to parallel fiber stimulation. We do not know the reason for this but we presume that it is related to the artificial nature of the direct parallel fiber stimulation compared with the more natural activation of parallel fibers carrying motor commands and proprioceptive feedback during ventilation or fin movements. It is also possible that in some cases our parallel fiber stimulation electrode was not positioned well to activate a sufficient number of parallel fiber inputs for a given AEN.

In some experiments, shortening the coupling period from 1 min to 30 s, while maintaining a 2 min rest period, over a period of five cycles also resulted in the development of a cancellation signal. However, a 3 min continuous coupling did not result in the development of a cancellation signal in those same cells (data not shown). These results indicate that for episodic coupling, reduction of the total coupling period from 5 to 2.5 min can still result in the development of a cancellation signal, and suggests that perhaps, under episodic conditions, it is possible that less total coupling time may be required than for continuous coupling. However, this observation is at this point still anecdotal and requires careful testing. If confirmed, the data would suggest that under episodic conditions some form of memory consolidation takes place during the resting periods. Memory consolidation refers to the process by which recent memories are crystallized into long-term memories. In many neural systems, a newly acquired memory is easily disrupted; however, it can become more resistant to disruption through memory consolidation (Brashers-Krug et al., 1996). In skates, under episodic coupling, the newly acquired cancellation signal during the coupling periods might be continuously consolidated during the resting period, like the off-line improvement of memory in some other systems (Robertson et al., 2004).

Storage and active reversal of a cancellation signal

The adaptive filter is based on the cancellation signal inputs to AENs that can be stored without change for long periods of time between bouts of a behavior, but can be rapidly changed (updated) to accommodate changes in reafference associated with that behavior. Bidirectional change at parallel fiber synapses has been demonstrated in the electrosensory lobe of mormyrid electric fish (Han et al., 2000) and in the mammalian cerebellum (Coesmans et al., 2004; Jörntell

and Ekerot, 2003). In the dorsal nucleus of skates, we have shown that after a cancellation signal is developed it can last for at least 3 h in the absence of further parallel fiber stimulation (or passive fin movement, as the case may be). However, when the coupled parallel fiber inputs are activated in the absence of the previously associated electrosensory input, the cancellation signal is lost within 2 to 10 min. These results demonstrate that the cancellation signal can be updated rapidly to accommodate changes in reafference associated with ongoing behaviors.

The mechanisms underlying the reversal of cancellation signals in the dorsal nucleus are still unclear. In the cerebellum, parallel fiber activation alone can induce parallel fiber long-term potentiation, which reverses the parallel fiber long-term depression induced by the coactivation of parallel fibers and climbing fibers (Coesmans et al., 2004). In the dorsal nucleus, evidence indicates that parallel fiber stimulation alone can induce parallel fiber long-term potentiation, which can reverse the cancellation signal (Bertetto, 2007).

The cancellation signal is a more robust indicator of AEN plasticity

Compared with the decrease in the AEN response to the external electrosensory stimuli during coupling, the cancellation signal as noted above is a more robust indicator of AEN plasticity. Only 43% of the AENs that developed significant cancellation signals also exhibited a significant decrease in the response to the external electrosensory stimuli. We presume that under our experimental conditions the external electrosensory stimulation may be too strong in some cases to be effectively suppressed by the cancellation signal; only after removal of the external stimuli is the plasticity evidenced as cancellation signals against the background firing rate.

CONCLUSIONS

In this study, both natural and artificial stimuli were used to demonstrate the subtraction of predictable sensory features during episodic associations. The incremental development of the cancellation signal in the dorsal nucleus indicates that the change in the synaptic strength of parallel fiber–AEN synapses is acquired incrementally, is preserved in the parallel fiber–AEN synapses in the absence of activation of the parallel fiber, and can be reversed when the association of parallel fiber and AEN activity no longer exists.

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