Balanced ionotrophic receptor dynamics support signal estimation via voltage-dependent membrane noise

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†Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada; ‡Department of Mathematics, University of Utah, Salt Lake City, Utah; ∥Department of Physics, University of Ottawa, Ottawa, Ontario, Canada; and *Brain and Mind Institute and Center for Neural Dynamics, University of Ottawa, Ottawa, Ontario, Canada

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Marcoux CM, Clarke SE, Nesse WH, Longtin A, Maler L. Balanced ionotrophic receptor dynamics support signal estimation via voltage-dependent membrane noise. J Neurophysiol 115: 530–545, 2016. First published November 11, 2015; doi:10.1152/jn.00786.2015.—Encoding low-frequency signals in pyramidal neurons of the weakly electric fish Apteronotus leptorhynchus, an animal that can accurately encode even minuscule amplitude modulations of its self-generated electric field. We demonstrate that slow NMDA receptor (NMDA-R)-mediated excitatory postsynaptic potentials (EPSPs) are able to summate over many interspike intervals (ISIs) of the primary electrosensory afferents (EAs), effectively eliminating the baseline EA ISI correlations from the pyramidal cell input. Together with a dynamic balance of NMDA-R and GABA-A-R currents, this permits stimulus-evoked changes in EA spiking to be transmitted efficiently to target electrosensory lobe (ELL) pyramidal cells, for encoding low-frequency signals. Interestingly, AMPA-R activity is depressed and appears to play a negligible role in the generation of action potentials. Instead, we hypothesize that cell-intrinsic voltage-dependent membrane noise supports the encoding of perithreshold sensory input; this noise drives a significant proportion of pyramidal cell spikes. Together, these mechanisms may be sufficient for the ELL to encode signals near the threshold of behavioral detection.

electric fish; signal detection; stochastic resonance; NMDA receptor; correlations; spike train

LOW-LEVEL SENSORY SYSTEMS employ a balance of excitatory and inhibitory inputs to principal cells that, in turn, convey estimates of stimulus parameters (e.g., stimulus intensity) to higher-level brain areas. The excitatory input may involve both AMPA (AMPA-R) and NMDA (NMDA-R) receptors, and the inhibitory input may involve both fast (GABA-A/glycine) and slow (GABA-B) channels. The effects of this specific balance of excitatory and inhibitory currents on stimulus encoding are currently unknown, as is the connection to the specific complement of receptor types. This scenario arises naturally in the electrosensory system of gymnotiform fish, animals that can encode a large range of stimulus intensities extending down to barely detectable (Knudsen 1974; Nelson and MacIver 1999). We use the simplicity of electrosensory stimuli to show that the balance of excitatory (NMDA-R) and inhibitory (GABA-A) transmission, coupled with membrane noise, enables an accurate encoding of electrosensory signal amplitude.

Individual Apteronotus leptorhynchus emit a constant high-frequency electric organ discharge (EOD, species range: ~700-1,000 Hz). The EOD generates an electric field around the fish sensed by ~15,000 electoreceptors that drive electroreceptor afferents (EAs). The EOD drives EA discharge in a probabilistic manner with a mean frequency of 200 spikes/s (Gussin et al. 2007). Objects with conductivity greater (e.g., prey) or less (e.g., rocks) than the ambient water perturb the field to generate a spatially localized electric image—electrically “bright” or “dark” patches on the skin. Behavioral studies (Nelson and MacIver 1999) have shown that the electrosense is essential for prey capture. Detection can occur with prey further than 3 cm from the fish’s body (Nelson and MacIver 1999), which translates to a <1-μV increase over a baseline EOD amplitude of ~1.3 mV (Chen et al. 2005; Nelson and MacIver 1999). In a prey detection time window of 200 ms, these ultraweak stimuli cause the average EA to increase its discharge by ~1 spike relative to a baseline of 40 spikes (Bastian 1981a; Gussin et al. 2007; Nelson et al. 1997).

Baseline EA discharge is not completely random but exhibits negative interspike interval (ISI) serial correlations (SCs)—i.e., a long ISI is followed by a shorter one and vice versa (Chacron et al. 2001; Gussin et al. 2007; Ratnam and Nelson 2000). These SCs reduce EA spike count variability over the 200-ms detection window (Chacron et al. 2001; Ratnam and Nelson 2000) and can therefore improve the fish’s ability to encode prey signals via a rate or spike count code (Chacron et al. 2005). Detailed calculations suggest that, even with this reduction in variability, the small increase in spike count produced by the weakest prey signals is not sufficient for prey detection (Gussin et al. 2007; Maler 2009b). Several more sophisticated detection models that utilize some form of temporal coding have been proposed. These theories all use stimulus-induced deviations from expected ISI correlations to improve signal encoding over the limits imposed by simple trial-based spike counts. The proposed mechanisms include temporal filtering plus integration of EA spike trains (Goense and Ratnam 2003) or continuously computing conditional probabilities of successive ISIs via short-term plasticity (Ludtke and Nelson 2006). It is, however, difficult to devise experimental tests of these theoretical mechanisms. Nesse et al. (2010) demonstrated that, in theory, an encoding/decoding mechanism that matched pre- and postsynaptic kinetics could utilize the SC between only two successive ISIs to encode...
weak signals. Our results below are a first step toward confirming this theory.

Glutamatergic EAs terminate in three topographic maps within the electro-sensory lobe (ELL): the centromedial (CMS), centrolateral (CLS), and lateral (LS) segments (Krake and Maler 2014). The CMS and CLS are both strongly responsive to the spatially localized low-frequency signals associated with, e.g., prey, while the LS is more specialized for processing spatially diffuse electrophysiological signals (Krake and Maler 2014). In all maps the EAs drive two classes of output pyramidal neurons (Clarke et al. 2015; Krake and Maler 2014; Maler 1979, 2009a) as illustrated in Fig. 1. EAs terminate directly onto AMPA-R- and NMDA-R-rich ON-type pyramidal cells (previously described as E cells) and GABAergic interneurons (Bastian 1981b; Berman and Maler 1998; Maler et al. 1981; Maler and Mugnaini 1994). These interneurons in turn inhibit the ON cells. ON cells typically detect conductive objects. OFF-type pyramidal cells (previously described as I cells) receive indirect EA input via the inhibitory interneurons and therefore typically respond to nonconductive objects (Bastian 1981b; Berman and Maler 1998; Maler et al. 1981; Maler and Mugnaini 1994).

In this study we used ELL slices to investigate the cellular mechanisms by which A. leptorhynchus can encode low-frequency prey signals. To get discernible synaptic responses in ON and OFF cells, we had to stimulate with pulse patterns derived from moderate to strong signals (>3 μV, see methods). Our results directly pertain to the cellular mechanisms by which such signals are encoded in the firing rate of EAs; they do not, however, directly address the mechanisms by which the weakest signals are detected.

We focus on four specific questions of EA and ELL ON cell physiology: 1) Are EA-negative ISI SCs transmitted to ON cells as negative SCs of evoked excitatory postsynaptic potential (EPSP) amplitudes? 2) What are the respective roles of AMPA-R and NMDA-R components of the EA-evoked ON cell EPSPs in transmitting information about local low-frequency signals (e.g., prey)? 3) Is the disynaptic GABA-mediated inhibition of ON cells essential for transmitting such information, or does it serve to simply prevent saturation of the ON cell excitatory EA input? 4) Can membrane noise aid in signal encoding, or is it merely an unavoidable contamination that limits encoding of low-frequency signals? We now elaborate on these individual questions.

We have previously shown that the AMPA-R component of the EA to ON cell synapses exhibits short-term presynaptic depression with rapid recovery (Khanbabaie et al. 2010). As we discuss in detail below, this should lead to a negative SC of the AMPA-R peaks at each EA-to-ON cell synaptic contact. These correlations might, over a short timescale (<200 ms), interfere with the signal-induced modulations of synaptic release at this site, i.e., be a synaptic source of high-frequency noise. We hypothesize that the EA correlations are removed by postsynaptic dynamics and analyze this possibility in detail. As a second focus of this section, we note that the Nesse et al. (2010) analysis shows that a matching of pre- and postsynaptic dynamics can eliminate the EA SCs. Although our data cannot prove this model, they could lend preliminary support to it; they could also disprove it if the SCs persist in the ON cell EPSP amplitudes.

2) In ELL slices, the AMPA-R component of the EA-evoked EPSP (ON cell) can trigger spiking (Berman and Maler 1998), suggesting that these receptors are important for signal encoding. This conclusion appears to be contradictory to the depression of the AMPA-R component of the evoked EPSPs when EAs are driven above their baseline frequency (Khanbabaie et al. 2010). We hypothesize that the slow NMDA-R component of the evoked EPSP will then be critical for encoding slow signals (e.g., prey) and test this idea.

3) EAs contact local GABAergic interneurons and evoke disynaptic inhibitory postsynaptic potentials (IPSPs) in ON cells (Berman and Maler 1998; Maler and Mugnaini 1994). We consider two possible roles of this inhibition. First, it may merely prevent the high-frequency excitatory EA input to the ON cells from saturating their response. Second, the disynaptic inhibition may be a critical component of signal encoding by ON cells. We address these hypotheses in two ways. First, we examine ON cell stimulus encoding in the absence of inhibition (pharmacological blockade). Second, we examine signal encoding in OFF cells. OFF cells are not directly contacted by EAs but do receive disynaptic inhibition from the same GABAergic interneurons as ON cells. We therefore reason that if OFF cells can encode low-frequency signals, it would support our second hypothesis, that inhibition is required for signal encoding in ON cells.

4) The first in vitro study of ELL reported that ON and OFF cells exhibited membrane noise (Mathieson and Maler 1988). This noise was voltage dependent and might therefore be expected to increase with stimulus-evoked excitation of ON cells. This noise might first appear to be counterproductive for signal encoding. We therefore studied this noise in greater detail and, specifically, tried to connect it to EA-evoked ON cell depolarization. Our data led us to hypothesize that the membrane noise may drive ON cell spiking via the conversion of subthreshold smooth NMDA-R-dependent synaptic input into spike trains (Stacey and Durand 2001). This is an ingredient of the stochastic resonance (SR) signal enhancement effect (Longtin 1993; McDonnell and Ward 2011), here for low-frequency signals such as prey. Although it is not possible experimentally to vary noise levels alone to verify that SR is at play, our results show recruitment of noise near threshold, and thus point to noise-driven firing to assist the detection and encoding of stimuli. A major implication of our results on SR is that the responses of pyramidal cells with overlapping receptive fields should not be correlated, since their internal noise sources are likely independent. We explore this possibility in some detail (see discussion).

Both the CMS and CLS are very sensitive to the electro-sensory signals associated with prey (Krake and Maler 2014). Our in vitro analysis of how prey signal mimics are transmitted across the EA-to-ON cell synapses was, for technical reasons, confined to the CMS (see methods). Our in vivo and in vitro analysis of the contribution of noise to stimulus amplification was, again for technical reasons, done mainly in the CMS (see methods).

METHODS

In vitro recordings. The weak electric fish A. leptorhynchus (male and female, 10–15 cm in length) was used in these studies. Adult fish of both sexes were deeply anesthetized in oxygenated water with 0.2% 3-aminobenzoic ethyl ester (tricaine methanesulfonate,
bath applied 2-amino-5-phosphonopentanoic acid (APV, 100 μM; Sigma-Aldrich), and transverse slices were prepared as previously described (Harvey-Girard et al. 2010). In brief, fish were transferred to a foam-lined holder and their gills were superfused with water containing the anesthetic, whereupon the ELL was removed. The ELL was then immersed in ice-cold artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 3 KCl, 0.75 KH2PO4, 2 CaCl2, 1.5 MgSO4, 24 NaHCO3, and 10 d-glucose) containing 1 mM kynurenic acid and transferred to a vibratome (Technical Products International, St. Louis, MO), where 350-μm slices were taken from the transverse plane of the ELL (Maler 1979). The ELL slices were transferred to an interface slice chamber with flowing oxygenated ACSF for a minimum of 1 h at room temperature before recordings began.

**Stimulation and recording procedures.** Intracellular recordings were obtained from pyramidal cells of the CMS of the ELL with sharp microelectrodes (80–120 MΩ). We used the CMS because EA afferent fibers to this map are confined to compact narrow bundles (Lannoo et al. 1989); we could usually place the bipolar stimulating electrodes over most of the extent of such bundles and thereby evoke strong, readily measured EPSPs. The greater dispersal of the EA afferent fibers in the CLS and LS maps made it far more difficult to find a stimulation site that evoked strong, consistent responses. We selected mainly ON-type pyramidal cells since they receive direct input from the EAs onto their basal dendrites and this greatly simplifies our analysis (Berman and Maler 1998; Maler 1979; Maler et al. 1981). We identified ON cells by stimulating the EAs: ON cells exhibit short-latency EPSPs in response to such stimulation, while OFF cells exhibit IPSPs (Berman and Maler 1998; Harvey-Girard and Maler 2013). In a few cases, in order to evaluate whether their inhibitory input contributed to stimulus encoding by ON cells, we also recorded from OFF cells that are in receipt of the GABA-A receptor (GABA-A-R)-mediated disynaptic inhibition from the same interneurons as the ON cells (Berman and Maler 1998; Maler 1979; Maler et al. 1981; Maler and Mugnaini 1994). For studies of synaptic transmission between the EAs and downstream pyramidal cells, all active conductances were blocked by routinely filling the pipettes with cesium acetate, lidocaine N-ethyl bromide (QX-314, 100 mM in 3 M CsAc; Alomone Labs, Jerusalem, Israel), and tetraethylammonium (TEA, 50 mM; Sigma-Aldrich) (Berman et al. 2001; Khanbabaie et al. 2010). As previously reported (Khanbabaie et al. 2010) cells stopped spiking in response to depolarizing current in <5 min after impalement, and experiments were commenced at this time. In this case, the ON cells effectively become passive reporters of the EA-evoked synaptic potentials. To evaluate the role of NMDA-R in ON cell responses we bath applied 2-amino-5-phosphonopentanoic acid (APV, 100 μM; Tocris, Bristol, UK) to block the NMDA-R-mediated components of the EA-evoked EPSP (Berman and Maler 1998; Khanbabaie et al. 2010). We first recorded the control response and then applied APV, while delivering single EA stimulating pulses every few seconds. Once the late phase of the EPSP had completely disappeared (at least 10 min), we initiated our stimulus protocols. For the APV experiments we therefore always compare the same cell for control and blockade conditions. Given the long times required for these interventions, we were not able to hold the cells long enough for washout. In some cases, we also included picrotoxin (PTX, 100 μM; Sigma-Aldrich) in the recording pipette to block the disynaptic GABAergic input to all pyramidal cells. Again, we gave stimulating pulses to the EAs to determine when the evoked IPSP was fully blocked; as previously reported (Khanbabaie et al. 2010) this occurred at around 3 ms of excised recorded membrane potential. The afferent input contributed to stimulus encoding by ON cells, we also recorded from OFF cells that are in receipt of the GABA-A receptor (GABA-A-R)-mediated disynaptic inhibition from the same interneurons as the ON cells (Berman and Maler 1998; Khanbabaie et al. 2010). Two stimulation patterns were used in this study, both derived from in vivo recordings of EA activity (Gussin et al. 2007). We chose EA recordings with moderate mean frequencies of 115 spikes/s and 128 spikes/s because higher-frequency EAs often produced very short interpulse intervals (IPIs < 3 ms); in these cases, the stimulus artifact of the second pulse obscured the peak of the evoked EPSP evoked by the first pulse.

We used both the baseline discharge and evoked responses of these EAs as stimuli. In the case of baseline discharge, the EAs are driven by the constant amplitude of the fish’s EOD and variations in the spike train IPIs are due to the internal dynamics of the receptor and its afferent fiber. We also used pulse trains derived from one of the EA’s spiking responses to 0- to 4-Hz random amplitude modulations (RAMs) of the fish’s EOD (Gussin et al. 2007). Low-frequency amplitude modulations are typical of electrolocation and are commonly experienced during navigation and prey capture (Nelson and McIver 1999). To average across recordings from many pyramidal cells, we used a single short stretch of stimulus lasting 1 s and repeated both strong (~80 μV) and moderate (~5 μV) amplitude modulations. In preliminary tests we found that stimulation for >2 s resulted in clear rundown of the evoked EPSPs. We therefore chose to use a minimal stimulus duration of 1 s to avoid the artificial reduction of EPSPs toward the end of the stimulus period. We compared the evoked pyramidal cell membrane potential fluctuations to the external sensory stimulus using a correlation measure as done in previous in vivo studies (Bastian et al. 2004; Chacron et al. 2003). Although these stimulus pulse patterns were derived from in vivo
recordings of EAs, our stimulation method causes synchronous activation of all EAs, whereas in vivo such low-frequency stimuli would evoke increased but uncorrelated discharge across the EA population contributing to the receptive field of the recorded neuron (Benda et al. 2006; Maler 2009a). This effect of this difference is analyzed further below.

To characterize the intrinsic pyramidal cell noise, we recorded ON cells from both the CMS and CLS maps (Krähe and Maler 2014). The recording pipette was filled with 2 M potassium acetate only. In these cases we bath applied 6-cyano-7-nitroquinoline-2,3-dione (CNQX, 1 mM; Tocris) and APV to block all synaptic input to pyramidal cells (Berman and Maler 1998). We again used single EA stimulating pulses and initiated recording when we could not record any synaptic response (at least 10 min). In these cases, we could not perform a control case because membrane fluctuations before complete synaptic blockade could result from either membrane or synaptic noise. After drug application, any remaining membrane fluctuations could then be attributed to cell-intrinsic sources. Studies examining the intrinsic membrane fluctuations (noise) of ELL pyramidal cells used 2-s holding currents to maintain the impaled cell at various membrane potentials (RESULTS). We started by applying sufficient holding current to bring the cell above spike threshold. For the cell in Fig. 6 spike threshold was at approximately −65 mV, consistent with the pyramidal cell threshold in CMS (approximately −63 to −67 mV, Mehaffey et al. 2008). For the illustrated cell we first depolarized by 5 mV to −60 mV (Fig. 6B) to induce strong membrane noise and robust spiking; in order to compare levels of depolarization (above spike threshold) across cells, this suprathreshold level of depolarization was set to 0 as in Fig. 6A. From the above threshold membrane potential, we then stepped the injected current so as to produce −5-mV hyperpolarizations, bringing the cell down to −20 mV below the initial holding potential. Therefore, different holding currents were used for each cell to preserve this relative relationship to spike threshold.

In vivo recordings. Fish were anesthetized, and the caudal cerebellum, overlying the ELL, was exposed. After general anesthesia was stopped, a local anesthetic was applied to the wound margins. Fish were immobilized with a size-dependent dose of pancuronium bromide, injected intramuscularly, and were respirated with a constant flow of aerated water for the duration of the experiment. The fish were transferred into a large tank of 27°C water with the electrical conductivity kept between 100 and 120 µS/cm, and a custom holder was used to stabilize the head during long-term recordings. Fish were given time to acclimatize before data acquisition and were monitored closely for signs of stress.

Single-unit extracellular recordings were obtained from the ELL map of the ELL for direct comparison of pyramidal cell spiking to the in vitro data described above. One reason the ELL map was preferred for these studies is that the receptive fields of CLS pyramidal cells are much larger than those of CMS and therefore much easier to localize (Maler 2009a, 2009b). Pyramidal cells with receptive fields near the fish’s dorsum (at the water’s edge) were excluded from the analysis because of their proximity to the air-water interface, where boundary effects warp the electric field. Once a suitable cell was located and its firing rate and type (ON or OFF) verified with local steps in the electric potential, long sections of baseline activity were recorded and used to compute ISI SCs. The serial ISI correlation coefficients for lag $j$ [SC($j$)] are defined as $SC(j) = \frac{<I_jI_{j+1}>}{\text{Var}(I)}$, where $<.>$ represents the average value and $\text{Var}(I)$ the variance of the ISIs. An average SC was determined for five ON and five OFF cells from three different fish. Analysis was performed with custom MATLAB scripts. All procedures were reviewed and approved by the Animal Care Committee at the University of Ottawa and followed Society for Neuroscience guidelines.

RESULTS

Elimination of electroreceptor afferent interspike interval serial correlations by slow NMDA-R-mediated EPSPs. It has previously been shown that the baseline activity of EAs exhibits a negative ISI correlation at lag 1, indicative of sensitivity to the timing of the last two action potentials (Ratnam and Nelson 2000). The effect of synaptic transmission on such correlations has not been well studied. A theoretical analysis did demonstrate that presynaptic depression can suppress positive ISI correlations in incoming spike trains, resulting in much less correlated ISIs impacting the postsynaptic receptors (Goldman et al. 2002). Recent theoretical work has also shown that correlations in input currents (but not ISIs per se) can be transferred to output ISIs (Schwalger et al. 2015), but little is known about input-to-output transfer of ISI correlations. If an input spike strongly influences spiking probability then one might intuitively expect that ISI correlations are transferred, but the situation is less clear when many spikes from different neurons are required to fire the cell and when there is noise, which are both the situation of interest here. Our results below therefore advance our knowledge on ISI correlation transfer and also raise interesting questions in this context.

The baseline activity of target ELL pyramidal cells is effectively a renewal process with minimal or no ISI correlations (shown below in the in vivo section). The elimination of ISI correlations might be achieved at many levels of the ELL circuitry or via intrinsic properties of the pyramidal cells. In the absence of spiking and thus network feedback, our goal was to understand whether EA-to-CMS pyramidal cell synaptic dynamics can, by itself, remove the EA correlation structure. An earlier study established that blocking AMPA-R-mediated transmission with CNQX also blocks NMDA transmission (Berman et al. 1997). Therefore, we assume that AMPA transmission is crucial in sufficiently depolarizing pyramidal cell basal dendrites to unblock NMDA-R channels and is required for transmitting electrosensory signals from EAs to pyramidal cells. For experiments examining the mechanisms of decorrelation and signal transmission, we consequently tested the role of NMDA-R and GABA-A-R by first studying the intact system and then systematically eliminating the NMDA-R and GABA-A-R components.

ELL ON cells receive direct EA input onto their basal dendrites (Fig. 1) (Berman and Maler 1998). With intrinsic conductances pharmacologically blocked (see METHODS) but AMPA-R, NMDA-R, and GABA-A-R intact, stimulation of EA with a natural baseline discharge pattern induced rapid summation of evoked potentials to a plateau level (Fig. 2A, left). This is consistent with a previous study showing that temporal summation results in a plateaued potential when using a 200-Hz stimulus train (Khanbabaie et al. 2010). Stimulation of the EAs with these high-frequency stimulus trains evokes a complex sequence of IPSPs and EPSPs in the pyramidal cells, which vary greatly in amplitude (Fig. 2A, right). In some recordings it is possible to distinguish between the NMDA-R and AMPA-R components of the pyramidal cell EPSP (Fig. 2A). As previously shown (Berman and Maler 1998; Khanbabaie et al. 2010), the AMPA-R component appears as a rapidly rising, short-latency response that can decay before full activation of the NMDA-R, which has a longer latency and slower rate of rise. This produces a prominent “notch” in the
ON cell

OFF cell

descending/feedback
input to distal apical
dendrite

burst production

Na

NaP

K

Na

NaP

K

Ga

GJ

A

A

A

N

N

N

Glu

EA

EOD (prey)

EOD (rock)

Fig. 1. Summary diagram of the electrosensory lobe (ELL) circuitry that generates the ON and OFF cell responses. ON cells receive direct glutamatergic (Glu) synaptic input from electrosensory afferents (EAs) onto their basal dendrites; glutamate excites the ON cell via AMPA receptor (AMPA-R) (A) and NMDA receptor (NMDA-R) (N). The AMPA component of the EA-evoked excitatory postsynaptic potential (EPSP) shows strong short-term depression (down arrow beside A). The EAs also contact local GABAergic interneurons (G) that, in turn, synapse on the ON cell soma utilizing GABA-A receptors (GABA-A-R) (Ga). The net effect of this arrangement is that increases in electric organ discharge (EOD) intensity within the receptive field of the ON cell due to a conductive object, e.g., prey, will depolarize the ON cell and elicit increased spiking. Immunocytochemistry and physiological studies have shown that the soma and proximal apical dendrite of the ON cell express both fast (Na) and persistent (NaP) Na⁺ channels as well as K⁺ (K, 3) channels. The basal dendrite of the ON cell also expresses Na⁺ channels (immunocytochemistry), but it is not known whether these are the fast or persistent variety or both (therefore “Na?”). The OFF cell receives input from EAs only disynaptically via the same GABAergic interneuron; this inhibitory input generates the OFF cell receptive field center. Excitation of the OFF cell is via gap junction (GJ) input from ascending dendrites (AD) of the same interneurons; these, however, emanate from distant cells and therefore represent more distant body regions (receptive field surround). The GABAergic interneurons that inhibit the OFF cell also inhibit the ascending dendrites. The net effect of this circuitry is that a decrease in EOD intensity due to, e.g., a rock will reduce the direct inhibition of the OFF cell and, via the ascending dendrite and gap junction synapses, also permit excitation from distant regions of the fish’s skin. The OFF cell will therefore inhibit the electrosensory input and give a spiking response to nonconductive objects. The OFF cell also expresses the same Na⁺ and K⁺ channels as the ON cell. The circuitry illustrated in this figure was first demonstrated in Maler (1979) and Maler et al. (1981).

EA-evoked EPSPs vary in amplitude depending on the previous ISI length. After short ISIs, the NMDA-R component of the EPSPs summates and typically generates EPSPs with near-equal or larger amplitudes (Fig. 2A, right). This result stands at odds with our previous study using fixed-frequency or random stimulation (Khanbabaie et al. 2010), where, in the presence of NMDA-R and GABA-A-R antagonists, strong and fast presynaptic depression of the EA-evoked EPSP was observed following short-ISI stimulus pulses.

To resolve this issue, we repeated our earlier study by first blocking NMDA-R alone with APV. We note that this drug application does not merely block NMDA-R transmission at the EA-to-ON cell synapse; ELL granular interneurons also express NMDA-R (Harvey-Girard et al. 2007), and so this treatment will likely reduce disynaptic GABA-A inhibition as well as blocking NMDA-R transmission onto ON cells (Fig. 1). Under these conditions, we observed minimal temporal summation (Fig. 2B, left), and short ISIs often resulted in prominent depression of the evoked EPSP (Fig. 2B, right). This is not surprising since there is no longer the slow NMDA-R component capable of temporal summation, thus unmasking the previously characterized depressing AMPA-R component.

We next blocked GABA-A-R with intracellular PTX but left NMDA-R-mediated transmission intact (Fig. 2C, left). Again, we observed strong temporal summation of EPSPs to a plateau potential, and the EPSPs evoked after shorter ISIs were smaller; now, however, longer ISIs could also produce this effect (Fig. 2C, right). It appears that, with inhibition blocked, the NMDA-R component of the EPSPs can summate to a plateau potential and the peaks represent the combined AMPA-R and NMDA-R components.

With both NMDA-R and GABA-A-R blocked, temporal summation of successive EPSPs did not occur and no summation to a plateau potential was observed. As seen in Fig. 2D, left, short ISIs result in prominent depression that, as previously shown (Khanbabaie et al. 2010), recovers after only a single longer ISI. Therefore, the temporal summation of EPSPs evoked by short EA ISIs is due to slow NMDA-R currents, which effectively counteract the fast EA presynaptic short-term depression. Note that the dynamics of disynaptic inhibition (currently unknown) may complicate this effect.

We next computed the SCs at lag 1 between successive peak amplitudes of the evoked EPSPs. Two-tailed t-tests were performed to determine whether the mean SCs differed significantly from zero. The original EAs had negative ISI SCs (−0.44), but in the control case (AMPA-R, NMDA-R, and GABA-R intact) these negative ISI correlations were not reflected in any significant correlations between successive EPSP peak amplitudes (Fig. 2E; correlation = 0.083, SD = 0.16, n = 10, P = 0.13, 2-tailed t-test). Blocking either NMDA-R (correlation = −0.11, SD = 0.075, n = 6, P = 0.0046, 2-tailed t-test) or both NMDA-R and GABA-A-R (correlation = −0.12, SD = 0.066, n = 5, P = 0.013, 2-tailed t-test) resulted in the reappearance of negative SCs in the peak amplitudes, while blocking inhibition with PTX application alone did not have a significant effect (correlation = −0.065, SD = 0.19, n = 5, P = 0.5037, 2-tailed t-test). Indeed, the correlation coefficient for the control condition differs significantly from both cases in which NMDA is blocked (P = 0.0188, 1-way ANOVA with Tukey post hoc comparisons) but not when inhibition alone is blocked. For the APV and APV + PTX cases, the small AMPA-R-mediated EPSP peaks merely reflect the depression due to a lack of NMDA-R-mediated temporal summation (Khanbabaie et al. 2010). On average, the unmasked depression results in higher peaks being followed by...
smaller ones, and vice versa, constituting an expression of the negative ISI correlations at the level of synaptic responses. The absence of a significant effect in the case of blockade of inhibition by PTX is presumably due to the residual NMDA-R-dependent temporal summation that masks the AMPA-R depression. Note that in all cases the serial EPSP peak correlation did not reach in vivo SC values for the afferent ISIs.

We conclude that, under control conditions, the postsynaptic dynamics of combined direct excitatory plus disynaptic inhibitory EA input onto E-cell dendrites is matched to the EA ISI structure, in the sense that SCs between successive EPSP peak amplitudes are eliminated. These uncorrelated peak amplitudes are left to represent the stimulus, consistent with the Nesse et al. (2010) coding scheme but not proving it. The NMDA-R-mediated component of the EPSP is most important for this effect because its time course extends over several ISIs and the resulting temporal summation produces a positive correlation that counterbalances the negative correlation imparted by short-term depression of the AMPA-R component.

Signal encoding by pyramidal cells with excitation plus inhibition. An in vivo study (Bastian et al. 2004) has shown that the EA input to an ON cell (summed EPSPs/IPSPs) can faithfully reconstruct a low-frequency stimulus presented within its receptive field. Larger functional networks (Chacron et al. 2011) were active in these experiments and might conceivably contribute to the observed stimulus reconstruction. Here we aimed to determine the synaptic requirements for this reconstruction in the absence of these networks. To estimate reconstruction accuracy, we used a spike sequence recorded from an EA in response to a RAM stimulus (Fig. 3A; see
METHODS) to drive the EAs of E cells. We used stimulus-evoked responses from the same EAs that were used for our analysis of the ON cell response to baseline EA discharge. We then computed the cross-correlation coefficient between the evoked membrane potential and the original RAM stimulus. Again, we blocked voltage-dependent conductances to study the synaptic potentials in isolation in the E cells. Despite the fact that our stimulation protocol induced nonphysiological synchronous EA input, the ON cell membrane potential was still able to reconstruct moderate amplitude signals in vitro (Fig. 3B), provided both NMDA-R and GABA-A-R receptors were not blocked. The membrane potential clearly followed even the small modulations of the original stimulus.

Signal reconstruction is significantly impaired with NMDA-R blocked by APV but with inhibition intact; in particular, the response to the small modulations is now absent, although the response to the large dip in the RAM (trough) is still evident (Fig. 3C). At the maxima of the amplitude modulations, pyramidal cells are receiving increased excitatory input; however, with NMDA-R blocked, there is no temporal summation and the AMPA-R-mediated EPSPs become depressed. This is due to fast AMPA-R-mediated short-term depression (Khanababae et al. 2010) acting in concert with the GABA-A disynaptic inhibition as described above. It is not surprising that temporal summation of EPSPs is required to encode a low-frequency signal and that slow NMDA-R-mediated synaptic currents are responsible for this encoding. Therefore, with NMDA-R currents intact we expected efficient and possibly better than the control signal coding in the absence of GABA-A inhibition.

Surprisingly, upon block of inhibition with PTX, pyramidal cell encoding of amplitude modulations was almost completely eliminated (Fig. 3D). It appears that, in the absence of inhibition, the NMDA-R-mediated EPSPs summate to a saturated plateau potential and can no longer encode the variations in EA input. When both NMDA-R and GABA-A-R were blocked there was no modulation at all in response to the EA stimulation. AMPA-R alone are not capable of encoding even strong sensory input (Fig. 3E).

As summarized in Fig. 3F, good stimulus reconstruction was only obtained when both NMDA-R and GABA-A-R were intact (RAM-response cross-correlation = 0.53, SD = 0.091, n = 10). The mean cross-correlation in the APV (cross-correlation = 0.10, SD = 0.19, n = 5), PTX (cross-correlation = 0.18, SD = 0.10, n = 6), and APV + PTX (cross-correlation = −0.14, SD = 0.22, n = 5) cases were significantly different from control (1-way ANOVA, P < 0.001). Two-tailed t-tests revealed that the control (P = 0.001) and APV (P = 0.0080) conditions were significantly different from 0, while PTX (P = 0.3038) and APV + PTX (P = 0.2322) were not.
ANOVA, \( P \leq 0.001 \); Tukey post hoc). Two-tailed \( t \)-test revealed that the control (\( P \leq 0.001 \)) and APV (\( P = 0.008 \)) conditions were significantly different from 0 while PTX (\( P = 0.30 \), 2-tailed \( t \)-test) and APV + PTX (\( P = 0.23 \), 2-tailed \( t \)-test) were not. We conclude that the relative proportions of excitation and inhibition in the in vitro control case are required to encode electrosensory signals by ON cells. The NMDA component of the evoked EPSP must summate to permit an encoding of the slow timescale of the input RAM (0–4 Hz). By itself, NMDA summation will result in a saturated response that no longer follows the stimulus fluctuations. Fast disynaptic inhibition (Berman and Maler 1998; Khanbabaie et al. 2010) is required to prevent saturation and keep the NMDA-R excitation within its dynamic range.

To get a deeper appreciation of these effects, we examined the ON cell responses to the regions in Fig. 3, designated as “trough” and “peak,” of the low-frequency stimulus (Fig. 4). It is noteworthy that, in all treatment conditions, the EPSP peaks evoked at the local stimulus trough are larger than those evoked at the local peak. This is simply due to the fact that the decrease in stimulus intensity during a trough (below the baseline EOD amplitude) causes a reduction in EA discharge (Gussin et al. 2007) and this in turn results in longer than average EA ISIs. These long ISIs do not cause depression at EA synapses (Khanbabaie et al. 2010). In contrast, the shortened ISIs in the EAs during a stimulus peak cause strong depression and therefore reduced EPSP peak amplitudes.

In the control case (Fig. 4A), the EPSPs during a stimulus trough are large with clear AMPA-R- and NMDA-R-mediated EPSPs, cleanly separated by strong hyperpolarizing events that are likely caused by disynaptic GABA-A inhibition. Although the peak EPSPs are large (~5 mV), they occur while the cell is hyperpolarized (Fig. 4A, left) and these EPSPs rarely trigger spikes in response to sensory input that falls below the baseline EOD amplitude (Bastian et al. 2004). During the peak response, much smaller fluctuations of the membrane potential (~1 mV) are observed riding on the slow NMDA-R-mediated depolarization (Fig. 4A, right). Similar results are obtained after application of the drugs (Fig. 4, B–D), the notable differences being that the EPSPs at the peak are more prominent than for the no-drug condition and that blocking inhibition with PTX application (with or without APV) application results in less variability in EPSP amplitude at both the peak and the trough.

Two important issues are raised by these results. First, it is not clear whether the disynaptic GABA-A-R-mediated inhibition is merely acting as a tonic inhibition that prevents NMDA-R-mediated saturation of the responses to the RAM stimulus or plays a more active role in stimulus coding. Second, it is not clear to what extent the remaining potential fluctuations in peak

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Fig. 4. Pyramidal cell responses evoked from stimulation of EAs using in vivo-derived EA spiking responses to RAMs. The “peak” and “trough” were the sections of the response defined by the named bars in Fig. 3. Individual EPSPs at the peak (right) and trough (left) of the RAM signal were examined. A, left: when intrinsic conductances are blocked, EPSPs have distinct AMPA-R (arrow)- and NMDA-R-mediated components. A, right: at stimulus peak, small membrane potential fluctuations are seen riding on the top of the slow NMDA depolarizations. B–D: under all conditions, EPSPs at the stimulus trough are larger in amplitude than those evoked at the stimulus peak.
response are due to the artificial synchronous stimulation of the EAs. We deal in turn with these issues in the following sections.

Fast disynaptic inhibition can encode slow signals. Since the absence of disynaptic inhibition prevented signal reconstruction (Fig. 3D), we wanted to determine whether GABA-mediated synaptic transmission contributed to the coding of these signals or simply prevented the NMDA-R component of the summing EPSPs from saturating. This was achieved by examining the RAM stimulus-evoked response of OFF cells (see Fig. 1 for OFF cell circuitry), which receive only inhibitory GABA-A input from their receptive field center and are excited by spatially averaged surround input, generated by gap junction input from granular cell dendrites (Bastian et al. 2002; Berman and Maler 1998; Maler 1979; Maler et al. 1981). The OFF cells, as expected, inverted the response to the RAM signal (Fig. 5). Remarkably, they were able to accurately follow even the small amplitude modulations (Fig. 5). A stimulus-response cross-correlation value of $-0.64$ (SD = 0.17, $n = 5$) was attained, and, although sign inverted, its absolute value was not significantly different from that obtained for E cells ($P = 0.53$). Blocking inhibition with PTX abolished the OFF cell’s response to EA stimulation as expected from a previous study (Berman and Maler 1998; Harvey-Girard and Maler 2013). The cell’s membrane potential simply randomly drifted up and down, and the negative correlation between the OFF cell response and the RAM signal (cross-correlation = 0.057, $n = 1$) was eliminated, demonstrating that its response did indeed arise from disynaptic GABA-A-mediated inhibition. The same GABAergic interneurons send projections to both ON and OFF cells (Maler and Mugnaini 1994). Since these projections convey information sufficient for stimulus encoding in the OFF cells, we conclude that they also convey the same information to the ON cells. These results demonstrate that the ability of an ON cell’s membrane potential to encode RAM signals is likely due to the dynamic balance of NDMA-R-mediated excitation and GABA-A-mediated disynaptic inhibition.

Computational analyses suggest that asynchronous in vivo synaptic potentials are smoother than those evoked by synchronous in vitro stimulation and are unlikely to evoke spikes. An unavoidable caveat of our in vitro stimulation protocol is the synchronous activation of multiple EAs that would otherwise fire asynchronously in vivo. EAs are probabilistic encoders, each firing at a given phase of the EOD waveform but skipping an intermittent number of EOD cycles (Gussin et al. 2007). In vivo, populations of EAs that converge onto a pyramidal cell (25 for CMS map) (Maler 2009a) will fire independently when driven by low-frequency stimuli (Benda et al. 2006). Under baseline conditions or when driven by low-frequency prey mimic signals, various subsets of the EA population will discharge in every single cycle of the high-frequency EOD but will not all discharge synchronously. However, for our in vitro experiments the strong pulses used to reliably stimulate the EA fibers in slice are derived from one EA spiking response to a RAM stimulus (Figs. 3–5). As a result the stimulated population is entrained to the activity patterns of our exemplar unit (see METHODS), firing synchronously within a given EOD cycle and producing an absence of spikes in many others. For example, in Fig. 4A, right (control, peak response), the stimulus pulses skip two to four EOD cycles. The strong stimulus likely evokes a response in most or all of the 25 EA afferents contacting the ON cell and generates a maximal response. The stimulus used for the in vivo recording that generated this EA spike train would result in a peak EA probability of discharge of $\approx 0.33$ (Gussin et al. 2007). Therefore, in vivo, we would expect that approximately eight (one-third) of the EA afferents discharged on each EOD cycle and evoked a weaker (one-third of maximum in vitro) EPSP. Intuitively, one would expect the asynchronous case to generate smoother summed synaptic potential because of the “filling in” of each EOD cycle with smaller EPSPs and the temporal summation of evoked responses. We lack a detailed understanding of EA-evoked disynaptic inhibition in ELL, and cannot therefore directly model the effect of natural synaptic input on the smoothness of the summed ON cell response. Below we provide a simple approximate statistical analysis of the heights of the EPSPs that illustrates the magnitude of our hypothesized smoothing effect.

We assume that 25 EAs converge onto the CMS ON cell (Maler 2009a) and, during the peak stimulus response (Figs. 3 and 4), have a mean discharge probability = 0.33. In the artificial in vitro case, with stimulation by the one EA exemplar, on each EOD cycle the input is 0 or 25 EAs. This is a discrete density, with 0 having a probability of 2/3 and 25 a probability of 1/3. The first moment (i.e., mean) of this density is $\mu_1 = \sum \lambda x P(x) = 0(2/3) + 25(1/3) = 8.33$, and the second moment is $\mu_2 = \sum \lambda (x - \mu_1)^2 P(x) = 20(2/3) + 625(1/3) = 161.67$. The variance is $\sigma^2 = \mu_2 - \mu_1^2 = 161.67 - 8.33^2 = 81.83$. The computational analyses suggest that asynchronous in vivo synaptic potentials are smoother than those evoked by synchronous in vitro stimulation and are unlikely to evoke spikes. An unavoidable caveat of our in vitro stimulation protocol is the synchronous activation of multiple EAs that would otherwise fire asynchronously in vivo. EAs are probabilistic encoders, each firing at a given phase of the EOD waveform but skipping an intermittent number of EOD cycles (Gussin et al. 2007). In vivo, populations of EAs that converge onto a pyramidal cell (25 for CMS map) (Maler 2009a) will fire independently when driven by low-frequency stimuli (Benda et al. 2006). Under baseline conditions or when driven by low-frequency prey mimic signals, various subsets of the EA population will discharge in every single cycle of the high-frequency EOD but will not all discharge synchronously. However, for our in vitro experiments the strong pulses used to reliably stimulate the EA fibers in slice are derived from one EA spiking response to a RAM stimulus (Figs. 3–5). As a result the stimulated population is entrained to the activity patterns of our exemplar unit (see METHODS), firing synchronously within a given EOD cycle and producing an absence of spikes in many others. For example, in Fig. 4A, right (control, peak response), the stimulus pulses skip two to four EOD cycles. The strong stimulus likely evokes a response in most or all of the 25 EA afferents contacting the ON cell and generates a maximal response. The stimulus used for the in vivo recording that generated this EA spike train would result in a peak EA probability of discharge of $\approx 0.33$ (Gussin et al. 2007). Therefore, in vivo, we would expect that approximately eight (one-third) of the EA afferents discharged on each EOD cycle and evoked a weaker (one-third of maximum in vitro) EPSP. Intuitively, one would expect the asynchronous case to generate smoother summed synaptic potential because of the “filling in” of each EOD cycle with smaller EPSPs and the temporal summation of evoked responses. We lack a detailed understanding of EA-evoked disynaptic inhibition in ELL, and cannot therefore directly model the effect of natural synaptic input on the smoothness of the summed ON cell response. Below we provide a simple approximate statistical analysis of the heights of the EPSPs that illustrates the magnitude of our hypothesized smoothing effect.
moment is \( \mu_2 = \sum x^2 P(x) - (25)^2/3 = 208.3 \). The variance of the EPSP height can then be calculated as \( \sigma^2 = \mu_2 - \mu_1^2 = 138.94 \). In the natural case, on each EOD cycle a fraction of the 25 EAs will independently discharge. This is a binomial distribution by synaptic noise could not be excluded. Since these large depolarizations (blips) became apparent and these blips were seen to co-occur with spiking (Fig. 6B). To investigate the membrane noise in more detail, we computed histograms of the membrane potential at different levels of depolarization. The membrane noise histograms were well fit by the Gaussian distribution at relatively hyperpolarized levels (Fig. 7A). However, at more depolarized levels (near threshold) the distribution has a higher variance (Fig. 7, B and C) and becomes both more peaked (kurtotic) and positively skewed to the right (Fig. 7, B and D). The positive skew is due to an increase in the occurrence of the large blips. When we included voltage-gated ion channel blockers in the pipette the variance was low and independent of membrane potential (Fig. 7C, inset; \( n = 1 \)), conclusively identifying the noise as intrinsic rather than a result of synaptic conductances.

Voltage-dependent membrane noise triggers spiking. Previous studies of cortical (Azouz and Gray 2000, 2003) and ELL pyramidal cells (Chacron et al. 2007) have shown that spiking is evoked by rapid depolarization of the membrane potential, i.e., by steep increases that can be localized in time with the first or second temporal derivative. It is often assumed that AMPA-R-mediated currents provide the necessary rapid depolarization to elicit spiking. In an ELL slice, the rising phase of a single EA-evoked EPSP (mostly AMPA-R) will evoke a spike (Berman and Maler 1998; Khanbabaie et al. 2010). EAs normally discharge continuously at a high rate (Gussin et al. 2007), and under these conditions the AMPA-R-mediated component of the EPSP is slightly depressed (Khanbabaie et al. 2010). We therefore hypothesize that, in vivo, it is the NMDA-R and not AMPA-R component of the stimulus-evoked EPSP that evokes most spiking in ON cells. Furthermore, our experimental results and statistical analysis suggest that the response to slow increases in EOD amplitude are smooth and lack the rapid depolarizing events (i.e., no large second derivative) required to evoke spikes (Chacron et al. 2007). This prompted us to consider how the slow NMDA-R EPSPs could possibly drive spiking.

Early work in the ELL described the presence of membrane noise that increased in amplitude with depolarization (Mathieson and Maler 1988); this noise was attributed to voltage-gated ion channels likely selective for Na\(^+\), although a contribution by synaptic noise could not be excluded. Since these rapid membrane potential fluctuations were able to elicit spikes in pyramidal cells in vitro (Mathieson and Maler 1988), we decided to investigate whether noise could compensate for the depressed AMPA-R component of the EA-evoked EPSP and drive the fast upstrokes in membrane potential required for spiking in pyramidal cells.

We treated the ELL with CNQX and APV to block synaptic transmission onto ON cells and interneurons and therefore eliminated synaptic input as the source of rapid fluctuations in membrane potential. We then recorded from CMS pyramidal cells without any channel blockers (i.e., no QX-314 or cesium ion in the pipette) and applied depolarizing current steps; because synaptic transmission was blocked, we were not able to determine whether we were recording from ON or OFF cells. As previously described (Mathieson and Maler 1988), the pyramidal cell membrane potential was noisy and the amplitude of the membrane potential fluctuations increased dramatically with depolarization (\( n = 5 \); Fig. 6A). Spike threshold varied across cells but was typically near \(-65 \text{ mV} \). As the membrane potential approached spike threshold, the presence of rapid, large depolarizations (blips) became apparent and these blips were seen to co-occur with spiking (Fig. 6B).

Fig. 6. Recordings of spontaneous pyramidal cell membrane potential at various holding potentials. Recordings were taken with extracellular 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and APV to block synaptic noise. A: amplitude of membrane potential fluctuations increases dramatically with depolarization to spike threshold (around \(-65 \text{ mV} \), here normalized to 0). Near threshold, rapid large depolarization elicits spiking (*, spikes truncated). B: blips elicit spikes (arrow) when they reach spike threshold (dotted line). Here we give the absolute membrane potentials for this particular neuron—traces (top, \(-60 \text{ mV} \); bottom, \(-65 \text{ mV} \)) are an expanded version of the normalized top 2 traces in A (0, \(-5 \)).
We hypothesize that many stimulus-evoked spikes in ON cells are elicited by the voltage-dependent blips, which would ride on top of the summating NMDA-R component of the PSPs evoked by the EAs. To investigate this possibility, we turned to the CLS of the ELL and repeated the noise experiments described above (n = 5). We switched to the CLS map because preliminary experiments had shown that the blip events (right tail of the distribution shown in Fig. 7B for the CMS map) were far more prominent, allowing us to determine blip occurrence with greater fidelity and temporal accuracy. The overall membrane noise characteristics were otherwise identical to the CMS map: CLS pyramidal cells display fluctuations whose variance and skewness increase with depolarization. Inset: when voltage-gated ion channels were blocked, variance was reduced and became independent of membrane potential, demonstrating an intrinsic source of noise. D: skew increases dramatically at potentials at or above spike threshold.

Figure 8A shows an example membrane potential trace from a CLS neuron, demonstrating clear blips and the occasional spike; the inset shows that an action potential may trigger directly off the peak of a blip (indicated by the dashed arrow). Furthermore, the blip waveform has an onset slope nearly identical to the AMPA-R component of an EPSP (Fig. 8A, gray, overlying the excited blip), suggesting that these noisy fluctuations are sufficient to trigger spikes when they occur near the action potential threshold. Using the same derivative criteria as used above to identify spikes, we isolated the times at which blips occurred and computed interblip interval (IBpI) statistics including the SC coefficients of IBpIs and the IBpI histogram. The IBpIs had means ranging between 0.39 s (SD = 0.23) and 0.22 s (SD = 0.25) for the three CLS cells where blips could be cleanly extracted. The IBpI showed no SC (Fig. 8Bi), indicating that the blips originate from a renewal process. We found that the IBpI distributions were consistent with the renewal process being either a Poisson (Fig. 8C, top left) or a more general gamma (Fig. 8C, bottom left) process, depending on the neuron.

To determine whether the statistical patterns of blip generation might influence the statistical patterns of pyramidal cell spiking, in vivo extracellular recordings were obtained from CLS pyramidal neurons in the absence of a stimulus (n ON cells = 5, n OFF cells = 5). Examination of the data showed that there were no significant differences between ON and OFF cells, and the data were therefore pooled. The mean ISIs of the 10 cells ranged between 0.13 s (SD = 0.13) and 0.039 s (SD = 0.025). The mean ISIs are significantly smaller than the mean IBpIs, presumably because of the cells being driven more strongly by the EAs and feedback in vivo, rather than by intracellular current injection in vitro. As illustrated in Fig. 8Bi, this small sample suggests that many in vivo ISIs are not significantly correlated at any lag, demonstrating that some ON and OFF cell spiking follows a renewal process. Because there are some apparently contradictory results in the literature concerning ELL pyramidal cell serial ISI correlations (see Discussion), we also examined the distribution of baseline ISI SCs in a much larger sample of previously recorded CLS ON and OFF cells (Clarke et al. 2014). We had a sample of 50 ON cells (firing rates of 6.3–35.4 spikes/s) and 41 OFF cells (firing rates of 5–30.7 spikes/s). Preliminary analysis showed that these serial ISI correlations were not significantly different in the two populations, and so the data were pooled for a sample of n = 91 cells. As illustrated in Fig. 8Bi, the ISI correlations are all low (mean 0.003; ± 0.059) and include both small positive as well as negative correlations ranging between −0.1 and +0.1. In contrast, we also illustrate the EA serial ISI correlations taken from Ratnam and Nelson (2000). These have a mean of −0.52 ± 0.14 (Fig. 8Bii, solid line labeled “EA”) and a range of −0.23 to −0.82; the lower value (−0.82) is off the scale of the figure, and a dashed line indicates the maximum correlation (−0.23) observed. It is clear, in going from EAs to pyramidal cells, that there is a massive reduction in ISI.
of the ELL pyramidal cells, even in vivo when the ON or OFF renewal point process, which is conserved in the spiking output. Furthermore, we hypothesize that blips form a basis for a signals by ON cells, i.e., blips are the basis for ON cell SR. and transmission of sub- and perithreshold low-frequency sig- nalestimation of EPSP peaks by the natural mixture of synaptic currents, the renewal blip process ensures an efficient encoding of stimulus amplitude modulations centrally, including for signals that lie below or in the vicinity of the spiking threshold.

**DISCUSSION**

Our in vitro study reveals how excitatory and inhibitory synaptic transmission to ON pyramidal cells and their intrinsic noise combine to encode electrosensory signals transmitted by the EAs. 

**Elimination of EA ISI correlations.** The EA ISI correlations are not transmitted to serial correlations of EPSPs peaks because of the NMDA-R component of the EPSPs; the AMPA-R component, on its own, will transmit the correlation (Fig. 2E). This is expected because the AMPA-R EPSP component shows a rapidly recovering depression after short ISIs (Khanbabaie et al. 2010). Therefore a short ISI will evoke a depressed second AMPA-R EPSP; the following longer ISI (due to the negative ISI correlation at lag 1) will evoke a larger AMPA-R EPSP. A sequence of a long ISI followed by a short ISI will, by the same reasoning, evoke a large followed by a small AMPA-R EPSP. In this manner, AMPA-R transmission will, on its own, preserve the ISI negative SCs in the EA as negative correlations of the EPSP peak amplitude (AMPA-R component).

The EA synapses onto ON cells utilize NMDA-R (Berman et al. 2001; Harvey-Girard et al. 2007) with long evoked EPSPs (Berman and Maler 1998) (>100 ms), greatly exceeding the mean baseline EA ISIs (~5 ms) (Gussin et al. 2007). The NMDA-R-evoked EPSP timescale is better matched to the low firing rates (mostly <20 Hz or >50 ms and ~10 EA ISIs) of our sample of ON/OFF cells; again the long ON cell ISIs would be expected to reduce or eliminate the effect of EA ISI correlations. We hypothesize that temporal summation of EPSPs over many ISIs due to NMDA-R effectively eliminates EA negative ISI correlations. 

An earlier study (Chacron et al. 2007) showed, in a small sample, that ON and OFF cells with a low firing rate (<20 Hz) showed minimal serial ISI correlations, consistent with our observations on a much larger sample (Fig. 8Bii). These authors did show that pyramidal cells with a higher firing rate (>40 Hz; in our sample all rates were 20 Hz) showed negative serial ISI correlations (~0.1 to ~0.3). Chacron et al. (2007) attributed this to an intrinsic mechanism (threshold fatigue) and not to the EA input, and this result is therefore also consistent with our analyses. We hypothesize that the negatively correlated fluctuations in AMPA-R potential amplitudes constitute high-frequency synaptic noise that might potentially corrupt the low-firing rate ON cell response to low-frequency prey signals. The NMDA-R component therefore eliminates this noise source and also matches the ON cell response to the low-frequency input associated with, e.g., prey signals.

Theoretical analyses in Nesse et al. (2010) predict that the negatively correlated EA ISIs are caused by quasi-independent peaks of the adaptation variable intrinsic to an EA. Furthermore, the quasi-independence of the adaptation variable could be recovered postsynaptically provided that the synaptic kinetics were matched to those of the EA adaptation process. This representation in terms of independent variables could, in
theory, implement an efficient encoding strategy. The elimination of postsynaptic negative SCs is a signature of such matching and is thus at least consistent with the Nesse et al. (2010) theory. A similar effect has been observed at the single-neuron level in cortex, although it is implemented by an entirely different biophysical mechanism (Pozzorini et al. 2013). Their analysis strongly suggested that power-law spike frequency adaptation performs temporal whitening of inputs. We have provided a direct example of temporal decorrelation (whitening) at the synaptic level, here implemented by the combined activity of NMDA-R and AMPA-R currents.

Electrosensory signal encoding. Behavioral experiments have shown that A. leptorhynchos can detect weak prey signals (<1 μV) (Knudsen 1974; Nelson and Maclver 1999). Our results suggest that the dynamic balance between NMDA-R-mediated temporal summation and GABA-A-mediated inhibition is needed to encode these signals. Blockade of NMDA-R activity eliminates this coding. The importance of NMDA-R transmission is made clear by examining EA synaptic responses during the peaks of the RAMs. The short ISIs induce presynaptic depression of the EPSP AMPA-R component (Khanbabaie et al. 2010), making it unlikely that they will trigger many spikes in vivo. We hypothesize that the AMPA-R component merely permits activation of the NMDA-R and does not contribute to signal encoding in our CMS ON cell population.

The NMDA-R component of the EPSP does not depress. This is likely due to two factors: temporal summation of these long EPSPs may mask depression and the relative affinity (half-maximal effective concentration, EC_{50}) of glutamate for the AMPA-R (EC_{50} = 500 μM) vs. NMDA-R (EC_{50} = <2 μM) (Dingleidine et al. 1999). EA afferents are glutamatergic (Wang and Maler 1994). Glutamate concentrations at the synaptic cleft reach ~1 mM (Clemens et al. 1992), with extrasynaptic concentrations reaching 190 μM (Dzubay and Jahr 1999). We hypothesize that the EA presynaptic depression is due to a reduction of the cleft glutamate concentration below that required for AMPA-R activation but still adequate to activate NMDA-R. The longer time course and positive voltage dependence of the summating NMDA-R EPSP can then effectively encode and amplify low-frequency signals extending over many EA ISIs.

The sensitivity to prey signals inferred from our study is likely higher in vivo. Our in vitro stimulation, based on the response of a few EAs, resulted in the synchronous activation of pyramidal cells. In vivo encoding in ELL would arise via asynchronous responses of heterogeneous EAs and better follow small stimulus fluctuations. For practical reasons (see methods), our synaptic analysis was confined to the CMS. ON-type pyramidal cells in the CMS receive ~25 EA inputs, while those in the CLS receive ~100 inputs (Maler 2009a). CLS ON cells are thus more sensitive to prey mimics in their receptive fields (Chacron et al. 2011; Krahne and Maler 2014; Marsat et al. 2012), although both maps respond equally sensitively to moving prey mimics (Khosravi-Hashemi and Chacron 2014).

Role of GABA-A-mediated inhibition in signal detection. Blockade of GABA-A transmission alone prevents both ON and OFF cells from encoding signals. This might be due to prevention of NMDA-R EPSP saturation. Saturation does not, however, account for the excellent OFF cell responses to stimuli in their receptive field center relayed by disynaptic GABA-A synaptic input (Berman and Maler 1998; Maler et al. 1981; Maler and Mugnaini 1994). Because OFF cell circuitry is complex (Maler et al. 1981; Maler and Mugnaini 1994), we offer no further speculation on the mechanism(s) by which OFF cells effectively encode signals (Fig. 1). We simply conclude that ON cell encoding of prey signals requires a balance of direct EA input and disynaptic inhibitory input. Detailed computational analyses will be required to understand the underlying dynamics.

Somatic amplification of signal input via voltage-dependent stochastic resonance. The ON cell response to stimulus-evoked EA spike patterns was typically small (<2 mV) and comparable to the responses seen in vivo (Bastian et al. 2004). These small depolarizations are not expected to reach spike threshold from the typical in vitro resting membrane potential (Mehaffey et al. 2008). However, in vivo, these small compound EPSPs do evoke a strong spiking response (Bastian et al. 2004), suggesting that some amplification intervenes between synaptic response and spike output. We have shown that somatic persistent Na⁺ channels amplify EPSPs arising in distal apical dendrites (Berman et al. 2001) and hypothesize that the same mechanism will amplify EA input to the ON cell basal dendrites.

We have discovered voltage-dependent membrane noise that is steeply activated near spike threshold (Mehaffey et al. 2008). The fluctuations have a sharply rising slope resembling that of AMPA-R EPSPs and trigger spiking. Although the precise nature of the underlying stochastic biophysical dynamics is not known for ELL pyramidal neurons, similar voltage-dependent noise has been noted in other systems (Jacobson et al. 2005). Our initial studies suggested that the ELL pyramidal cell noise was due to Na⁺ channels (Mathiesen and Maler 1988), while a recent theoretical analysis has suggested that the stochastic closure of K⁺ channels is the main contributor to membrane noise (O’Donnell and van Rossum 2014). We have shown (Fig. 1) that both Na⁺ and K⁺ (K,3) channels are distributed over the soma and proximal dendrites of ON and OFF cells (Fig. 1) (Deng et al. 2005; Turner et al. 1994) and that both fast and persistent Na⁺ channels are present on the soma and on proximal apical dendrites (Berman et al. 2001; Turner et al. 1994). The biophysical substrates for membrane noise are therefore available in ELL pyramidal cells. The origin of the blips is more difficult to determine. Turner et al. showed that the Na⁺ channels on the somatic and dendritic membranes of ELL pyramidal cells were clustered in small “hot spots” (Turner et al. 1994). An untested but interesting idea is that blips might in part be generated by the cooperative interactions of tightly colocalized Na⁺ channels.

Whatever the source of the ON cell membrane noise, we hypothesize that it implements a voltage-dependent SR effect (SRVD). Through this effect, noise due to blips would provide the crucial amplification step that transforms subthreshold/ perithreshold, input-evoked smooth synaptic responses into ON cell spiking output that correlates with the input. The higher parts of the input signal elicit more noise via the voltage dependence, allowing them to become encoded into spikes, albeit with some stochasticity. In the absence of a sensory input, this voltage-dependent noise underlies the stochastic firing of the ON cell, and, as is usual for SR, noise would likely not benefit the encoding of large signals. It is not possible at
present to experimentally confirm that ON cells use SR to encode low-frequency signals. The key test, using intracellular QX-314 application to block noise, would also block spiking. There is, however, supporting evidence for our conjecture. We previously demonstrated that the ON cell membrane potential accurately followed a 4-Hz stimulus within its receptive field center (Bastian et al. 2004). Figure 4 this paper shows that fast depolarizations ride on top of the slow 4-Hz modulation and drive spiking. We suspect, but cannot prove, that these fast fluctuations seen in vivo are due to the membrane noise that we analyzed in vitro; although clearly not conclusive, these data are at least consistent with our SRV_{TD} theory.

There are three consequences of our hypothesis that SR_{TD} drives spiking to weak low-frequency signals. First, since spiking is driven by intrinsic noise rather than the rising phase of EPSPs, it should not be phase locked to the EOD. This is indeed observed for all CMS and CLS ON neurons (Krahe et al. 2008) and strongly supports our hypothesis. We note that this is not simply due to all ELL target cells being unable to follow the high-frequency discharge of EAs but, rather, a design feature of these ON cells. This conclusion is based on two exemplars. First, one class of ELL interneuron (ovoid cell) is very strongly phase locked to the EOD (Bastian et al. 1993). This is likely due to this cell type having little (Harvey-Girard et al. 2007) or no (Bottai et al. 1997) NMDA-R expression and therefore being driven by EA-evoked AMPA-R EPSPs. Second, a large subset of LS ON cells (precise identity unknown) is also phase locked to the EOD (Krahe et al. 2008); neither the mechanism nor the consequences for sensory coding are known in this case. Based on analogy with the ovoid cell we predict that, in this cell class, 1) EA-evoked AMPA-R EPSPs will drive spiking, 2) these cells will have minimal membrane noise, and 3) they will be most responsive to high-frequency amplitude modulations.

The second consequence is that, as shown above, pyramidal cell discharge will have renewal statistics in the absence of sensory input, with the same distribution as that of the IBplS (gamma). Their baseline spike trains are now an ideal basis for inhomogeneous rate coding.

The third consequence is that the spiking response of ON cells receiving overlapping EA input should not display correlations due to common input, i.e., noise correlations. This exact result has been reported by Krahe et al. (2002) for CMS cells driven by RAMs of the EOD with a cutoff frequency of <20 Hz, providing strong support for our hypothesis. Litwin-Kumar et al. (2012), recording from the CLS and LS and delivering electrosensory input (RAM, 120 Hz cutoff) to neighboring pyramidal cells’ receptive fields, did find noise correlations. The correlations peaked at time delays near 100 ms, consistent with common EA input driving spiking via slow (i.e., NMDA-R driven) excitatory input and noise and not consistent with spiking driven directly by fast synaptic events (i.e., AMPA-R). Again, this result is supportive of our hypothesis. Chacron and Bastian (2008), recording from the CLS and LS neurons (RAM, 120 Hz cutoff), reached partially contradictory conclusions. Correlations were observed upon stimulation within the neighboring ON cells’ overlapping receptive field, but these were mostly signal (not noise) correlations. This result is consistent with that of Krahe et al. (2002) and Litwin-Kumar et al. (2012) and supportive of our hypothesis. Chacron and Bastian (2008) also reported that there were correlations of baseline spiking activity of neighboring ON (and OFF) cells over short time windows. This result is apparently not consistent with our hypothesis. However, baseline EA discharge will include long ISIs evoking a large (nondepressed) AMPA-R component. We hypothesize that, at least for CLS and LS cells, baseline discharge can be weakly correlated because it is triggered by the AMPA-R component of the EPSP. Sensory stimulation, by shortening ISIs, causes depression of AMPA-R EPSPs; noise correlations are reduced because spiking is then evoked by membrane noise riding on slow NMDA-R EPSPs.

We hypothesize that ON (and likely OFF) cell discharge in the CMS and CLS maps, in response to low-frequency input confined to their receptive fields, will be generated by intrinsic noise (blips). The response of cells with overlapping receptive fields will therefore be uncorrelated. This, in turn, will enable averaging the independent responses by downstream neurons and therefore enhance the detection and estimation of the weakest, e.g., prey signals. Clearly, further experiments are required to verify these hypotheses.

Our results also point to a role for noise in signal encoding—facilitating encoding in a voltage-dependent manner. Blips are the main feature of this membrane noise and enable spiking where it would not otherwise occur. Noise-assisted signal encoding as in the SR effect (Gammaitoni et al. 1998; Hanggi 2002; Longtin 1993) falls under the broader heading of stochastic facilitation (McDonnell and Ward 2011). It assumes that the stimulus-evoked EPSPs are subthreshold, as expected for weak stimuli, or generally stimuli that sit below threshold because of net bias inputs from the circuitry. For our study, where signals are slow and aperiodic, SR is referred to as aperiodic SR (Collins et al. 1995). Noise-assisted stimulus encoding has been demonstrated in vitro in hippocampal cells with current injection (Stacey and Durand 2001). Our study reveals that this effect is likely present here because of noisy blips arising near threshold. Because of the voltage dependence of the rate of occurrence of these blips, and consequently of the noise variance (Fig. 7 and Fig. 8C), the noise is amplified just where it is needed, namely, when subthreshold activity reaches spiking threshold. Therefore not only does our study show that spikes are associated with noise blips and thus noise assists stimulus encoding at the second stage of a sensory system, it also relies on the voltage dependence of this noise.

Let us put this result in a broader context. Chacron et al.’s modeling study (2000) predicted that EAs operate in the suprathreshold regime for baseline firing, meaning that firings would still occur in the absence of noise. The same goes for encoding of zero-mean amplitude modulations into a modulation of baseline activity. Thus noise in these receptors does not help the encoding of subthreshold stimuli, and SR is not at work; noise is nevertheless thought to help by linearizing the EA input-output firing rate function, smoothing over nonlinear phase locking effects (Knight 1972).

Our results suggest that the next stage (ELL) is poised to detect any small modulations of baseline EA firing using noise. We cannot carry out the required classic experiment to prove that SR is at work, i.e., we cannot show that the input-output correlation of a pyramidal cell peaks for an intermediate noise level by adding and suppressing noise. The reason is that we cannot vary the noise level alone—at the very least the noise level covaries with the mean of the membrane potential, i.e., it.
is voltage dependent. Nevertheless, the fact that the noise increases right at the limit of detection of the signal suggests that the noise-induced firing helps encode the signal. In fact, upward excursions of the signal will recruit more blips, with the result that the (stochastic) rate of firing is modulated by the signal.

Hence, postsynaptically to the EAs, it appears that noise helps the encoding process, in contrast to the EA level where the signal appears to modulate an already suprathreshold baseline firing pattern. It is also clear that too much noise at the second stage will degrade the encoding, as in the SR effect. It is tempting to further investigate whether this suprathreshold-followed-by-subthreshold encoding is a more general design principle of sensory systems, and whether an optimal noise level associated with a $SR_{VD}$ is at play here and in other systems.

The ISI decorrelation process supported mainly by the NMDA-R synaptic component appears to combine with noise-induced firing to ensure a sensitive encoding of naturalistic stimuli. The true statistics of the input spikes from multiple EAs to ON cells is not fully known, and the summed spike train may already have altered SCs compared with single EAs (Lindner 2006). The interaction of the summed EA spike trains and postsynaptic dynamics remains a critical but challenging subject for future investigation.

Conclusions. 1) The EA negative ISI correlations are not transmitted to successive EPSP peak amplitudes by the NMDA-R component of the EA to ON cell synapses. We hypothesize that the ISI correlations would otherwise be transmitted as variable amplitude peaks of the AMPA-R component and act as a high-frequency noise source. This elimination of EPSP correlations is consistent with the theoretical optimal coding predictions of Nesse et al. (2010), which require that the effective timescale of the postsynaptic response be similar to the EA adaptation timescale giving rise to the negative ISI correlations. 2) The response of CMS ON cells to the low-frequency signals produced by, e.g., prey, is driven primarily by the dynamic balance of the slow NMDA-R component of the EA-evoked EPSPs and disynaptic GABA-A mediated inhibition. 3) The evoked spiking response of ON cells is caused by intrinsic voltage-dependent noise riding on the NMDA-R component of the EA to ON cell EPSPs; this noise has renewal statistics. This has several important consequences: First, the noise acts in a voltage-dependent manner to generate spikes near threshold; this suggests that SR is at play to express weak or perithreshold signals in ON cell spiking responses. Second, ON cell spiking is not phase locked to the EOD. Third, ON cell baseline discharge also has renewal statistics and is therefore ideally suited for inhomogeneous rate coding. Finally, neighboring ON cells with overlapping receptive fields therefore have uncorrelated spiking responses to, e.g., prey signals. Their outputs are therefore independent and can be effectively summed by their downstream midbrain target neurons.

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AUTHOR CONTRIBUTIONS

REFERENCES


