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Differential and History-Dependent Modulation of a Stretch Receptor in the Stomatogastric System of the Crab, *Cancer borealis*

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Birmingham, John T., Cyrus P. Billimoria, Timothy R. DeKlotz, Raj A. Stewart, and Eve Marder. Differential and history-dependent modulation of a stretch receptor in the stomatogastric system of the crab, *Cancer borealis*. *J Neurophysiol* 90: 3608–3616, 2003. First published August 27, 2003; 10.1152/jn.00397.2003. Neuromodulators can modify the magnitude and kinetics of the response of a sensory neuron to a stimulus. Six neuroactive substances modified the activity of the gastropyloric receptor 2 (GPR2) neuron of the stomatogastric nervous system (STNS) of the crab *Cancer borealis* during muscle stretch. Stretches were applied to the gastric mill 9 (gm9) and the cardio-pyloric valve 3a (cpv3a) muscles. SDRNFLRFamide and dopamine had excitatory effects on GPR2. Serotonin, GABA, and the peptide allatostatin-3 (AST) decreased GPR2 firing during stretch. Moreover, SDRNFLRFamide and TNRNFLRFamide increased the unstimulated spontaneous firing rate, whereas AST and GABA decreased it. The actions of AST and GABA were amplitude- and history-dependent. In fully recovered preparations, AST and GABA decreased the response to small-amplitude stretches proportionally more than to those evoked by large-amplitude stretches. For large-amplitude stretches, the effects of AST and GABA were more pronounced as the number of recent stretches increased. The modulators that affected the stretch-induced GPR2 firing rate were also tested when the neuron was operating in a bursting mode of activity. Application of SDRNFLRFamide increased the bursting frequency transiently, whereas high concentrations of serotonin, AST, and GABA abolished bursting altogether. Together these data demonstrate that the effects of neuromodulators depend on the previous activity and current state of the sensory neuron.

INTRODUCTION

The mathematical relationships or "codes" that relate the activity of sensory neurons to characteristics of an appropriate stimulus have been investigated for the better part of a century (Rieke et al. 1997). Adrian and Zotterman's (1926) seminal study of isolated individual muscle fibers and stretch receptors suggested the idea of a simple *rate* or *frequency code*, in which there is a one-to-one relationship between stimulus amplitude and firing (spike) rate. Because of spike rate adaptation and other activity-dependent effects, a rate code may be a poor description of the code used by most neurons to describe many stimuli. For instance, some stretch-sensitive neurons in the chordotonal organ that monitor the joint between the tibia and femur in crustacean and insect legs more faithfully describe velocity or acceleration rather than position (Bush 1965; Field and Matheson 1998; Matheson 1990). While the computational implications of a sensory neuron's temporal dynamics have only recently been considered (Fairhall et al. 2001), there has been significant progress made on a similar problem: understanding the role of synaptic dynamics on information transfer across a synapse (Goldman 2000; Goldman et al. 2002).

A sensory neuron's code can also be modified by the presence of neuromodulatory substances (Pasztor and Bush 1987, 1989; Pasztor and MacMillan 1990). Neuromodulation can modify a neuron's input-output relationship through effects on the sensory transduction mechanism and/or the sensory neuron's cellular excitability. For example, the increased sensitivity (decreased threshold stimulus) in the visual systems of the horseshoe crab (Kass and Barlow 1984; Renninger et al. 1989), the moth *Deilephila elpenor*, and the owlfly *Ascalaphus macaronius* (Hamdorf et al. 1989), in response to exogeneous octopamine application, is due in large part to a direct effect on photoreceptor pigments. On the other hand, the adrenaline-induced increase in the differential response or *gain* of olfactory receptors from the newt *Cynops pyrrhogaster* results from an enhancement of a sodium current and the reduction of a T-type calcium current via a common cAMP-dependent pathway (Kawai et al. 1999).

Neuromodulation, not surprisingly, can modify a sensory neuron's dynamics. Application of octopamine to insect mechanoreceptors can result in a selective enhancement of the neuron's tonic (position) versus the phasic (velocity) responses (Matheson 1997; Ramirez et al. 1993) and modification of the kinetics of sensory adaptation (Zhang et al. 1992). Exposing one of the lobster's mechanoreceptors to a peptide from the FLRFamide family switches it between two qualitatively different modes of firing (tonic vs. bursting) (Combes et al. 1997) with even more dramatic coding implications. What has not yet been studied is the opposite process: how a sensory neuron's activity can affect the time course and magnitude of a neuromodulatory effect.

To address this and other questions, we studied the effect of neuromodulation on a muscle stretch receptor known as the gastropyloric receptor 2 (GPR2) (Katz et al. 1989), found in the stomatogastric nervous system of the crab *Cancer borealis*. The stomatogastric system produces the gastric mill motor pattern that controls movement of teeth within the stomach and

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the pyloric motor pattern that controls the muscles of the pylorus, which is a filtering apparatus. Both motor patterns are modulated by a large number of peptides and amines that are either released by modulatory projection and sensory neurons or that circulate in the hemolymph as hormones (Blitz et al. 1999; Christie et al. 1995; Harris-Warrick et al. 1992; Marder 1987; Marder and Calabrese 1996; Marder et al. 1995; Nusbaum et al. 2001; Skiebe 2001). Many of these same neuromodulators also have actions at the neuromuscular junctions of stomach muscles and on their nerve-evoked contraction amplitudes (Gutovitz et al. 2001; Jorge-Rivera and Marder 1996; Jorge-Rivera et al. 1998; Lingle and Marder 1981; Meyrand and Marder 1991; Sharman et al. 2000; Weimann et al. 1997).

In this paper, we describe the effects of some of these same neuromodulatory substances on the GPR2 response to square-wave muscle stretches. The vast majority of measurements in studies of neuromodulators have been made when the preparations were in one of two states: previously unstimulated or fully adapted. Instead, we studied how the magnitude of the neuromodulatory effects varied, not only with modulator concentration and stretch amplitude, but also with the preparation's recent stimulation history. The results suggest that the efficacy of neuromodulatory effects on GPR2 depends both on the amplitude of the stimulus and also on the recent history of the neuron. Preliminary reports of some of these results have been presented in abstract form (Billimoria et al. 2002) and in a short conference paper (Birmingham 2001).

METHODS

Animals and solutions

Adult crabs of the species *C. borealis* were obtained from local seafood suppliers and kept stored in aerated aquaria at 12–15°C. We used physiological saline with the following compositions (in mM): 440 NaCl, 11 KCl, 13 $CaCl_2$, 26 $MgCl_2$, 5 maleic acid, and 11 Trizma base, pH 7.4–7.5.

The modulators allatostatin III type A (AST, Bachem, Torrance, CA), buccalin A (a gift of F. Vilim, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York), crab cardioactive peptide (CCAP, Bachem), *C. borealis* tachykinin-related peptide (CabTRP, a gift from M. P. Nusbaum, Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA), corazonin (Sigma, St. Louis, MO), ala13-orcokinin (synthesized by University of Illinois Protein Science Facility, Urbana, IL), *Drosophila* tyrosine^{1}-norleucin^{16} pigment dispersing factor (PDF, a gift of D. Nässel, Stockholm University, Department of Zoology, Stockholm), γ-aminobutyric acid (GABA, Sigma), pilocarpine (Sigma), proctolin (Sigma), red pigment concentrating hormone (RPCH, American Peptide Company, Sunnyvale, CA), dopamine (Sigma), serotonin (Sigma), octopamine (Sigma), TNRNFLRFamide (American Peptide Company), and SDRNFLRFamide (American Peptide Company) were dissolved in distilled water at 10^{-2} or 10^{-3} M and stored at −20°C until they were diluted into saline before use.

Physiology

The GPR2 neurons in the crab *C. borealis* are a pair of bilateral stretch-sensitive neurons (Katz et al. 1989) that provide sensory input to the stomatogastric nervous system, a small well-studied neural network that generates rhythmic stomach movements used for digestion (Harris-Warrick et al. 1992). GPR2 innervates two muscles of the animal's foregut, gm9 and cpv3a (nomenclature from Maynard and Dando 1974), and responds to both passive and nerve-evoked stretch (Katz et al. 1989). Neuromuscular preparations were dissected according to Birmingham et al. (1999). The stomach was removed from the animal, slit ventrally from the esophagus to the midgut, and pinned flat in a dissecting dish. Preparations consisting of the cpv3a and gm9 muscles and the lateral ventricular (*lvn*) and gastropyloric (*gpn*) nerves were removed and placed flat in 5-ml silicone elastomer (Sylgard)-coated (Dow Corning, Midland, MI) petri dishes. During recording sessions the preparations were continuously superfused (2–3 ml/min) with saline cooled to 10–12°C. Extracellular measurements of the activity in the *gpn*, which contains the GPR2 cell body, were made using glass suction electrodes, amplified by an AM-Systems 1700 differential amplifier (Carlsborg, WA) and recorded using an Axon Instruments (Union City, CA) Digidata interface board. Spike times were extracted using the Datamaster program developed in the laboratory of E. Marder by W. Miller and C. Howe and were analyzed using routines written in Matlab (The MathWorks, Natick, MA).

Stretching the muscle

The basic procedure for stretching the muscle has been previously published (Birmingham et al. 1999; Katz et al. 1989). Measurements were made using either the gm9 or cpv3a muscle. The muscle's origin was pinned to the Sylgard-coated dish, and the insertion was attached to a Grass force-displacement transducer (Model FT03, Quincy, MA) with No. 6 suture thread. The transducer in turn was attached either to the lever arm of a chart recorder pen motor or to an audio speaker. The pen motor or speaker was driven and hence the muscle stretched using a square waveform. Muscle displacements were calibrated visually to ±0.02 mm using an eyepiece with a reticule. Before each experiment, the system was adjusted so that the muscle was completely extended but under no tension. The muscle was stretched until a threshold response was obtained and then slightly relaxed.

Statistics

In experiments where one concentration of modulator was used, a paired *t*-test was used to test for statistical significance. When multiple concentrations of a given modulator were used, a one-way ANOVA was employed. For those experiments that included multiple conditions without and with the presence of modulator, a two-way ANOVA was used. A three-way ANOVA was used to determine if the effect of the modulator differed in the early versus the late part of the response for multiple amplitude stretches. Pairwise comparisons were made with the Student-Newman-Keuls method. For all statistical tests, significance with respect to control was indicated on figures using the following symbols: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars on plots correspond to SEs.

RESULTS

GPR2 activity is decreased by serotonin, allatostatin, and GABA and increased by SDRNFLRFamide, TNRNFLRFamide, and dopamine.

Figure 1*A* shows that the response of a GPR2 neuron to a 10-s, 0.48-mm square-wave stretch of the cpv3a muscle was modified in the presence of serotonin in a concentration-dependent manner. As the serotonin concentration was increased, the total number of GPR2 spikes generated during the stretch decreased. Figure 1*B* is a dose-response curve that summarizes the effect of serotonin on the mean spike rate (spike number/stretch duration) elicited during these stretches. For each experiment ($n = 4$), the spike rate was measured in control saline and in four concentrations of serotonin. To facilitate compar-

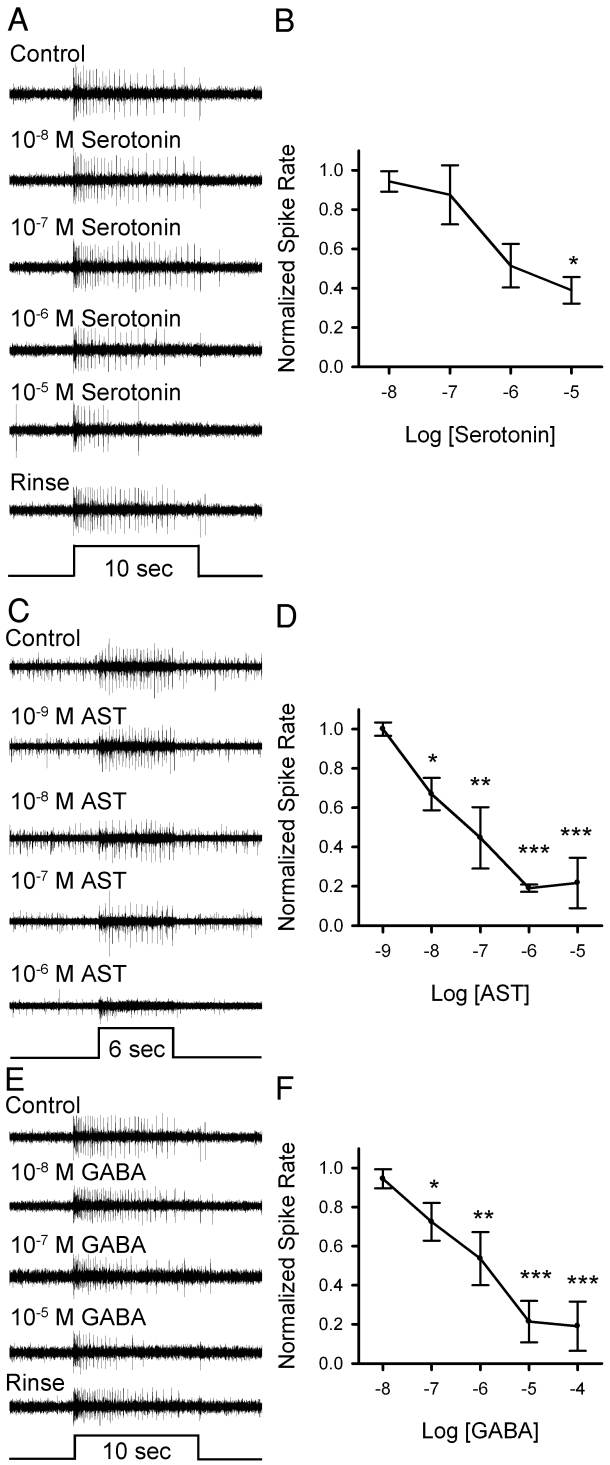


FIG. 1. The effects of serotonin, allatostatin-3 (AST) and GABA on gastropyloric receptor 2 (GPR2) activity. *A*: extracellular recordings of GPR2 spikes (recorded on the *gpn*) show a dose-dependent effect of serotonin in response to 10-s duration 0.48-mm square-wave muscle stretches. *B*: summary data for 4 serotonin dose-response experiments. *C*: responses in AST to 6-s, 0.48-mm stretches. *D*: summary dose-response curve for 4 AST experiments. *E*: responses in GABA to 10-s, 0.48-mm stretches. *F*: summary dose-response curve for 8 GABA experiments. Significant changes compared with control in *B, D,* and *F* calculated with a 1-way ANOVA using spike rates prior to normalization.

ison, the spike rate for each concentration was normalized to the control value for that preparation, and the average over experiments was plotted. The effect of 10^{-5} M serotonin was significant (1-way ANOVA, $P = 0.014$). Stable effects of each of the three inhibitory modulators were seen after 6 min of application. Early experiments were done with normal saline rinses between each dose until the response returned to near control levels, but these washes were eliminated when it was determined that they did not affect the results.

Figure 1, *C* and *D,* shows the results of a study of the effects of the peptide AST on a GPR2 response to a 6-s, 0.48-mm muscle stretch. A control response and the response in several concentrations of AST are shown in Fig. 1*C*. Figure 1*D* summarizes the results of four experiments. The threshold and saturation concentrations were $\sim 10^{-8}$ and 10^{-6} M, respectively. All concentrations tested $\geq 10^{-8}$ M produced significant effects (1-way ANOVA).

GABA was the third neuroactive substance that decreased the GPR2 response. The control response and the response in several concentrations of GABA are shown in Fig. 1*E*. Figure 1*F* summarizes eight experiments in which the threshold and saturation concentrations were $\sim 10^{-7}$ and 10^{-5} M, respectively. All concentrations tested $\geq 10^{-7}$ M produced significant effects (1-way ANOVA). For AST and GABA, the decrease in GPR2 activity during a stretch was nondesensitizing and persisted for hours.

Figure 2*A* shows that 10^{-7} M SDRNFLRFamide increased the number of action potentials in response to a 6-s, 0.48-mm square-wave stretch. Figure 2*C* shows that the average spike rate during the stretch increased significantly ($n = 4$; paired *t*-test, $P = 0.012$) in the presence of 10^{-7} M SDRNFLRFamide. Dose-response experiments were not done because long-lasting desensitization occurred within 2–3 min of the onset of the firing rate increase. Peptide application at 10^{-6} M to naïve preparations produced high-frequency spontaneous firing that obscured the stretch response altogether. Figure 2, *B* and *C,* shows that 10^{-4} M dopamine also increased the spike rate ($n = 7$; paired *t*-test, $P = 0.006$) in response to a 6-s, 0.48-mm stretch. SDRNFLRFamide (10^{-7} M) significantly increased the spontaneous firing rates in the absence of stretch ($n = 5$; $P = 0.0197$), while 10^{-4} M dopamine did not ($n = 6$; $P = 0.457$).

The effects of these modulators on the GPR2 activity during a stretch could be caused by a direct effect on muscle tension at a given length or by direct action on the stretch-sensitive neuron. Therefore we conducted a series of experiments in which we measured muscle tension in the presence and absence of modulators over the range of muscle lengths used in this study. The effects of 10^{-6} M AST, 10^{-4} M GABA, and 10^{-7} M SDRNFLRFamide on the relationship between muscle length and steady-state tension were tested for stretches with amplitudes between 0 and 0.64 mm (0.08 mm increment). No significant effect of any modulator was observed (all $n = 3$; paired *t*-test, AST, $P = 0.784$; GABA, $P = 0.654$; SDRNFLRFamide, $P = 0.534$.) Hence, it appears that each modulator modified the GPR2 response to stretch rather than simply changing the relationship between muscle tension and stretch.

More than 15 neuroactive substances have physiological effects in the stomatogastric nervous system of the crab *C. borealis* (Christie et al. 1995; Marder and Weimann 1992; Marder et al. 1995, 1997; Nusbaum and Beenhakker 2002;

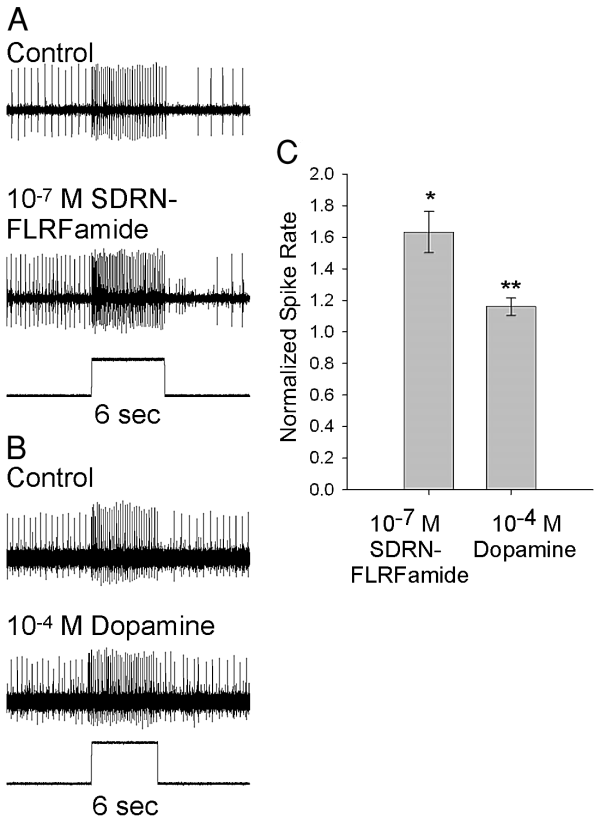


FIG. 2. The effects of SDRNFLRFamide and dopamine on GPR2 activity. *A*: extracellular traces of GPR2 activity in response to 6-s, 0.48-mm square-wave muscle stretches show effect of 10^{-7} M SDRNFLRFamide application. *B*: response in 10^{-4} M dopamine to same stimulus. *C*: 4 experiments show a significant increase in GPR2 activity (paired *t*-test, $P = 0.012$) in 10^{-7} M SDRNFLRFamide. Seven experiments show a significant increase in activity in 10^{-4} M dopamine ($P = 0.006$).

Skiebe 2001). We found no significant effect on the GPR2 response to bath applications of the following substances: 10^{-6} M ala13-orcokinin ($n = 3$), 10^{-6} M buccalin A ($n = 3$), 10^{-6} M CabTRP ($n = 4$), 10^{-6} M CCAP ($n = 3$), 10^{-6} M corazonin ($n = 3$), 10^{-6} M PDF ($n = 3$), 10^{-4} M pilocarpine ($n = 3$), 10^{-6} M proctolin ($n = 3$), 10^{-6} M RPCH ($n = 3$), and 10^{-4} M octopamine ($n = 5$). The effect of TNRNFLRFamide proved difficult to characterize. In three of three experiments, applications at 10^{-8} M had no observable effect, while application at 10^{-7} M resulted in high-frequency firing, an effect that desensitized within minutes. Interestingly, lobster neuromuscular preparations exhibited irreversible desensitization when exposed to 5×10^{-8} M TNRNFLRFamide (Worden et al. 1995).

AST and GABA have amplitude-dependent neuromodulatory effects

Figure 1 shows that serotonin, AST, and GABA all decreased the number of spikes in response to a stretch stimulus of a relatively long duration (6–10 s). We next measured the effects of AST and GABA on square-wave stretches of different amplitude when the neuron was in a fully recovered state. By "fully recovered," we mean when the preparation was sufficiently rested such that its response was independent of the previous stimulus history. For a preparation to recover fully after a single 10-s large-amplitude stretch such as the ones shown in Fig. 1 required ~10 min. Therefore to reduce the recovery time, we reduced the stretch duration to 1 s. The required recovery time was ~1 min for stretches with amplitudes ≤0.64 mm. We also limited our investigation to AST and GABA because prolonged exposure (~15 min) to high concentrations (10^{-4} M) of serotonin resulted in desensitization, and these experiments each required more than an hour of exposure to the neuromodulator. There was no evidence of desensitization with AST or GABA.

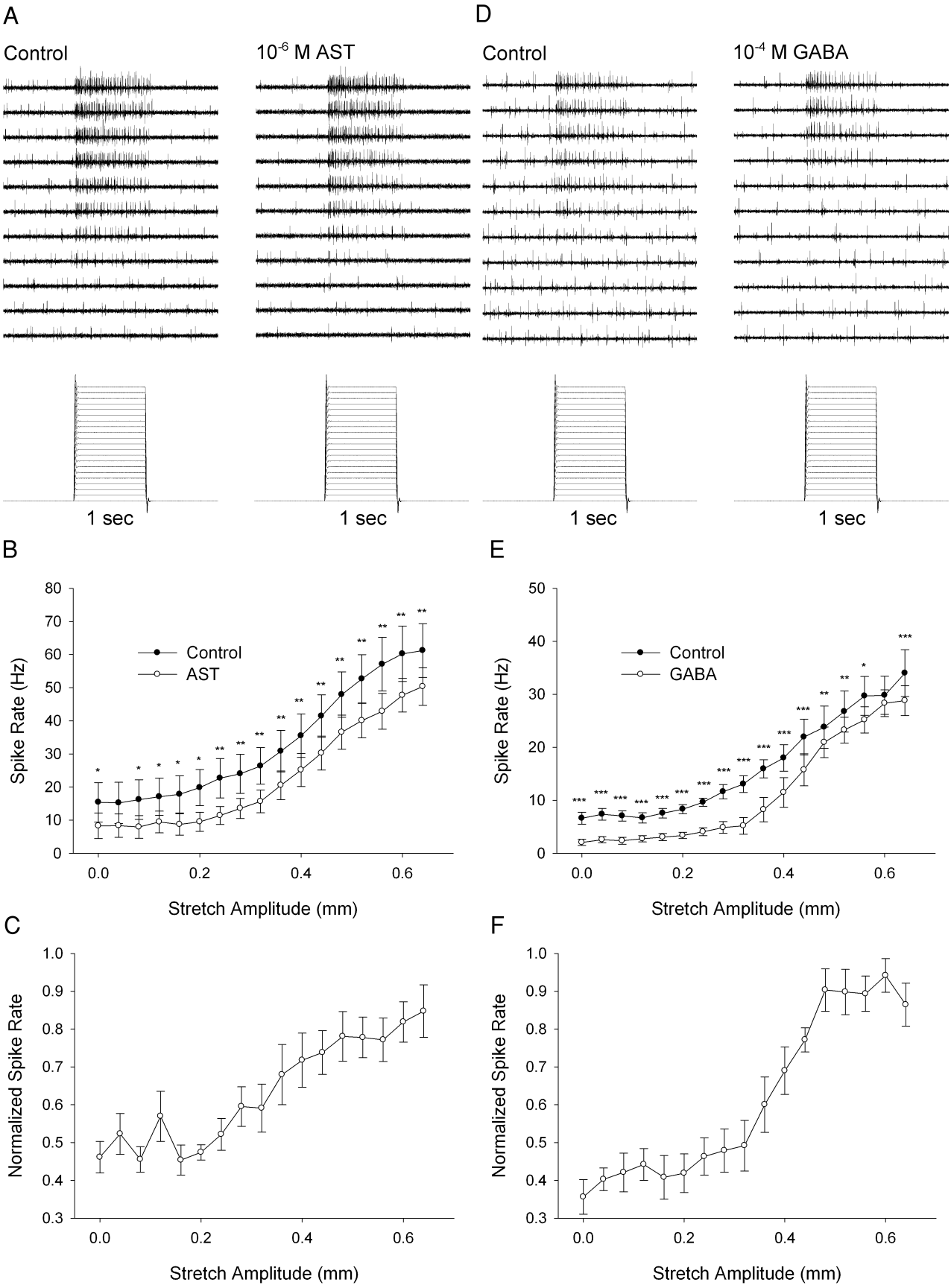
Figure 3*A* shows responses to a family of 21 square-wave stretches ranging from 0 to 0.64 mm in control saline and 10^{-6} M AST. Every other trace is shown because of space constraints. Figure 3*B* summarizes the relationship between the average firing rate and stretch amplitude ($n = 6$). The difference in the mean firing rates between the control and AST responses was statistically significant (2-way ANOVA, $P = 0.012$) over the full range of stretches, and the effects of AST did not depend on the stretch amplitude ($P = 0.077$). The Student-Newman-Keuls test was used to make pairwise comparisons of the individual mean responses for each stretch amplitude, and significance is indicated on the plot. AST decreased the spontaneous firing rate of GPR2 in the absence of stretch and for all but one stretch amplitude. Because the reduction in spike rate (in Hz) is relatively independent of the control spike rate, the net effect of AST is a larger percentage decrease in spike rate for smaller stretches (Fig. 3*C*).

Figure 3, *D* and *E,* shows raw traces and a summary of a similar set of experiments studying the effect of 10^{-4} M GABA on the dependence of firing rate on stretch amplitude. The difference in the mean firing rates between the control and in GABA was statistically significant ($n = 7$; 2-way ANOVA, $P < 0.001$). Moreover, the effects of GABA did depend on the stretch amplitude ($P = 0.019$). The Student-Newman-Keuls test was used to make pairwise comparisons, and significance is indicated on the figure. Like AST, GABA significantly decreased the spontaneous GPR2 firing rate in the absence of stretch ($P < 0.001$). Figure 3*F* shows that the percentage decrease in spike rate in GABA decreased with increasing stretch amplitude with GABA having almost no effect on the response to the largest stretches. Thus the effects of both AST and GABA were more pronounced at low amplitudes and low spike rates.

The control GPR2 response to square-wave stretches adapts over both short (tenths of a second) and longer (seconds) time scales (Birmingham et al. 1999). To investigate whether the magnitude of the effects of AST and GABA changed as the firing rate decreased during the stretch, we broke the 1-s stretch into two 500-ms segments and did a three-way ANOVA. We found no statistical difference between the effect of AST or GABA for the first 500 ms as compared with the second 500 ms (AST, $P = 0.693$; GABA, $P = 0.905$).

Modulatory effects of AST and GABA are history dependent

The data shown in Fig. 3 reflect the neuromodulatory effects of AST and GABA on the GPR2 response to a particular well-defined stimulus when the preparation was fully recovered. To explore the effects of neuromodulation along another dimension in the stimulus parameter space, we made repeated measurements at a single stretch amplitude without allowing



the preparation to recover fully. Under control conditions, 99 1-s, 0.64-mm square-wave stretches (trials) were presented to a fully recovered preparation with 1-s rest between each trial. The preparation was then allowed to recover fully, and the set of stretches was repeated an additional two times. The modulator was then applied, and after a time sufficient for the effect of the modulator to stabilize (≥15 min), the protocol was repeated. A very large-amplitude stretch was chosen specifically because the effects of AST and GABA are initially very small but become larger with trial number for these amplitudes (Fig. 3, *C* and *F*). Figure 4*A* shows the average spike rate during each 1-s stretch for the first 30 stretches in three experiments in both control saline and 10^{-6} M AST. As trial number increased, both the control and AST rates decreased but the rate in AST decreased further as is shown in Fig. 4*C* where the ratio of the rates is plotted as a function of trial number. Figure 4*B* shows the results when using the same experimental protocol with 10^{-4} M GABA ($n = 7$). The effects of GABA were also more pronounced late in the sequence of trials (Fig. 4*C*). The Student-Newman-Keuls test was used to make pairwise comparisons, and significance is indicated on the figure. It must be emphasized that the downward trends in spike rate in Fig. 4 do not reflect the time course of the application of the modulator but rather show the effects of intertrial adaptation on modulator action. Thus again, the effects of both AST and GABA were more apparent at lower spike rates.

GPR2 bursting and spiking are affected by a similar set of neuromodulatory substances

Two qualitatively different modes of GPR2 activity have been observed (Birmingham et al. 1999; Katz et al. 1989). In addition to the *spiking* mode that has been discussed thus far, in a minority of preparations, GPR2 operates in a *bursting* mode, (Katz et al. 1989), an example of which is shown in Fig. 5*A, top*. We wished to determine whether the substances that affected the GPR2 spiking response also affected GPR2 bursting. In the bursting preparations tested, muscles were unstretched. Figure 5*A, middle,* shows that 10 min after bath application of 10^{-6} M AST, the bursting had ceased. The effect was reversible as shown in Fig. 5*A, bottom*. Application of 10^{-4} M GABA or 10^{-5} M serotonin abolished bursting in a similar fashion (traces not shown). Application of 10^{-6} M SDRNFLRFamide, on the other hand, reversibly increased the rate of bursting (Fig. 5*B*). A summary of experiments involving the four modulators is presented in Fig. 5*C*, where the activity of bursting neurons is shown for 3 min before and 10 min after the application of each neuromodulator. Each dot (●) represents the start time of a burst, and each row reflects a separate experiment. The burst duration is not represented. Although the initial burst rate varied considerably from preparation to preparation, the application of the three inhibitory

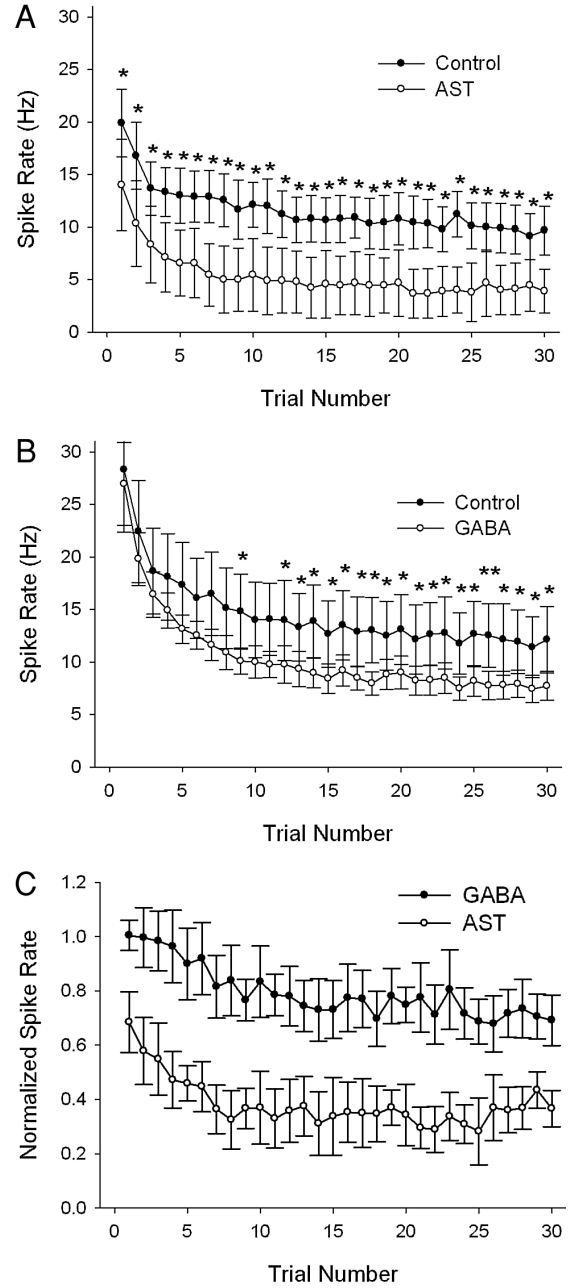


FIG. 4. GPR2 spike rate in AST and GABA as a function of previous stretch history. Fully recovered preparations were given a series of 1-s duration 0.64-mm stretches (trials) with 1-s rest between each trial. *A*: average GPR2 spike rate for 3 experiments in control and in 10^{-6} M AST as a function of trial number. Pairwise comparisons between control and modulated conditions in *A* and *B* were made with the Student-Newman-Keuls method and significant differences are indicated. *B*: summary of analogous experiments with 10^{-4} GABA ($n = 7$). A log transformation was used in the 2-way ANOVA to correct for differences in variance between the control and GABA data. *C*: normalized AST and GABA spike rates as a function of trial number.

FIG. 3. The effects of AST and GABA on GPR2 response as a function of stretch amplitude. Stretches were 1 s in duration with sufficient time between stretches (1 min) to allow for recovery from spike rate adaptation. *A*: extracellular recordings (*top*) showing GPR2 responses in control saline and in 10^{-6} M AST for stretch amplitudes (*bottom*) between 0 and 0.64 mm. *B*: summary data for 6 experiments showing mean spike rate as a function of stretch amplitude for control and 10^{-6} M AST. *C*: spike rate in 10^{-6} M AST normalized with respect to control as a function of stretch amplitude. *D*: GPR2 responses in control and 10^{-4} M GABA over the same range of amplitudes. *E*: summary data for 7 experiments for control and 10^{-4} M GABA. *F*: spike rate in 10^{-4} M GABA normalized with respect to control as a function of stretch amplitude. Pairwise comparisons between control and modulated conditions in *B* and *E* were made with the Student-Newman-Keuls method and significant differences are indicated.

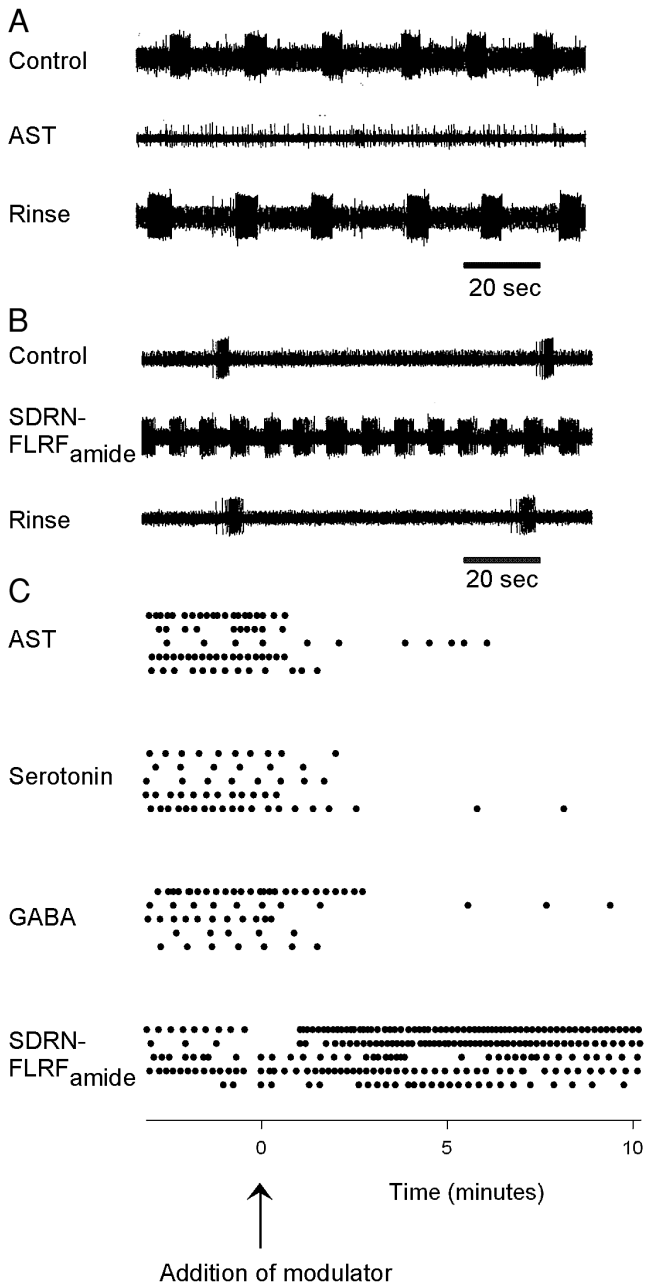


FIG. 5. The effects of neuromodulators on GPR2 bursting mode. *A*: 10^{-6} M AST in most cases abolished spontaneous bursting. *B*: 10^{-6} M SDRNFLRFamide increased the rate of spontaneous bursting. *C*: the effects over time of 10^{-6} M AST ($n = 5$), 10^{-5} M serotonin ($n = 5$), 10^{-4} M GABA ($n = 5$), and 10^{-6} M SDRNFLRFamide ($n = 5$) on the rate of bursting. ●, the start time of each burst.

modulators eliminated or drastically slowed the bursting. The four experiments in which SDRNFLRFamide was applied showed excitatory effects on the rate of bursting. Figure 5*C* reveals that there was desensitization to SDRNFLRFamide similar to that seen when applying this modulator to a preparation in the spiking mode. Application of TNRNFLRFamide to the AGR (anterior gastric receptor) stretch receptor in the STNS in the lobster *Homarus gammarus* switched that neuron from a spiking to bursting mode (Combes et al. 1997). No neuromodulatory substance was discovered in our preparation that switched GPR2 from spiking to bursting or vice versa.

DISCUSSION

Many studies of coding properties of individual sensory neurons have been done using invertebrate nervous systems, which are attractive because of their relative simplicity. Among the best-studied invertebrate sensory neurons whose response is modified through the effects of neuromodulators are muscle stretch receptors (Combes et al. 1997; el Manira et al. 1991; Matheson 1997; Pasztor and Bush 1987; Ramirez and Orchard 1990; Strawn et al. 2000; Zhang et al. 1992). Previously we showed that GPR2 encodes stretch amplitude in both the spiking and bursting modes (Birmingham et al. 1999). Here we show that modulation alters the encoding of stretch in an amplitude- and history-dependent manner.

Two of the neuromodulators studied here, AST and serotonin, are found as cotransmitters in the GPR2 neuron itself (Katz and Harris-Warrick 1989–1991; Katz et al. 1989; Skiebe and Schneider 1994). Although there are no reports of distal release of cotransmitters by GPR2, the high concentrations required for an effect on GPR2 argue against a hormonal source of the modulators and suggest that autoreceptors to GPR2's cotransmitters could be responsible. If this were the case, modulator release would probably necessitate a high-frequency GPR2 spike train similar to that required for serotonin release by GPR in the STG (Katz and Harris-Warrick 1989–1991). The resulting reduction in sensitivity could act as a form of gain control to limit GPR2's firing rate. Modulation of sensitivity in a crustacean stretch receptor via autoreceptor action has been previously reported for the lobster oval organ (Pasztor and Bush 1989). In that system, release of the peptide proctolin resulted in increased rather than decreased sensitivity to stretch.

Inhibition of the crayfish abdominal stretch receptor by a single GABA-immunoreactive neuron is well documented (Elekes and Florey 1987), and we suggest that GABAergic modulation of GPR2 may occur in a similar fashion. GABA is found in several projection neurons in the stomatogastric nervous system of *C. borealis* (Blitz et al. 1999; Swensen et al. 2000). Each of the two modulatory proctolin neurons (MPNs) sends a pair of axons through the stomatogastric ganglion (STG), with one fiber projecting down each *lvn* at least as far as the dorsal branch of the *lvn* which is just anterior to the *gpn* and its innervated muscles (Swensen et al. 2000). MPN and GPR both influence the gastric mill rhythm (Beenhakker and Nusbaum 2001, 2002; Blitz and Nusbaum 1997), and it is likely that their activities are related. Electron microscopic examination of GABA-sensitive muscles posterior to GPR2 revealed inhibitory nerve terminal profiles (Sharman et al. 2000), and we suggest that the most likely source of GABAergic modulation of GPR2 is one of the MPN neurons.

TNRNFLRFamide and SDRNFLRFamide are found in the neuropil of the crab STG (Marder 1987; Weimann et al. 1993) and in the pericardial organs and can be released by depolarization (Li et al. 2003). Dopamine has similarly been localized in the pericardial organs (Cooke and Goldstone 1970). Our assumption is that hormonal release from the pericardial organs is the likely source of the peptides and dopamine.

The spontaneous spike rate of sensory receptors in the absence of a stimulus can be modified by neuroactive substances (e.g., Grosmaitre et al. 2001). Our data show that the spike rate of unstretched GPR2 spiking mode neurons increased and

decreased in the presence of excitatory and inhibitory modulators, respectively (Figs. 1–3 and 5). A simple change in the spontaneous firing rate should result in a shift in the frequency versus amplitude curves (Fig. 3, *B* and *E*) while preserving their shapes. This may entirely account for the effect of AST (Fig. 3*B*) and to a lesser extent the effect of GABA (Fig. 3*E*). The effects on the spontaneous rate and on the unstretched bursting mode preparations make it unlikely that actions on stretch-activated channels alone could explain the data. Modulation of the spike threshold or of other conductances influencing the neuron's activity, on the other hand, could influence both the spontaneous firing rate and the change in rate produced by the stretch.

However, it is extremely important to remember that from the perspective of a postsynaptic cell, a change in spontaneous firing rate may be as important as modulation of stretch-activated channels. The postsynaptic target cannot differentiate between spontaneously occurring action potentials and those specifically activated by the stretch. A final understanding of the effect of the modulation of GPR2 will require an understanding of how it fires in vivo and how its dynamics of firing influence its release of all of its cotransmitters.

Neuromodulatory effects are dependent on spike rate

Figures 3 and 4 show that the effects of the neuromodulators AST and GABA are more pronounced at lower spike rates. This is true whether the lower spike rate is due to a lower amplitude stretch (Fig. 3) or due to spike rate adaptation (Fig. 4). Amplitude-dependent modulatory effects have been reported in the locust chordotonal organ. Application of 10^{-4} M octopamine resulted in a significant (~25%) increase in tonic spiking at flexed tibia angles when the firing rate was highest, but no significant increase at extended angles (Matheson 1997). We are not aware of any other studies of the influence of spike rate adaptation on the magnitude of a neuromodulatory effect on a sensory neuron. What do these data mean for the encoding of muscle stretch? Not only can neuromodulators alter the firing rate in response to a given stretch, but the efficacy of the modulator depends on the stretch amplitude and the history of activity. These data suggest that a given GPR2 firing rate is in effect a degenerate code and that it is not unambiguously signaling muscle stretch but is integrating information about history of activity, neuromodulatory environment, and dynamics of movement. The challenge of the future will be to understand how the postsynaptic targets decode complex discharges produced by such a modulated sensory neuron.

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