Currents Contributing to Decision Making in Neurons B31/B32 of *Aplysia*

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1Leslie and Susan Gonda (Goldschmied) Multidisciplinary Brain Research Center, 2Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel; and 3Center for Neurobiology and Behavior and 4Department of Psychiatry, Columbia University, New York, New York

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Hurwitz I, Ophir A, Korngreen A, Koester J, Susswein AJ. Currents contributing to decision making in neurons B31/B32 of *Aplysia*. *J Neurophysiol* 99: 814–830, 2008. First published November 21, 2007; doi:10.1152/jn.00972.2007. Biophysical properties of neurons contributing to the ability of an animal to decide whether or not to respond were examined. B31/B32, two pairs of bilaterally symmetrical *Aplysia* neurons, are major participants in deciding to initiate a buccal motor program, the neural correlate of a consummatory feeding response. B31/B32 respond to an adequate stimulus after a delay, during which time additional stimuli influence the decision to respond. B31/B32 then respond with a ramp depolarization followed by a sustained soma depolarization and axon spiking that is the expression of a commitment to respond to food. Four currents contributing to decision making in B31/B32 were characterized, and their functional effects were determined, in current- and voltage-clamp experiments and with simulations. Inward currents arising from slow muscarinic transmission were characterized. These currents contribute to the B31/B32 depolarization. Their slow activation kinetics contribute to the delay preceding B31/B32 activity. After the delay, inward currents affect B31/B32 in the context of two endogenous inactivating outward currents: a delayed rectifier K⁺ current (Iₖ₉ᵥ) and an A-type K⁺ current (Iₖ₉ₐ), as well as a high-threshold noninactivating outward current (Iₗ₃₄). Hodgkin-Huxley kinetic analyses were performed on the outward currents. Simulations using equations from these analyses showed that Iₖ₉ᵥ and Iₖ₉ₐ slow the ramp depolarization preceding the sustained depolarization. The three outward currents contribute to braking the B31/B32 depolarization and keeping the sustained depolarization at a constant voltage. The currents identified are sufficient to explain the properties of B31/B32 that play a role in generating the decision to feed.

**INTRODUCTION**

Organisms decide to initiate a behavior in the context of continuously changing and often ambiguous stimuli. Neural circuits must evaluate such stimuli and make discrete decisions (Glimcher 2004). The circuit organizing *Aplysia* consummatory feeding behaviors is an excellent model system for exploring some of the cellular processes underlying choice and decision making and the modulation of these processes. Before each consummatory act, *Aplysia* decide on whether to respond as well as on the nature and the intensity of a response (for review, see Elliott and Susswein 2002).

*Aplysia* feeding is a particularly appropriate behavior for examining cellular processes that underlie decision making. The decision on whether or not to respond is made by a small group of protraction-phase neurons the properties and interconnections of which have been explored (Baxter et al. 1997; Cropper et al. 2004; Dembrow et al. 2003, 2004; Elliott and Susswein 2002; Hurwitz et al. 1994, 1996, 1997, 1999a,b, 2000, 2003, 2005; Jing and Weiss 2001, 2002, 2005; Serrano and Miller 2006). In addition, neural correlates of feeding behaviors (termed buccal motor programs) can be monitored in a reduced preparation (Hurwitz et al. 1996; Jing et al. 2003; Susswein et al. 1996; Weiss et al. 1986) in which the biophysical properties of relevant neurons and muscles are readily characterized (Gardner 1989; Serrano and Miller 2006; Jacklet and Tieman 2004; Kozak et al. 1996; Scott et al. 1997; Trudeau et al. 1993; Ye et al. 2006). The feeding behavior controlled by the neuronal circuit (Elliott and Susswein 2002; Horn et al. 2004; Hurwitz and Susswein 1992; Kupfermann 1974; Lum et al. 2005; Neustadter et al. 2007; Ye et al. 2006), and its modulation by changes in motivational state (Hurwitz et al. 2006; Kupfermann 1974; Kupfermann et al. 1991), or by learning and memory (Botzer et al. 1998; Lechner et al. 2000; Susswein et al. 1986), have been examined in detail. Circuitry and modulation of feeding also have been examined in a number of related animals, allowing evolutionary and adaptive comparison between species (for reviews, see Chase 2002; Elliott and Susswein 2002).

*Aplysia* consummatory behaviors transport food from the environment into and out of the mouth and gut via a protraction of the toothed radula, which is followed by retraction (Kupfermann 1974). The decision to initiate a consummatory behavior is made when the animal decides to protract because protraction is invariably followed by retraction (Kupfermann 1974). A delay period often precedes the firing of protraction-phase neurons in response to an adequate stimulus. During the delay, the neurons are exquisitely sensitive to additional inputs that can cause a program to be initiated earlier or that can cause it to be delayed or blocked. The protraction-phase neurons behave as though they weigh their options and choose whether or not to respond in the few seconds preceding a full-blown protraction. Once protraction is initiated, it is difficult to stop the full protraction-retraction sequence (Susswein et al. 2002), indicating that initiation of protraction represents a choice to respond to a stimulus with a buccal motor program.

This paper examines some of the subcellular processes that contribute to the seeming ability of a group of key protraction-phase neurons to choose and decide. We focus on the B31/B32 neurons. These are two pairs of neurons—one pair in each of the bilaterally symmetrical buccal ganglia. The four cells have indistinguishable biophysical properties. The two cells in each hemi-ganglion are strongly electrically coupled to one another.

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(Susswein and Byrne 1988) and more weakly coupled to other protraction-phase neurons (Hurwitz et al. 1997; Susswein and Byrne 1988). They are monosynaptically excited by contralateral protraction-phase neurons, such as B63 and B34 (Hurwitz et al. 1997). They and other protraction-phase neurons are also monosynaptically excited (Hurwitz et al. 2003) by command-like cerebral ganglion neurons that can initiate feeding programs (Church and Lloyd 1994; Morgan et al. 2000; Rosen et al. 1991). During protraction, neurons B31/B32 display a sustained plateau-like depolarization of \( \sim 40 \text{ mV} \) from the rest potential (Susswein and Byrne 1988). The sustained depolarization is preceded by a delay, which is followed by a slow ramp depolarization. Aspects of B31/B32 activity that are examined in this study include: the delay in initiation of B31/B32 activity and the ramp depolarization, which are neural correlates of a time window during which the system decides on whether or not to respond, and the sustained depolarization of B31/B32, which is a neural correlate of a full commitment to protraction. Because the activity of B31/B32 is a major determinant of radula protraction (Hurwitz et al. 1994, 1996), examining the processes underlying B31/B32 activity provides a rare opportunity to directly relate cellular processes to behavior.

Depolarization of B31/B32 before and during the sustained depolarization is driven by fast and slow chemical and electrical excitatory postsynaptic potentials (EPSPs) from other protraction-phase neurons (Dembrow et al. 2004; Hurwitz et al. 1997, 1999a, 2003; Jing and Weiss 2001, 2002; Kabotyanski et al. 1998), operating on a background of endogenous voltage-dependent currents. We examined how the combination of evoked and endogenous currents gives rise to the ability of B31/B32 to decide. We have identified three outward currents activated by depolarization of B31/B32 and have examined their contributions to the activity of B31/B32 during a buccal motor program. Two of the currents display inactivation and participate in shaping the sustained depolarization and the ramp that precedes it. An additional nonactivating current is activated during the sustained depolarization. This current produces a relatively small effect on B31/B32 activity.

**Methods**

Quantitative data on outward currents were entirely from a set of experiments performed in Israel. A preliminary set of experiments were performed in New York in somewhat different conditions. A number of the figures presented in the following text are from the preliminary data, but all of the quantitative data are derived from the second set of experiments. Data from the two sets of experiments were very similar. Differences in procedures in the two sets of experiments are noted in the following text.

**Animals**

In both Israel and in New York, experiments were performed on *Aplysia californica* (80–250 gram) purchased from Marinus (Long Beach, CA) and Marinus Scientific (Garden Grove, CA).

**Dissection**

Animals were anesthetized with isotonic MgCl\(_2\) (25–50% of the body weight) prior to dissection. The buccal ganglia were then removed from the animals and placed in a chamber containing 50% filtered artificial seawater (ASW) and 50% isotonic MgCl\(_2\). In the preliminary set of experiments, the ganglia were pinned and then exposed to 0.5% glutaraldehyde in ASW for 1 min to kill sheath muscle. The ganglia were then re-exposed to 50% ASW 50% MgCl\(_2\), and the connective tissue sheath overlying the neurons was surgically removed. In the quantitative set of experiments, the ganglia were desheathed without first killing the sheath muscle. After the desheathing, the bathing solution was replaced with ASW.

**Experimental conditions**

The preliminary set of experiments was performed at 17°C using 1–5 M\( \Omega \) electrodes filled with 1 M KCl in a chamber filled with 0.5 ml ASW. Data presented in Figs. 2, 3, 4, and 8 are from this set of experiments. All experiments from which the quantitative data were derived were performed at room temperature (23°C) using 5–10 M\( \Omega \) electrodes filled with 10% 1 M KCl and 90% 2M potassium acetate. During this set of experiments, neurons adjacent to B31/B32 were killed to reduce possible problems arising from electrical coupling to adjacent neurons (e.g., Hurwitz et al. 1997; Susswein and Byrne 1988). Data presented in Figs. 4B, 5 and 6 are from this set of experiments.

Axoclamp 2 or 2A (Axon Instruments, Foster City, CA) current/voltage clamps were used for voltage- and current-clamp experiments. The current headstage was a model HS-2 (Axon Instruments) and Igor 4.01 (Wavemetrics, Portland, OR). Voltage and current data were acquired and analyzed using PCs running the Clampex component of pClamp 8.0 software (Axon Instruments) with a sampling rate of 1 kHz (1st series of experiments) or 10 kHz (2nd series of experiments). A/D and D/A conversions were performed by Digidata 1200A (1st series) or 1322A (2nd series) digitizers (Axon Instruments).

**Drugs**

Drugs were added by preparing concentrations 100 times greater than that needed and then adding the drug to the bath to achieve the desired concentration. Tetrodotoxin (TTX) from Calbiochem or from Alomone was made up as a 6 mM stock solution that was kept frozen at –30°C. Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) were prepared freshly daily.

**Analysis**

Data were analyzed using the Clampfit component of PClamp 8.0 (Axon Instruments) and Igor 4.01 (Wavemetrics, Portland, OR). Current traces were analyzed assuming a Hodgkin-Huxley model (Hodgkin and Huxley 1952). The activation, inactivation and deactivation current traces were fit to the general equation

\[
I(t) = (\xi I_o - \xi I_{-} e^{-t/\tau})^p
\]

Where \( t \) is time, \( I_o \) is the steady-state current, \( I_{-} \) is the current at \( t = 0 \), \( \tau \) is the time constant of the exponential relaxation, and \( p \) is the number of gates in the Hodgkin-Huxley model. Because \( I_{-} \) is close to zero at the holding potential, the preceding equation simplifies to

\[
I(t) = I_o(1 - e^{-t/\tau})^p
\]

Correspondingly, tail currents were fitted to

\[
I(t) = I_o e^{-t/\tau}\gamma
\]

The normalized conductance was fitted to the Boltzmann equation

\[
G = \frac{G_{\text{max}}}{1 + e^{(-v-v_{\text{th}})/s}}
\]

Where \( G/G_{\text{max}} \) is the conductance normalized to its maximal value, \( v \) is membrane potential, \( h \) is the voltage at which \( G \) is half-maximal when \( P = 1 \), and \( s \) is the slope factor.

For the two inactivating K\(^+\) currents described in the following text, the steady-state voltage-dependent percent activation over the...
full voltage range of activation, and the maximal conductance ($g_{\text{max}}$) in response to depolarization, were determined by applying voltage steps in +10-mV increments from a holding potential of $-80$ to $+60$ mV. For analysis of $I_{K_{V}}$, the steps were maintained for 5 s, and for $I_{K_{A}}$, 1.5 s.

To determine the steady-state voltage-dependent percent inactivation over the full range of voltages, two protocols were used. For the membrane potential range from $-50$ mV (or $-100$ mV for $I_{K_{A}}$) to $-10$ mV, the neuron was prepulsed in 10-mV increments from the holding potential of $-80$ mV to the voltage of interest for 2.5 s for $I_{K_{V}}$ (2 s for $I_{K_{A}}$), before giving a test pulse to 0 mV. The peak current during the 0-mV test pulse was compared with the peak current in response to a 200-ms step from $-80$ to 0 mV for $I_{K_{V}}$ ($-100$ to 0 for 30 ms for $I_{K_{A}}$) with no preceding prepulse. For the membrane potential range of 0 to $+60$ mV, steady-state inactivation was directly measured from the decrease in current in response to the voltage steps used to measure activation. The steady-state voltage-dependent percent inactivation was measured at the end of a 5-s step to voltages ranging from 0 to $+60$ mV in 10-mV increments.

To determine the kinetics of the activation and inactivation of the outward currents, for voltages from $-20$ to $+60$ mV, the activation and inactivation kinetics of both $I_{K_{V}}$ and $I_{K_{A}}$ were derived directly from experiments in which the cell was stepped from the $-80$-mV holding potential to the relevant voltage. The activation kinetics of both currents at voltages more negative than $-20$ mV were estimated by measuring the deactivation of the channel. For the delayed rectifier, a conditioning voltage step to $+60$ mV was applied for 50 ms, followed by a step back to test values that ranged from $-20$ to $-100$ mV. For $I_{K_{A}}$, the procedure was identical except that the conditioning voltage step was maintained for 8 ms. The kinetics of removal of inactivation for both currents at voltages more negative than $-50$ mV were measured by maximally inactivating the conductance with a step from $-80$ to $+40$ mV for 50 ms for $I_{K_{V}}$ (8 ms for $I_{K_{A}}$), followed by a step of variable amplitude and duration. The steps ranged from $-50$ to $-120$ mV and varied over a range of durations: for $I_{K_{V}}$: 100, 200, 300, 400, 500, 1,000, 2,000 ms; for $I_{K_{A}}$: 10, 20, 50, 100, 500, 1,000, 2,000 ms. The voltage was then stepped to a test potential of $+40$ mV. The kinetics of inactivation-removal were determined by measuring the ratios of the peak currents measured at the test potential to those resulting from a step from $-80$ directly to $+40$ mV.

**Simulations**

Simulations were performed using Simulator for Neural Networks and Action Potentials (SNAP) (Ziv et al. 1994) Version 8.0 (snnap. uth.mmc.edu), which runs on JAVA, under Windows XP. The simulations contained a single B31/B32 soma and a B31/B32 axon. Parameters for the soma were obtained from the experimental data presented in the following text. Trial and error was used to fit the mean values derived from voltage-clamp experiments to standard equations provided by the SNAP program. Parameters for the axon were adjusted to cause it to respond with a pattern of spike-activity recorded in the soma similar to that recorded during a fictive buccal motor program. The soma and axon compartments were connected via a simulated electrical synapse. In some experiment, simulated current injections were performed. The wave form and amplitude of the current injections were designed to give rise to a voltage change in B31/B32 similar to that which occurs during a fictive buccal motor program. The amplitude of a series of small step depolarizations was varied by trial and error until the current injections gave rise to depolarization of B31/B32 with the desired amplitude and waveform.

**RESULTS**

The bilaterally symmetrical B31/B32 neurons are among a small group of protraction-phase neurons that are involved in deciding whether to initiate a buccal motor program that is a correlate of feeding behavior in an intact animal. Stimulating B31/B32 by current injection (Hurwitz et al. 1994; Susswein and Byrne 1988) initiates a buccal motor program. B31/B32 are electrically coupled to the bilaterally symmetrical cholinergic B63 neurons, from which they also receive excitatory chemical synaptic input (Hurwitz et al. 1997) (Fig. 1A). The stimulus for the program is generated by command-like neurons in the cerebral ganglion that monosynaptically excite
B31/B32, B63 and additional protraction phase neurons (Hurwitz et al. 2003; Jing and Weiss 2005; Rosen et al. 1991; Sanchez and Kirk 2000; Wu et al. 2003) (Fig. 1A). B31/B32 are also motor neurons. The B31/B32 axons excite the 12 muscles, the contraction of which makes a major contribution to radula protraction (Drushel et al. 1998; Hurwitz et al. 1994, 1996, 2000; Neustadter et al. 2007). During protraction, action potentials in the B31/B32 axons begin only after the initiation of the sustained depolarization of the soma (Hurwitz et al. 1994). The soma depolarization drives buccal motor programs, whereas the axon spikes cause one-for-one excitatory-junction potentials (EJPs) in the muscle (Hurwitz et al. 1996) that trigger muscle contraction (Hurwitz et al. 2000).

Characteristics of B31/B32 activity during buccal motor programs

B31/B32 activities before and during buccal motor programs were quantified. B31/B32 had a resting potential of −61.1 ± 1.1 (SE) mV (n = 17). Before a program was initiated, B31/B32 neurons displayed a gradual ramp depolarization, which ended with a sustained depolarization. The rate of depolarization was variable. In short-latency buccal motor programs (Fig. 1B) the ramp depolarization lasted from 1 to 3 s. In longer-latency programs, the ramp depolarization can last >10 s (Fig. 1C). During the ramp depolarization, B31/B32 displayed a series of fast depolarizations that arose from EPSPs, particularly from B63, but also from other neurons (Dembrow et al. 2003, 2004; Hurwitz et al. 1997). The EPSPs were also seen when B31/B32 was directly activated by injecting depolarizing current because B63 and other neurons driving B31/B32 are electrically coupled to B31/B32 and are thereby indirectly excited by depolarizing B31/B32 (Fig. 1, A and B). The EPSPs summed and gradually depolarized B31/B32 until a sustained depolarization was generated (Susswein et al. 2002). The sustained depolarization is a correlate of the protraction phase of a buccal motor program (Hurwitz et al. 1997). There were many small, fast depolarizations superimposed on the sustained depolarization. These are of heterogeneous origin. Some are axon spikes that fail to invade the soma (Hurwitz et al. 1994), whereas others are chemical and electrical EPSPs (Hurwitz et al. 1997). The mean membrane potential at the base of the sustained depolarization was −23.6 ± 1.8 (SE) mV and at the peak of the fast depolarizations was −15.3 ± 1.7 mV. Thus the mean amplitude of the fast depolarizations superimposed on the sustained depolarization was 8.3 ± 0.6 mV (n = 17).

Currents in untreated ganglia

Inward and outward currents. Currents underlying B31/B32 activity were examined under voltage-clamp conditions. In neurons held at −60 mV, voltage steps were applied every 30 s in 10-mV increments from −50 to 0 mV (Fig. 2A). These parameters cover the physiological range of the voltage changes during the protraction phase of the fictive buccal motor program.

Depolarizing voltage steps to −30 mV and above induced inactivating outward currents (Fig. 2B), which reached amplitudes of ≥300 nA at 0 mV. The currents declined within 3–4 s. Many current traces also showed fast inward transients of ≥10 nA in response to larger depolarizing voltage steps. These presumably reflect action potentials in the axon that fail to invade the soma.

Many current traces also displayed (Fig. 2B) slow inward deflections, followed by outward deflections, superimposed on the outward currents. The inward deflections were 10–30 nA in amplitude and 2–4 s in duration. They were followed by outward deflections of 20–60 nA (Hurwitz et al. 2005) that were maintained for 1–3 s. The inward-outward current sequences reflect currents that contribute to buccal motor programs in an unclamped B31/B32 (see Fig. 1) in which a sustained depolarization of B31/B32 is followed by repolarization. The buccal motor programs were generated in membrane regions that were not under good voltage-clamp control: they occurred at a variety of holding potentials and at variable latencies and were of variable duration, suggesting that they are not endogenous to B31/B32.

Currents after synaptic transmission is blocked

A 60 μM concentration of tetrodotoxin (TTX) was used to eliminate spiking in neurons presynaptic to B31/B32 and thereby eliminate currents caused by chemical synaptic transmission as well as eliminating B31/B32 axon spikes. In Aplysia and other gastropods, this concentration blocks fast voltage-gated Na+ channels and action potentials (Geduldig and Grünewer 1970; Gilly et al. 1997). TTX would not eliminate currents originating in electrically coupled cells or currents caused by transmitter released from presynaptic neurons as a result of TTX-insensitive inward currents.

In addition to eliminating the fast inward transients arising from axon spikes, TTX also blocked the slow inward currents
(n = 34), which presumably generate the sustained depolarizations in unclamped B31/B32 cells (Fig. 2C). These data are consistent with previous suggestions (Dembrow et al. 2004; Hurwitz et al. 1999a) that the sustained depolarization in B31/B32 is initiated by excitatory synaptic input.

Subtracting currents recorded in TTX from those in its absence provided a more detailed view of the inward-outward current sequences underlying a buccal motor program (Fig. 2D). These recordings showed that inward currents gradually increase in amplitude over 2–3 s and are maintained at a relatively constant value for a number of seconds before being terminated by an outward current that presumably arises via B64 activity.

In contrast to the effects of TTX on buccal motor programs, the inactivating outward currents were minimally affected by TTX (Fig. 2C), indicating that these are likely to be endogenous to B31/B32. Treatment with TTX also revealed the presence of an additional outward current in response to voltage steps to −10 and 0 mV after the early currents had inactivated. This current displayed an amplitude of 10–20 nA at 0 mV, and it showed no inactivation. In quiescent preparations showing relatively few buccal motor programs, the high-threshold noninactivating current was also seen without TTX treatment (not shown).

PHARMACOLOGY OF THE OUTWARD CURRENTS. In Aplysia, delayed rectifier K⁺ currents (I_{K-V}) are selectively blocked by TEA, whereas fast inactivating K⁺ currents (A-currents- I_{K-A}) are blocked by 4-AP (Byrne 1980a; Furukawa et al. 1992; Ma and Koester 1995). Both agents affected the recorded currents (TEA: n = 11; 4-AP: n = 6), indicating that the inactivating outward current is a mixture of a delayed rectifier and an A-current. In the presence of 40 mM TEA (Fig. 3A, 1 and 2), the peak current at 0 mV was reduced in amplitude, shifted somewhat earlier, and displayed a more rapid inactivation. I_{K-V} was isolated by subtracting the currents in 40 mM TEA from those in its absence (Fig. 3A). Similarly, I_{K-A} could be examined by subtracting currents in the presence of 10 mM 4-AP from those recorded without 4-AP (Fig. 3B). A third outward current, which showed no inactivation (herein named the maintained outward current), was unaffected by either TEA or 4-AP (see Figs. 2C and 4B). Applications of progressively larger doses of TEA and of 4-AP showed that the effects of TEA on the delayed rectifier approached saturation at concentrations between 20 and 40 mM, and the effects of 4-AP on the A-current reached saturation at concentrations of between 4 and 8 mM (Fig. 4A). The maintained outward current was observed when both I_{K-V} and I_{K-A} were blocked by TEA plus 4-AP.

KINETICS. The kinetics of the three outward currents were compared by placing on the same time-scale examples of the three currents in response to a voltage step from −60 to 0 mV (Fig. 4B). The A-current and the delayed rectifier were isolated by subtraction of currents in the presence of 4-AP and TEA, respectively, from control currents. The maintained current was isolated by inactivating I_{K-A} and I_{K-V} with a 3-s prepulse to −20 mV (which is just threshold for activating the maintained current—see following text) and then stepping to 0 mV. The time to the maximal activation of I_{K-A} was <10 ms. In contrast, the times to the maximal activation of both I_{K-V} and the maintained current were ~70 ms. Inactivation of the A-current led to a 90% decrease of peak I_{A} amplitude in <1 s, whereas inactivation of the delayed rectifier led to a 90% decrease in ~2.5 s (Fig. 3, A3 and B3). The maintained current displayed no inactivation in any experimental conditions.

**Quantifying the two inactivating outward currents**

A series of experiments was performed to quantify the reversal potentials as well as the time and voltage dependencies of the outward currents. All experiments were performed in TTX. The difference between runs with and without 40 mM TEA provided data on properties of the delayed rectifier. I_{K-A} was measured by adding 10 mM 4-AP to the bath that already contained TTX and TEA, and subtracting the values obtained with 4-AP from those in its absence. This protocol was used because 4-AP partially blocks I_{K-V}, and applying 4-AP before I_{K-V} is already blocked would over-estimate the contribution of I_{K-A}.

REVERSAL POTENTIALS. Tail current analysis was used to determine the reversal potentials of the delayed rectifier and the A-current. V_m was stepped from a holding potential of −80 mV to a conditioning pulse at 0 mV and then to a test pulse that varied in 10-mV increments from −90 to 0 mV. The potential at which the peak tail current (extrapolated to the start of the test pulse) went to zero was used to estimate the reversal potential of the current. The reversal potential of the A-current and the delayed rectifier were estimated in the presence of TEA and 4-AP, respectively. The conditioning pulse durations were 20 ms for the A-current and 150 ms for I_{K-V}. I_{K-A} reversed at −85 mV, whereas I_{K-V} reversed at −70 mV. The difference may arise because the I_{K-V} channel may be less selective to K⁺ ions that is the
I$_{K-A}$ channel (e.g., Bekkers et al. 2000). A second possibility is that the electrode is likely to slowly leak K$^+$ ions. Because 4-AP was applied on top of TEA, the reversal potential of I$_{K-A}$ is measured after that of I$_{K-V}$, perhaps causing a more negative apparent reversal potential for the 4AP-difference current. The reversal potential of the maintained outward current was also determined by tail-current analysis. Instead of using drugs, causing a more negative apparent reversal potential for the membrane potential because a Hodgkin-Huxley model using 3 activation and 2 inactivation variables (A and B) for I$_{K-A}$ are $-8$ and 25%, respectively, of their maximum values. As a result there is a significant I$_{K-A}$ window current during the sustained depolarization.

**TABLE 1. Values derived from the voltage-clamp experiments used to fit the data of the steady-state activation and inactivation to the graphs illustrated in Fig. 5**

<table>
<thead>
<tr>
<th>Current</th>
<th>$h$, mV</th>
<th>$s$</th>
<th>$p$</th>
<th>$h$, mV</th>
<th>$s$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I$_{K-V}$</td>
<td>6</td>
<td>14</td>
<td>1</td>
<td>$-31$</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>I$_{K-A}$</td>
<td>$-14$</td>
<td>20</td>
<td>3</td>
<td>$-30$</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>I$_{\text{maintained}}$</td>
<td>19</td>
<td>11</td>
<td>1</td>
<td>10000</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

For steady-state activation, $SS_A = 1/(1 + e^{hV-Vp})$. For steady-state inactivation, $SS_h = 1/(1 + e^{hV-Vs})$, where $V$ is the membrane potential (in mV), $h$ is the membrane potential at half-activation or half-inactivation when $p = 1$, $s$ is the slope, and $p$ is the exponent to which the expression is raised.

**FIG. 4.** Concentration dependence of blocking drugs and kinetics of the outward currents. A: difference currents recorded in response to a voltage step from a holding potential of $-60$ to $0$ mV in the presence of various concentrations of TEA (top) or 4-AP (bottom). The traces shown were obtained by subtracting the currents measured with the drugs from the currents measured in their absence. B: kinetics of the 3 outward currents as initiated by a voltage step to 0 mV. Two examples of the delayed rectifier are shown to display the variability. The A-current and the delayed rectifier were obtained via subtraction in 4-AP and TEA, respectively. The maintained current was obtained by preinactivating the other currents via a 3-s prepulse to $-20$ mV.

**FIG. 5.** Steady-state voltage-dependent activation (SS$_A$; •) and inactivation (SS$_h$; □) parameters for the conductances underlying the 3 voltage-gated outward currents. A: percent activation and inactivation for the delayed rectifier conductance. B: percent activation and inactivation for the A-current conductance. Note that the half-inactivation in Table 1 is shifted to more negative values of the membrane potential because a Hodgkin-Huxley model using 3 activation gates was used. Inset: percent activation for the maintained current over the range of voltages for which data were gathered. The values for the A-current and the delayed rectifier are difference currents from preparations treated with TEA or 4-AP, respectively, with the leakage current and the maintained current subtracted. Means ± SE are shown (in many cases error bars are obscured by the point displaying the mean). For the A-current, for each point in the SS$_A$ curve the average number of measurements is 4 (range: 2–6); for the delayed rectifier, for each point in the SS$_h$ curve the average number of measurements is 9 (range: 3–14); for SS$_h$ each point has 4 measurements; for the maintained current $n = 8$. For both inactivating outward currents, data were fit by the equations in Table 1.

The calculated mean $g_{\text{max}}$ of the delayed rectifier was 15 $\mu$S. Measured parameters for the steady-state activation and inactivation were fit to the values and equations in Table 1 and plotted in Fig. 5. The delayed rectifier conductance showed little activation at voltages below $-40$ mV and little inactivation at voltages below $-50$ mV (Fig. 5A). It was half-activated at a voltage of $+6$ mV and half-inactivated at a voltage of $-31$ mV. It was $\sim 13\%$ activated at $-20$ mV, the voltage of the sustained depolarization in B31/B32. At this voltage the steady-state inactivation was $>80\%$ (i.e., $h < 0.2$). Thus I$_{K-V}$ will be active during the ramp depolarization preceding the sustained depolarization (see Fig. 1) and will display a window of activation during the sustained depolarization.

The calculated mean $g_{\text{max}}$ of I$_{K-A}$ was 9.5 $\mu$S. Measured values were fit to the equations and parameters in Table 1 as illustrated in Fig. 5B. Threshold for the steady-state activation of I$_{K-A}$ was $-40$ mV (Fig. 5B). The A-current conductance was half-activated at $+12$ mV. Unlike the delayed rectifier, the A-current conductance displayed some inactivation even at $-80$ mV. It was half-inactivated at $-30$ mV. At $-20$ mV, the voltage of the sustained depolarization, the activation and inactivation variables (A and B) for I$_{K-A}$ are $-8$ and 25%, respectively, of their maximum values.
TABLE 2. Values derived from the voltage-clamp experiments used to fit the data on the time dependence (τ) of activation and inactivation to the graphs illustrated in Fig. 6

<table>
<thead>
<tr>
<th>Current</th>
<th>τ_{max}, s</th>
<th>τ_{min}, s</th>
<th>h_{1}, mV</th>
<th>s_{1}, mV</th>
<th>p_{1}, mV</th>
<th>h_{2}, mV</th>
<th>s_{2}, mV</th>
<th>p_{2}, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_{K-V}</td>
<td>0.014</td>
<td>0.003</td>
<td>20</td>
<td>7</td>
<td>1</td>
<td>-15</td>
<td>-6</td>
<td>1</td>
</tr>
<tr>
<td>I_{K-A}</td>
<td>0.02</td>
<td>0.000275</td>
<td>-88</td>
<td>30</td>
<td>1</td>
<td>-50</td>
<td>-5</td>
<td>1</td>
</tr>
<tr>
<td>I_{mained}</td>
<td>0.014</td>
<td>0.003</td>
<td>20</td>
<td>7</td>
<td>1</td>
<td>-15</td>
<td>-6</td>
<td>1</td>
</tr>
<tr>
<td>I_{K-V}</td>
<td>0.875</td>
<td>0.11</td>
<td>-15</td>
<td>6</td>
<td>1</td>
<td>-73</td>
<td>-9</td>
<td>1</td>
</tr>
<tr>
<td>I_{K-A}</td>
<td>0.33</td>
<td>0.0008</td>
<td>-80</td>
<td>70</td>
<td>1</td>
<td>-56</td>
<td>-16</td>
<td>1</td>
</tr>
</tbody>
</table>

The data are fitted to the following equation: τ = τ_{max} - τ_{min}/(1 + e^{V - V_1}/h_1)^{p_1}(1 + e^{V - V_2}/h_2)^{p_2} + τ_{min}, where τ_{max} and τ_{min} are the maximal and minimal time constants, V is the voltage, s_{1} and s_{2} are the slopes of the ascending and descending arm of the function, and h_{1} and h_{2} are the voltages of the half points of the ascending and descending arm of the function, and p_{1} and p_{2} are the exponents to which the expressions are raised. The measured values for I_{K-V} were used to calculate the activation kinetics of I_{mained}, because the two currents had similar activation kinetics (see Fig. 4B). I_{mained} did not display inactivation.

FIT OF OUTWARD CURRENTS. The formulas for the 3 outward currents in Tables 1–3 were used to simulate outward currents in a series of simulated voltage clamps, using the SNAPP simulation package (Ziv et al. 1994). Simulated currents (Fig. 7) were qualitatively similar to those observed experimentally, confirming that the values used provide a reasonable reconstruction of the currents in B31/B32.

TABLE 3. Parameters used, along with those shown in Tables 1, 2, 4, and 5, to generate the simulations

<table>
<thead>
<tr>
<th>Neuron</th>
<th>C_{ion} μF</th>
<th>Current</th>
<th>E_{rev} mV</th>
<th>g_{max} μS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>B31 soma</td>
<td>0.011</td>
<td>I_{K-V}</td>
<td>-70</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>B31 soma</td>
<td>0.011</td>
<td>I_{K-A}</td>
<td>-85</td>
<td>9.5</td>
<td>1</td>
</tr>
<tr>
<td>B31 soma</td>
<td>0.011</td>
<td>I_{mained}</td>
<td>-35</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>B31 soma</td>
<td>0.011</td>
<td>g_{leak}</td>
<td>-60</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>B31 axon</td>
<td>0.003</td>
<td>I_{Na}</td>
<td>-70</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>B31 axon</td>
<td>0.003</td>
<td>I_{K}</td>
<td>-70</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>B31 axon</td>
<td>0.003</td>
<td>g_{leak}</td>
<td>-60</td>
<td>0.0012</td>
<td></td>
</tr>
</tbody>
</table>

E_{rev} is the reversal potential for a channel, g_{max} is the maximal conductance, and p is exponent to which a function describing a current is raised (see equation in the following text). The B31/B32 soma and axon were each modeled as single, isopotential compartments that are electrically coupled to one another via g_{leak}. The parameters for the soma are derived from the voltage-clamp experiments. The parameters for the axon are not based on data. They were devised to create action potentials recorded in the soma similar to those recorded in the cell. The equivalent electrical circuit for each compartment consisted of a membrane capacitance (C_{m}) in parallel with a leakage conductance (g_{leak}) with its associated equilibrium potential (E_{leak}) and time-dependent conductances with their associated equilibrium potentials. E_{leak} was set at -60 mV, close to the resting potential of B31/B32. g_{leak} was derived from voltage-clamp steps that were subthreshold for activating the active currents. The membrane potentials (V_{m}) of the soma and axon compartment were given by the differential equation: dV_{m}/dt = (\Sigma I_{ion} + \Sigma I_{stim}) - I_{leak}, where C_{m} is the membrane capacitance of a given compartment, \Sigma I_{ion} is the sum of the ionic currents in that compartment, \Sigma I_{stim} is the sum of the electrical coupling currents in that compartment, and I_{stim} is an extrinsic stimulus, which can be applied to a compartment. Each ionic current was obtained by solving the equation I_{ion} = g_{ion}(v(t), v(t)) (V_{m} - E_{ion}), where g_{ion}(v(t)) is the voltage- and time-dependent conductance and E_{ion} is the reversal potential associated with each current. g_{ion}(v(t)) was represented by a Hodgkin-Huxley-type formulation and each ionic conductance was evaluated by solving the general equation: g_{stim} = g_{max} h_{stim} A_{min}(v(t)) B_{stim}(v(t)) (V_{m} - E_{ion}), where g_{stim} is the maximal value of g_{stim}, A_{min}(v(t)), B_{stim}(v(t)), and (V_{m} - E_{ion}) are functions describing the voltage- and time-dependent activation and inactivation, respectively, associated with g_{stim}. p is the power to which A_{stim} was raised. V_{m} is the membrane potential, and E_{ion} is the reversal potential for a given ionic conductance. A and B parameters for B31/B32 were those shown in Tables 1 and 2. A and B parameters for the axon are shown in Table 4. g_{Na} soma to axon 0.075 μS, g_{K} axon to soma 0.4 μS.
Muscarinic inward current

A previous study (Dembrow et al. 2004) suggested that the sustained depolarization in B31/B32 arises via muscarinic transmission. These authors bathed the buccal ganglia in the muscarinic agonist oxotremorine (10^{-5} M) and applied slow ramp depolarizations, providing an estimate of the amplitude of inward currents produced by oxotremorine but not of their kinetics. We examined B31/B32 currents in response to voltage steps in the presence of oxotremorine, to determine the amplitude and time course of these currents (Fig. 8A).

DEPOLARIZING VOLTAGE STEPS INDUCE INWARD CURRENTS. Currents dependent on oxotremorine were examined by subtracting currents measured in response to voltage steps in the presence of oxotremorine (2 × 10^{-5} M) from those in its absence in ganglia that were pretreated with TTX. This concentration was used because we found that 2 × 10^{-5} M was somewhat more effective than the concentration used in previous experiments by Dembrow et al. 2004 (10^{-5} M). Depolarizing steps induced both outward and inward currents (Fig. 8A).

During a voltage step, the amplitude of the oxotremorine-dependent inward current gradually increased (Fig. 8A) and then remained constant for as long as the voltage step was maintained. The time course for the activation of the inward currents is most evident in response to voltage pulses to −50, −40, and −30 mV. Voltage pulses of larger magnitude elicited early outward currents (see following text) which obscure the early portion of the inward current. The mean time to reach the maximal inward current amplitude was 2.8 ± 1.7 (SE) s (n = 5). There was no systematic change in the time to reach maximal net inward current amplitude as the size of the voltage steps was increased.

Data on the amplitude of the inward currents at the end of the voltage step were consistent with those of Dembrow et al. (2004). The peak amplitude grew as the amplitude of a voltage step was increased from −60 to −30 mV, reaching maximal amplitude of approximately −20 nA. The current amplitude then declined in response to steps of −20 and −10 mV. The estimated reversal potential of the net oxotremorine-dependent current at the end of the step was approximately −2 mV, and
a step to 0 mV induced a net outward current (Fig. 8B). These data suggest that the channels through which the inward currents travel have increasing open probabilities as the voltage steps increase in amplitude from −50 to −30 mV. Beyond −30 mV, the current amplitude decreases as its driving force decreases. The apparent reversal potential is likely to be somewhat more positive than the actual value because in these experiments neurons adjacent to the recorded cells were not killed. The B31/B32 neurons are electrically coupled to one another and to additional neurons (Susswein and Byrne 1988), further reducing the possibility of a good space clamp.

AMPLIFICATION OF THE INACTIVATING OUTWARD CURRENT. In addition to initiating an inward current, oxotremorine also produced an increase of a voltage-dependent, inactivating outward current (Fig. 8, A and C). At voltage steps to 0 mV, the oxotremorine dependent outward current reached a peak value of 52.6 ± 7.6 (SE) nA (n = 5). This is a 17.3 ± 6.4% increase over the peak outward current (combined A-current and delayed rectifier) recorded in the same cells in the absence of oxotremorine.

To determine whether the A-current and/or the delayed rectifier was affected by oxotremorine, the ganglia were treated with TEA or 4-AP. However, these agents blocked both the amplification of the outward currents induced by oxotremorine as well as the oxotremorine induced inward currents (not shown). These findings are consistent with previous reports (e.g., Adler et al. 1979; Brezina 1988a,b; Kehoe 1972) that K+ channel blockers also block ACh receptors.

Although we could not determine experimentally whether oxotremorine modulates $I_{K-V}$ or $I_{K-A}$, we were able to simulate the modulation by increasing the $g_{\text{max}}$ of $I_{K-V}$ from 15 to 18 $\mu$S, suggesting that such a modulation could underlie the effect of oxotremorine (data not shown).

Preliminary experiments examined the effects of different concentrations of oxotremorine on B31/B32. The threshold for activation of an inward current was $10^{-5}$ M. As the concentration was increased to $5 \times 10^{-4}$ M, the effects of oxotremorine on outward currents became progressively larger, and effectively masked the inward currents.

OXOTREMORINE-DEPENDENT DEPOLARIZATIONS IN THE PRESENCE OF TTX. How large are the voltage changes produced by an oxotremorine-dependent inward current? To examine this point, the effect of $2 \times 10^{-5}$ M oxotremorine was examined under current-clamp conditions when synaptic activity was blocked by TTX (Fig. 8D). In this condition, a brief depolarization induced a sustained depolarization of −20 mV from rest. The depolarization was maintained for well after the cessation of the stimulus, confirming that plateau-like potentials are observed in the presence of oxotremorine. The mechanisms underlying the waveform and the regenerative properties of the oxotremorine-dependent plateau-like depolarizations were not examined.

Contribution of inward and outward currents to B31/B32 activity

What are the amplitudes and waveforms of inward currents required to produce B31/B32 activity similar to that before and during a buccal motor program, against the background of the outward currents in the B31/B32 soma? Are inward currents induced by oxotremorine at the concentrations used by us and by Dembrow et al. (2004) sufficient to depolarize B31/B32 to the extent that it is depolarized during a buccal motor program? How do the outward currents in the B31/B32 neurons affect the activity of the neuron before and during a buccal motor program? To answer these questions, B31/B32 was simulated using the data described in the preceding text for the outward currents. The activity of B31/B32 during a buccal motor program was simulated by injecting depolarizing currents into the simulated neuron. The waveforms and amplitudes of the depolarizing currents were created specifically to produce depolarizations appropriate to those seen during buccal motor programs in B31/B32. This procedure may provide a small overestimate of the inward currents required to drive B31/B32 because it does not take into account the increase in conductance that results from opening channels that allow the flow of inward currents. However, a simulation driven by the application of a current injection devised to cause a depolarization similar to that caused during a buccal motor program has the advantage of making no assumptions about the kinetics or the biophysical properties of the processes underlying the inward currents, which are as yet incompletely characterized.

The SNNAP simulation package was used to create a simplified model of a B31/B32 neuron containing a soma with a leak current based on that measured in B31/B32, as well as the currents in the absence of oxotremorine. The effects of inward currents on the activity of the neuron before and during a buccal motor program were modeled by simulating direct injection of different current waveforms into the soma.

OUTWARD CURRENTS IN B31/B32 DO NOT CAUSE THE DELAY IN ACTIVITY. A series of square pulse inward currents were applied to the simulated neuron, to determine the current amplitude needed to depolarize the neuron to −20 mV, the level of the sustained depolarization during the protraction phase. This pulse was found to be −55 nA in amplitude (Fig. 9). In response to this stimulus, the simulated neuron was immedi-

### Table 4. Parameters for steady-state activation and inactivation of time- and voltage-dependent currents in the axon

<table>
<thead>
<tr>
<th>Current</th>
<th>$h$, mV</th>
<th>$s$</th>
<th>$p$</th>
<th>$h$, mV</th>
<th>$s$</th>
<th>$p$</th>
<th>$B_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{K_a}$</td>
<td>−35</td>
<td>4.8</td>
<td>1</td>
<td>−52</td>
<td>9.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>$I_k$</td>
<td>−26</td>
<td>8.8</td>
<td>1</td>
<td>−16</td>
<td>1.5</td>
<td>2</td>
<td>0.15</td>
</tr>
</tbody>
</table>

As noted, the parameters for the axon are not based on data. They were devised to create action potentials recorded in the soma similar to those recorded in the cell. As for the soma, $SS_A$ for $I_{K_a}$ and $I_k$ were derived using the formula: $SS_A = h/(1 + e^{(h-V_a)\gamma})$. The formula used for $SS_B$ of $I_{K_a}$ was: $SS_B = 1/(1 + e^{(h-V_b)\gamma})$. For $SS_B$ of $I_k$, the following formula was used: $SS_B = 1 - B/h/(1 + e^{(h-V_b)\gamma}) + B_n$. 

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activity that is observed preceding a buccal motor program. A
rents in B31/B32 cannot account for the delay in B31/B32
indicates that activation and inactivation of the outward cur-
to establishing this delay. However, the simulation in Fig. 9
Fig. 1), the inactivating currents in B31/B32 could contribute
currents stabilizing membrane potentials become inactivated.
response to an excitatory stimulus only after the outward
contribute to a delay in activity in other systems (Byrne 1982;
simulations described in the following text.
this modulation was relatively small, it was not included in the
simulated neuron was stimulated with a depo-
SIMULATION OF OXOTREMORINE-DEPENDENT CURRENTS. How
would the inward current activated by oxotremorine affect
B31/B32? The simulated neuron was stimulated with a depo-
larizing current similar in amplitude to the oxotremorine-
dependent inward current at −20 mV (20 nA) with a waveform
similar to that for smaller voltage steps (−50 to −30 mV) in
Fig. 8A (Fig. 10). This stimulus produced a gradual depolar-
over −4 s, which was followed by a sustained depolar-
approximately −40 mV (Fig. 10). Although the
time course of slow depolarization was similar to that seen in

As noted in the preceding text, the parameters for the axon are not based on data. They were devised to create action potentials recorded in the soma similar
to those recorded in the cell. Time-dependent activation used the equations shown in the preceding text for the soma. Inactivation was described using a single
exponent equation: \( \tau = \tau_{max} - \tau_{min}(1 + e^{\frac{-20}{s}})p + \tau_{min} \).
\( I_{Na} \) and \( I_{K} \) are denoted by \( h_{Na} \) and \( h_{K} \), respectively. The steps of
inward currents sufficient to depolarize B31/B32 to
−20 mV caused an immediate depolarization of the neuron and
firing in the axon. Activation and inactivation of the outward
currents modulated the amplitude of the depolarization during
the first 5 s but did not create a delay. This simulation, plus the
data in Figs. 2D and 8A showing a slow activation of inward
currents, indicates that the delay is likely to arise as a result of the slow activation of inward currents rather than from the
activation and inactivation of outward currents.

| Parameters used in the simulation for time-dependence of activation and inactivation of active conductances in the axon |
|---|---|---|---|---|---|
| \( \tau_{A_{\text{max}}}, \text{ms} \) | \( \tau_{A_{\text{min}}}, \text{ms} \) | \( h_{1A}, \text{mV} \) | \( s_{1A}, \text{mV} \) | \( p \) | \( h_{2A}, \text{mV} \) | \( s_{2A}, \text{mV} \) | \( p \) |
| \( I_{Na} \) | 0.0015 | 0 | −32 | 1.85 | 1 | −51 | −11.2 | 1 |
| \( I_{K} \) | 0.027 | 0 | −24 | 11.7 | 1 | | |

| Parameters used in the simulation for time-dependence of activation and inactivation of active conductances in the axon |
|---|---|---|---|---|---|
| \( \tau_{B_{\text{max}}}, \text{ms} \) | \( \tau_{B_{\text{min}}}, \text{ms} \) | \( h_{B}, \text{mV} \) | \( s_{B}, \text{mV} \) | \( p \) |
| \( I_{Na} \) | 0.1 | 0.002 | −39.2 | 3.5 | 1 |
| \( I_{K} \) | 0.2 | 0.02 | −24 | 8.3 | 1 |

As noted in the preceding text, the parameters for the axon are not based on data. They were devised to create action potentials recorded in the soma similar
to those recorded in the cell. Time-dependent activation used the equations shown in the preceding text for the soma. Inactivation was described using a single
exponent equation: \( \tau = \tau_{max} - \tau_{min}(1 + e^{\frac{-20}{s}})p + \tau_{min} \).
\( I_{Na} \) and \( I_{K} \) are denoted by \( h_{Na} \) and \( h_{K} \), respectively. The steps of
inward currents sufficient to depolarize B31/B32 to
−20 mV caused an immediate depolarization of the neuron and
firing in the axon. Activation and inactivation of the outward
currents modulated the amplitude of the depolarization during
the first 5 s but did not create a delay. This simulation, plus the
data in Figs. 2D and 8A showing a slow activation of inward
currents, indicates that the delay is likely to arise as a result of the slow activation of inward currents rather than from the
activation and inactivation of outward currents.

**Table 5.** Parameters used in the simulation for time-dependence of activation and inactivation of active conductances in the axon.
The inward current was then maintained at an amplitude of approximately $-56 \text{nA}$. The large increase in inward current required at the start of a short-latency program reflects the relatively slow inactivation kinetics of the delayed rectifier.

**CONTRIBUTION OF OUTWARD CURRENTS TO PROGRAMS.** How do the three active outward currents affect B31/B32 activity in fast and slow buccal motor programs? Do they affect the ramp depolarization or do they also affect the sustained depolarization? To determine the contribution of the outward currents to B31/B32 activity we examined the currents flowing through each channel during both short- and long-latency programs. We also selectively removed each of the currents (Fig. 13) to observe how these treatments affected B31/B32 activity.

Both $I_{K,V}$ and $I_{K,A}$ were activated during the ramp leading to the sustained depolarization, during both the slow and fast motor programs. Both currents also remained activated during the sustained depolarization throughout both slow and fast programs. The activity of these currents during the sustained depolarization was explained by their lack of complete inactivation at $-20 \text{ mV}$, allowing them to contribute to the sustained depolarization. Removal of either current produced a more rapid ramp depolarization, and also increased the sustained depolarization to $-0 \text{ mV}$ (Fig. 13), confirming that both currents contribute to shaping the ramp depolarization and the sustained depolarization.

Currents carried by $I_{K,V}$ were different during long- and short-latency motor programs. During long-latency programs, the inactivation of the delayed rectifier was relatively slow, and therefore a gradually increasing inward current could be applied until reaching the sustained depolarization. By contrast, during a short-latency program inward currents larger than $-20 \text{ mV}$ were required at the start of a short-latency program to drive B31/B32 preceding and during a buccal motor program. The stimulus elicits small increases in conductance of the delayed rectifier and the A-current.

**WAVEFORM AND AMPLITUDE OF INWARD CURRENTS DRIVING B31/B32 ACTIVITY.** The data in the preceding text (see Fig. 9) indicate that the delay in B31/B32 activity cannot be explained by the inactivating outward currents and is likely to be explained by a slow activation of inward currents. We examined the waveform and amplitude of inward currents that are required to drive B31/B32 preceding and during a buccal motor program by injecting a variety of currents into the simulated B31/B32 soma and observing the response. Stimuli were devised that were appropriate for eliciting B31/B32 activity patterns similar to those during both long- and short-latency buccal motor programs (see Fig. 1, B and C). Parameters were chosen to provide delays and ramp depolarizations similar to those in long- and short-latency fictive protractions, a sustained depolarization to approximately $-20 \text{ mV}$, and axon firing at $-20 \text{ Hz}$ that is expressed in the soma as spikes $-10 \text{ mV}$ in amplitude.

To simulate a long-latency buccal motor program, progressively more inward current had to be applied over $\sim 8 \text{ s}$. The inward current was then maintained at an amplitude of $-55 \text{nA}$ (Fig. 11). To create a short-latency buccal motor program, the inward current had to be applied more rapidly, over $\sim 5 \text{ s}$, reaching a peak of $-70 \text{nA}$ (Fig. 12). After $\sim 1 \text{ s}$, the inward current was then gradually reduced and was maintained at approximately $-56 \text{nA}$. The large increase in inward current required at the start of a short-latency program reflects the relatively slow inactivation kinetics of the delayed rectifier.

**FIG. 11.** Contribution of the 3 voltage-dependent outward currents to a long-latency buccal motor program. As in the preceding figure, B31/B32 was simulated with the 3 voltage-dependent outward currents and a leak current. Depolarization similar to that underlying a long-latency buccal motor program in B31/B32 was created by stimulating B31/B32 with an appropriate inward current. The long-latency program is characterized by a relatively slow ramp depolarization ($-6 \text{ s}$) and a sustained depolarization that is maintained for a relatively long time ($-10 \text{ s}$). The figure shows the depolarizing stimulus injected, and 3 voltage-dependent outward currents, as well as the voltage changes in the B31/B32 soma. The stimulus current gradually increases over $-8 \text{ s}$ and then remains at a constant value of $-55 \text{nA}$ throughout the sustained depolarization. The delayed rectifier current and the A-current flow during the ramp depolarization as well as during the sustained depolarization.
those during the sustained depolarization were needed to overcome the currents carried by $I_{K-V}$ and achieve sufficiently fast depolarization of B31/B32. As $I_{K-V}$ became inactivated, the current required to depolarize B31/B32 lessened. Removal of the maintained current produced very small (1–2 mV) increases in the amplitude of the sustained depolarization of B31/B32 (Fig. 13) as would be expected from a current that is just becoming activated at the −20 mV potential of the sustained depolarization.

CONTRIBUTION OF OUTWARD CURRENTS TO THE SUSTAINED DEPOLARIZATION. Inward currents acting on B31/B32 during the sustained depolarization operate against a background of a leak current plus three active outward currents. To depolarize B31/B32 to −20 mV, how much inward current is needed to overcome the effect of each of the four outward currents? To examine this question, a series of simulations similar to those in Fig. 9 were run. These examined the amplitude of the inward current required to depolarize B31/B32 to −20 mV in response to a rectangular current pulse, in the presence of different combinations of outward currents. In the presence of only the leak current, an inward current of 21 nA was sufficient to depolarize B31/B32 to −20 mV. The addition of $I_{K-V}$ to the leak required an additional 17 nA of inward current to cause a −20-mV depolarization. The addition of $I_{K-A}$ alone to the leak current required an additional 13 nA of inward current to achieve a depolarization to −20 mV. Inward currents required to depolarize B31/B32 when any two of the three outward currents were present, or when all three of the outward currents were included in the simulation, were consistent with the effects of each current alone. Thus the effects of the four outward currents on the sustained depolarization are additive. This stems from the fact that the outward currents contribute to the sustained depolarization after a few seconds, when their fast activation and inactivation have already occurred, and what remains are window currents that are relatively time insensitive. The relative contributions of the outward currents to B31/B32 depolarization would be difficult to assess during the earlier portions of the square pulse, since their amplitudes are changing. The leak current provides ∼40% of the outward current against which inward currents act, $I_{K-V}$ provides 32%,

![Fig. 12](http://jn.physiology.org/)

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Inward currents are dependent on synaptic transmission. Blocking spiking using TTX, and thus synaptic transmission by neurons that provide synaptic input to the B3/B32 neurons, blocked the B31/B32 sustained depolarization (Fig. 2), indicating that the underlying currents are not endogenous to B31/B32. This confirms previous findings by Hurwitz et al. (1999a) and by Dembrow et al. (2004) that TTX blocks the expression of a buccal motor program in B31/B32. In contrast, endogenous plateau potentials that underlie the sustained depolarization and firing of B64, a major retraction-phase interneuron, are not blocked by blocking synaptic transmission (Hurwitz and Susswein 1996). This is also true of plateau potentials in neurons that drive *Aplysia* respiratory pumping (Alevizos et al. 1989; Koester 1989). The finding that TTX blocks the sustained B31/B32 depolarization is somewhat surprising because depolarizing B31/B32 also depolarizes the electrically coupled B63, which might be expected to release transmitter onto B31/B32 and generate buccal motor programs even without spikes. Block of buccal motor programs by TTX suggests that the depolarization of B63 in TTX is not sufficient to release significant quantities of transmitter perhaps because the B63 to B31/B32 synapse may have a high-threshold for release of transmitter. This is consistent with previous data from *Aplysia* neurons showing that the threshold for presynaptic Ca\(^{2+}\) currents leading to transmitter release are only minimally activated at \(-20\) mV (Trudeau et al. 1993).

Inward currents arising via a number of mechanisms contribute to the depolarization of B31/B32 from its resting potential of \(-60\) mV to the sustained depolarization of \(-20\) mV. Spikes in B63 induce fast electrical EPSPs in B31/B32 (Hurwitz et al. 1997). In addition, fast facilitating cholinergic EPSPs that are blocked by hexamethonium are induced by firing B63 and B34 (Hurwitz et al. 2003). Release of ACh from B63 and B34 will also initiate inward currents dependent on muscarinic transmission. These currents are blocked by pirenzepine (Hurwitz et al. 1999a; Dembrow et al. 2004) and can be induced by oxotremorine, suggesting that they bind to receptors that are pharmacologically similar to M\(_{2}\) receptors in vertebrates (Cooper et al. 2003). Our data suggest that inward currents initiated by oxotremorine at the concentrations examined by Dembrow et al. (2004) and by ourselves can account for at most half of the depolarization of B31/B32 (Figs. 8–10) because the inward currents induced by oxotremorine are much smaller than those required to depolarize B31/B32 to \(-20\) mV. However, our studies and those of Dembrow et al. (2004) did not systematically examine a range of concentrations of the muscarinic agonist, and it is possible that sub-optimal concentrations were used. Preliminary experiments (unpublished) that examined concentrations ranging from \(10^{-7}\) M to \(5 \times 10^{-5}\) M oxotremorine found that inward currents are not seen at concentrations \(<10^{-5}\) M and that more concentrated solutions than those used by ourselves and Dembrow et al. (2004) induce larger outward currents at the expense of the inward currents, suggesting that the concentration used in our study (\(2 \times 10^{-5}\) M) may be close to producing the maximal response. Other transmitters may also contribute to the sustained B31/B32 depolarization. For example, B20 and B65 initiate buccal motor programs via release of dopamine and GABA, in part via their action on B31/B32 (Diaz-Rios and Miller 2005; Dias-Rios et al. 2002; Jing and Weiss 2002; Kabotyanski et al. 1998; Proekt et al. 2007; Teyke et al. 1993). In addition, a variety of peptides are released by cerebral-buccal interneurons (CBIs) presynaptic to protraction-phase interneurons (Koh et al. 2003; Morgan et al. 2000, 2002). In addition, during natural stimuli evoking a buccal motor program the slow effects of ACh on other protraction-phase neurons may add to
the direct effects on B31/B32 and thereby may cause additional depolarization.

The muscarinic receptor-generated current has features in common with those in other systems. The conductance is voltage-dependent, as has been shown in other systems (Fresschi and Livengood 1989; Klink and Alonso 1997; Trimmer 1994). The peak current is seen at ~30 mV and the reversal potential is approximately ~5 mV. The reversal potential is consistent with that of another voltage-dependent muscarinic current that is mediated by nonselective cation-permeant channels in rats (Haj-Dahmane and Andrade 1996).

Currents through transmitter-modulated, voltage-dependent conductances are also seen in other central pattern generators. In the lamprey locomotor system, glutamate-activation of reticulospinal and motor neurons via NMDA receptors leads to plateau depolarizations that are thought to be important at slow rates of locomotion (Grillner et al. 2001). In the lobster stomatogastric ganglion, muscarinic agonists allow depolarizing current pulses to initiate plateau depolarizations (Bul et al. 1994).

KINETICS OF THE INWARD CURRENTS DETERMINE THE DELAY. B31/B32 often responds to a depolarizing input after a delay. The delay could arise as a result of the slow activation of inward currents depolarizing B31/B32 or from the inactivation of endogenous voltage-gated K⁺ currents recruited by a stimulus, (e.g., Byrne 1982; Getting 1983). Our data indicate that the delay arises via the former mechanism and not the latter. Depolarization of B31/B32 from its rest potential is initially via fast cholinergic transmission from B63. This process requires summation and facilitation to produce a substantial depolarization of B31/B32 (Hurwitz et al. 2003; Sanchez and Kirk 2000), which requires time. When B31/B32 is sufficiently depolarized, a slow muscarinic inward current is activated. This had a slow rise time (~3 s - Fig. 8A, for voltage steps from ~50 to ~30 mV) that would also contribute to the delay. The slow activation of the muscarinic inward current probably stems from slow channel kinetics rather than from slow buildup in second-messenger concentrations. Because the experiments were performed in a constant concentration of oxotremorine, intracellular concentrations of second messengers initiated by oxotremorine were also presumably constant. A further demonstration that the delay is determined by the slow kinetics of inward currents rather than by the activation and inactivation of outward currents is provided by a simulation in which a B31/B32 neuron containing the inactivating outward currents was stimulated with a square-pulse of current that eventually produced a depolarization to ~20 mV (Fig. 9B). This stimulus elicited an immediate depolarization to ~30 mV and axon spiking.

Outward currents

Three separate outward currents were identified.

DELAYED RECTIFIER. The properties of the delayed rectifier in B31/B32 were similar to those previously described in other Aplysia neurons (Brezina et al. 1994; Byrne 1980a,b). I_{K, V} in Aplysia and in other mollusks displays prominent but fairly slow inactivation (Byrne 1980a,b; Connor and Stevens 1971; Thompson 1977). The classic function of I_{K, V} is to repolarize the membrane during an action potential (Hodgkin and Huxley 1952). In B31/B32 somata, I_{K, V} cannot have this function because the soma does not display fast action potentials. Inactivating outward currents can also cause a delay between an adequate stimulus and a response (e.g., Byrne 1982; Getting 1983). As noted in the preceding text, the delayed rectifier is unlikely to have this effect in B31/B32. The current is not activated until the neuron is depolarized to ~40 mV and beyond, whereas the membrane potential of B31/B32 is below this value during much of the delay. The delayed rectifier has two effects in B31/B32: when B31/B32 becomes sufficiently depolarized, the delayed rectifier slows the rate of depolarization and the delayed rectifier plays a role in braking the B31/B32 voltage at ~20 mV because I_{K, V} is only partially inactivated at this voltage (Fig. 7). Simulations in which the delayed rectifier is removed from B31/B32 showed a steeper depolarization preceding the sustained depolarization as well as a larger depolarization during the sustained depolarization (Fig. 13).

A-CURRENT. In many neurons, the steady-state activation and inactivation of A-currents is shifted to more hyperpolarized values with respect to the equivalent values of delayed rectifier currents (Brezina et al. 1994; Thompson 1977). In contrast, we found that the voltage-range of steady-state activation and inactivation of the A-current in B31/B32 was very similar to that of the delayed rectifier (Fig. 5). Three different A-currents, termed, I_{Afast}, I_{Aslow}, and I_{Adepol} have been described in Aplysia (Furukawa et al. 1992). The A-current in B31/B32 is not likely to be any of these. I_{Afast} and I_{Aslow} differ from one another in their kinetics. The A-current in B31/B32 displays kinetics similar to that of I_{Aslow}. However, I_{Adepol} is completely inactivated at voltages positive to ~50 mV, whereas the A-current in B31/B32 is only 20% inactivated (Fig. 5B) at this voltage. I_{Adepol} is activated and inactivated at more depolarized voltages, similar to those for the A-current in B31/B32. However, I_{Adepol} displays slower inactivation kinetics. In addition, I_{Adepol} is more sensitive to 4-AP than is the A-current in B31/B32: 1 mM 4-AP blocks I_{Adepol} (Furukawa et al. 1992) but produces only a 40% block of the B31/B32 current (Fig. 4A). Because B31/B32 has an A-current with relatively depolarized activation and inactivation kinetics, the current can be active when the neuron remains active at a sustained depolarization of ~20 mV. Even if the main effect of I_{K-A} were to regulate spike height or spike frequency, the spikes in B31/B32 ride on a sustained depolarization. More conventional A-currents would be completely inactivated at these potentials and would contribute little to regulation of the neuron activity. One may predict that I_{K-A} and I_{K-V} in other Aplysia neurons that display plateau-like potentials would also display voltage-dependent activation and inactivation at values that are relatively depolarized. Preliminary studies on neuron B64, which also displays a plateau potential (Hurwitz and Susswein 1997), revealed an A-current similar to that in B31/B32 (Saada 2006).

 Blocking I_{K-A} had effects similar to those of blocking I_{K-V} in long-latency programs (Fig. 13). For short-latency programs, the effect of I_{K-V} was stronger because its slow inactivation allows it to slow the rate of depolarization.

MAINTAINED CURRENT. This current has properties that differ significantly from the two inactivating outward currents. First, its reversal potential of ~35 mV indicates that channels are permeable to a number of ions. Second, the channel shows no...
inactivation. Third, threshold for activation of the channel is approximately \(-20\) mV, which is \(20\) mV more depolarized than the threshold for activating the other two outward currents. This current contributes somewhat to braking the maintained depolarization to \(-20\) mV.

**Summary of the events underlying B31/B32 activity**

Earlier data (Dembrow et al. 2003; Hurwitz et al. 1999b, 2003; Kabotyanski et al. 1988) suggest that the protraction phase of a buccal motor program begins with activity in the CB1 neurons of the cerebral ganglion, which monosynaptically excite protraction-phase buccal ganglia interneurons, in particular, B63. Firing in protraction phase interneurons that are presynaptic to B31/B32, such as B63, B34, B65, and B50, leads to synaptic excitation and depolarization of B31/B32 via electrical synapses and via facilitating and summating fast chemical synapses. B31/B32 depolarization in turn causes, via electrical coupling, an increased excitation and firing in the cells that are presynaptic to B31/B32, leading to their still greater excitation. When depolarization of B31/B32 reaches \(-50\) mV and beyond, the combined depolarization of B31/B32 and the activation of muscarinic receptors together activate the voltage- and transmitter-dependent slow muscarinic inward current, which summates with the fast and slow EPSPs, to cause still greater depolarization of B31/B32. Additional processes are also likely to participate in the depolarization of B31/B32. When B31/B32 depolarizes beyond \(-40\) mV, \(I_{K-V}\) and \(I_{K-A}\) will slow the rate of depolarization. When B31/B32 depolarizes to \(-20\) mV, the depolarization is constrained to remain at a plateau level by a decrease in the driving force of some inward currents as they become closer to their equilibrium potential as well as by the sustained activation of the \(I_{K-V}\), \(I_{K-A}\), and the maintained outward current. The sustained depolarization in the B31/B32 soma drives axon spikes at a frequency of \(\sim 20\) Hz. These spikes in turn cause contraction of the I2 muscle, which produces radula protraction. The sustained B31/B32 depolarization also drives additional protraction phase neurons via electrical coupling. These eventually activate B64 the activity of which terminates protraction and drives retraction.

**Decision making**

Studies in mammals (Schall 2005), as well as in invertebrates (Briegman et al. 2005), have examined some of the neurophysiological processes underlying choice and decision making. The activity of B31/B32 has an important role in deciding whether or not Aplysia respond to food. Data on the currents that contribute to B31/B32 activity may provide general insights into how decisions are made in other organisms.

CHOICE. The choice to initiate a behavior has been investigated in the frontal eye fields. Firing of many neurons in this area is related to the decision to initiate a saccade (Bruce and Goldberg 1985; Hanes et al. 1998). During the delay between a stimulus and a saccade, the subject decides on whether or not to respond. The time from an adequate stimulus to a saccade varies (Hanes and Schall 1996). The firing rate preceding the choice varies with the time needed to choose (Hanes and Schall 1996). For a slow choice, the firing frequency increases more slowly than when the choice is made more quickly (Hanes and Schall 1996). In addition, a stimulus that countermands the choice slows the firing frequency and lengthens the time needed to choose (Hanes and Schall 1996). The firing frequency reached at the choice point is not dependent on the time required to choose.

The properties of the slow depolarization of B31/B32 correspond in many ways to those of the frontal eye field neurons involved in choice. During the slow depolarization, B31/B32, and the other cells causing the slow depolarization, are exquisitely sensitive to depolarizing and hyperpolarizing inputs (Susswein et al. 2002), which can affect how quickly B31/B32 reaches the sustained depolarization or whether it reaches it at all. In the mammalian system, stronger or more frequent depolarizing stimuli produce a faster depolarization and a decreased latency to the sustained B31/B32 depolarization. In intact animals, a faster depolarization might arise as a result of environmental stimuli that allow the system to choose more rapidly. Weaker stimuli produce a longer latency to respond or in some cases initiate a depolarization that is insufficient to cause a sustained depolarization (Susswein et al. 2002), indicating a more difficult choice or a choice not to respond. Mammalian neurons involved in choice have been recorded via extracellular electrodes. A graded depolarization similar to that preceding the B31/B32 sustained depolarization, and that is controlled by inward and outward currents comparable to those in B31/B32, could underlie the firing in mammalian neurons with spike frequency controlled by the amplitude of the ramp depolarization.

Experiments on other regions of the brain examined neural activity when animals choose between different responses (Huk and Shadlen 2005; Roitman and Shadlen 2002; Schall 2003; Sugrue et al. 2005). Neurons having a role in choice fire preceding a particular decision. They fire less or not at all prior to the choice of a nonpreferred decision. B31/B32 activity is not related to choice between the different consummatory responses (Hurwitz et al. 1996) but rather to the decision of whether or not to initiate any response. Choice between the different consummatory responses is made by differentially recruiting different interneurons to fire along with B31/B32 during a buccal motor program (Hurwitz et al. 1997; Jing and Weiss 2001, 2002; Morgan et al. 2000; Nargeot et al. 1999; Proekt et al. 2007). Nonetheless, the delay in the initiation of protraction in B31/B32 may be a correlate of an increase in processing time needed to decide on the appropriate consummatory response and to recruit the combination of interneurons required to produce it. If the delay in B31/B32 is part of an adaptation designed to allow time for another CPG element to decide on the correct motor pattern, the latency to the sustained depolarization of B31/B32 should be controlled by factors affecting choice.
uated among different categories of neurons in separate areas of the brain.

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