FIRING PROPERTIES AND Na⁺-DEPENDENT PLATEAU POTENTIALS OF NEURONS IN NUCLEUS PRINCIPALIS TRIGEMINI OF THE GERBIL

by

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ABSTRACT

We investigated the electrophysiological properties of neurons in the nucleus principalis trigemini (PrV), using whole-cell recordings in in vitro slice preparations of brainstem. We identified three groups mainly by their firing properties: type 1 neurons were spontaneously active and able to discharge action potentials in doublets or bursts; type 2 neurons, depolarized by current pulses, fired action potentials in a nonadapting (tonic) pattern; and the less commonly encountered type 3 neurons also fired in such patterns but with biphasic afterhyperpolarizations. Neurobiotin staining and reconstruction did not reveal significant morphological differences between types 1 and 2 neurons which were multipolar, with dendritic trees distributed mainly along one axis. Type 3 neurons had more expansive and circular dendritic arborizations.

Hyperpolarization beyond -75 mV or down to the K+ reversal potential due to current pulse injection, resulted in an inward rectification which was expressed as a sag in the voltage responses of types 1 and type 2 neurons. A rebound subthreshold depolarization or spike burst was evident on termination of a pulse. In type 1 neurons, the application of Cs+ (2 mM), a blocker of a
hyperpolarization-activated cation current ($I_H$), eliminated the voltage sag and the dependence of the rebound spike-latency on membrane voltage, but did not alter the main features of the rebound response. We attribute the inward rectification to the activation of an $I_H$-like current.

Depolarization by current pulse injection into type 1 neurons, hyperpolarized with DC to prevent firing, occasionally evoked "plateau potentials". This feature, not observed in types 2 or 3 neurons, consisted of an initial oscillatory burst of 3 or 4 spikes that decreased in amplitude, and then plateaued for a variable duration, followed by an abrupt repolarization. We always observed these voltage shapes on depolarizing current pulse injection during perfusion with Ca$^{2+}$ free media, with or without the Ca$^{2+}$-channel antagonists, Co$^{2+}$ or Cd$^{2+}$, and during external tetraethylammonium (TEA) application.

An analysis of the depolarizing voltage responses evoked by current pulses in type 1 neurons during blockade of persistent and transient Na$^+$ conductances with TTX (600 nM) and K$^+$ conductances with TEA (10 mM) and 4-aminopyridine (4-AP; 0.5 mM), revealed the presence of inward rectification. This had a peak activation near
the plateau itself and was completely blocked by Ni$^{2+}$ (600 μM). These observations are consistent with the activation of a transient Ca$^{2+}$-conductance. Hence, we propose that a Ca$^{2+}$-dependent K$^+$ conductance mechanism controls the generation of the plateau potential.

The application of TTX, as low as 0.6 nM, increased the latency to onset and decreased the duration of the plateau potential, without greatly affecting action potentials. In a concentration-dependent manner, TTX enhanced the negative slope of the plateau, as it descended towards an abrupt terminal repolarization. Higher concentrations of TTX (e.g., 60 nM for 6 min) abolished the plateau potential before completely blocking action potential genesis. Low [Na$^+$]-perfusion, however, simultaneously reduced the amplitudes of plateau potentials and fast spikes. Evidently, small changes in a persistent Na$^+$ conductance can produce marked changes in firing behavior of type 1 neurons.

A long-lasting hyperpolarization followed current pulses producing the plateau potential. Indeed, subthreshold or suprathreshold depolarization in Ca$^{2+}$ free ACSF with Co$^{2+}$ (1 mM) also evoked a hyperpolarization at the offset of the current pulse.
This hyperpolarization was blocked by TTX (5 nM and 300 nM) and varied with changes in the duration of the plateau potential. A semiquantitative analysis revealed that the magnitude of the hyperpolarization depended on the neuronal depolarization. We conclude, therefore, that Na\(^+\) entry during a depolarization can increase a K\(^+\) conductance in type 1 neurons.

From our studies, we conclude that plateau potentials represent the contributions of persistent and transient Na\(^+\) conductances, high threshold Ca\(^{2+}\)-dependent rectification, as well as Ca\(^{2+}\)- and Na\(^+\)-dependent K\(^+\) conductances. The ability to fire bursts as part of Na\(^+\)-dependent plateaus is an unusual property in neurons of primary sensory nuclei. In nucleus principalis trigemini, burst responses to mechanical stimuli represent a normal output of neurons that likely are subject to intra- and extracellular messenger regulation.
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1. INTRODUCTION

The nucleus principalis trigemini (PrV) is the primary central station in the lemniscal pathway mediating somatosensory signals from facial regions to the cortex. In mammals, the spinal trigeminal nucleus, consisting of subnuclei oralis, interpolaris, and caudalis, constitutes a second neuron system in the sensory pathway to the cortex (Olszewski 1950). In the gerbil, the PrV is large, well-defined and distinct from the spinal nucleus, situated laterally in the brainstem and just rostrally to the bifurcation of the trigeminal root (Ramon y Cajal 1910).

The PrV receives the primary sensory input from short ascending fibers of the fifth nerve and, as an output, sends information mostly by way of the medial lemniscus to the ventroposteromedial nucleus of the thalamus (VPM; Jones 1985; Torvik 1957; Williams et al. 1994). The PrV also receives afferent fibers from sensorimotor regions of the cerebral cortex (cat: Brodal et al. 1956), red nucleus (cat: Edwards 1972), periaqueductal gray and dorsal raphe nuclei (rat: Li et al. 1993). Hence, neuronal
operation and sensory transduction in the PrV are likely subject to various modes of control.

The peripheral input, although multimodal, conveys a predominance of tactile sensory afferents to the PrV, analogous to the dorsal column nuclei. In the PrV of rodents which have well-developed whiskers, barrelets occupy a large portion of the neuropil, representing afferents from individual vibrissae. Their discharge patterns convey information about vibrissal deflection, topographically in a point-to-point projection, to the barreloids in VPM, en route to the cortical barrel field (Belford and Killakey 1979a; Ma and Woolsey 1984; Van der Loos 1976; Woolsey 1970). Developmentally, such maps are present first in PrV at birth, and later in VPM and cortex (Belford and Killakey 1979b; Erzurumlu and Killakey 1983; Killakey and Belford 1979).

Peripheral stimulation produces single unit responses in PrV neurons that involve a variety of firing properties. Following electrical stimulation of the skin, the patterns of impulses from rapidly or slowly adapting afferent fibers, however, cannot account for the phasic and tonic response modes of PrV neurons (Darian-Smith 1960). The input-output transformations, therefore, infer the
existence of different cell classes. Darian-Smith (1960) described nonmonotonic stimulus-response curves for the phasic responses of certain neurons, showing that an increase in stimulus intensity beyond the strength needed for maximum firing probability, reduced the spike rate. A classical interpretation is that this reduction represents an inhibition over time (phasic adaptation) and space ('surround' in receptive fields). This implies a presence of distinct projection neurons and interneurons (Mountcastle 1984). On the other hand, different response patterns can reflect various contributions of membrane properties, according to a neuron's repertoire of voltage- and ligand-gated conductances.

In view of the above, the PrV should contain functionally different types of neurons. Despite a detailed knowledge of the morphology, connectivity, development and spike firing patterns in PrV, there is a surprising absence of information about the electrophysiological properties of its neurons. In this work, we report on three distinct cell types with certain membrane properties that are not known to occur in neurons of other primary sensory relay stations. For example, an interaction of a persistent Na\(^+\) conductance with Ca\(^{2+}\) and K\(^+\) conductances is particularly
interesting because it endows the PrV neuron with a bursting capability limited by a long-lasting inhibition.

2. METHODS

Using isoflurane, we deeply anesthetized Mongolian gerbils (Meriones unguiculatus) aged between 11 and 17 days (P11 to P17). After decapitation, the brain was removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) for 1-2 min. The brain was cut into two with a quasi-sagittal (i.e., a 20 to 30° horizontal deviation from the ideal sagittal plane) incision and further trimmed to form a block containing brainstem and caudal cerebellum. For making slices (with a Vibratome), oriented rostro-laterally to ventro-caudally, we used the tissue block that contained the larger portion of the brainstem. The cut slices were between 300 and 350 µm in thickness. Slices, containing a visually identifiable principal trigeminal nucleus (PrV), were submerged into ACSF at room temperature (23 °C) and allowed to recover for at least 1 h. The ACSF contained (in mM): NaCl, 125; KCl, 2.5; CaCl₂, 2; MgCl₂, 1; glucose, 25; NaHCO₃, 25; NaH₂PO₄, 1.25 and was saturated with 95% O₂ and 5% CO₂ which maintained the pH near 7.4. Low [Na⁺] solutions
were made by partially substituting NaCl with choline chloride. For ‘Na-deficient’ perfusion, the [NaCl] in the 1/4, 1/8, and 1/16 NaCl solutions were, respectively, 57.5, 42.1, and 34.1 mM. For Ca$^{2+}$ free ACSF, 2 mM CaCl$_2$ was omitted or substituted with 1 mM CoCl$_2$, or 1 mM CdCl$_2$.

For whole-cell recording (Blanton et al. 1989; Ströhmann et al. 1994), we used patch electrodes, pulled from thin-walled borosilicate glass with filament (WP Instruments) and filled with a solution containing (in mM): K-gluconate, 115; ethylene glycol-bis(β-aminoethylether) $N,N,N',N'$-tetraacetic acid (EGTA), 10; $N$-2-hydroxyethylpiperazine-$N'$-2-ethanesulfonic acid (HEPES), 10; MgATP, 4; NaGTP, 0.3; and KCl, 20. The pH was adjusted to 7.25 with KOH. The signals, recorded in the current-clamp mode (Axoclamp-2B, Axon Instruments), were filtered at 3 kHz and sampled at 5-10 kHz with a data acquisition board (16 bit resolution, National Instruments Corp.) in a Macintosh Quadra 950 computer running custom-made data acquisition and processing programs which were developed with Labview instrumentation software (National Instruments) (Appendix 1). The electrode resistances ranged from 7
To 9 MQ and access resistances were > 100 MQ. All experiments were conducted at room temperature (22-24 °C).
3. Results

3.1 Identification of nucleus principalis trigemini (PrV) neurons

In quasi-sagittal sections of the brainstem, the nucleus principalis trigemini (PrV) appeared under translucent or reflected light as an opaque, pale ovoid structure, elongated in the dorsoventral direction. Its location was ventral to the inferior cerebellar peduncle. Anatomical landmarks that helped in its identification were the sensory root of the trigeminal nerve which was rostral and ventral to the PrV, as well as the cigar shaped, spinal trigeminal nucleus which caudally descended in the brainstem. We also studied frozen sections, cut from these brain slices and Nissl-stained with cresyl violet (n = 6), to confirm the identity of the PrV and surrounding structures. Neurobiotin staining of recorded neurons provided confirmation of their location within the PrV (Figure 1, A-C).
Figure 1. Camera lucida drawings of neurobiotin-stained neurons in the nucleus principalis trigemini. A: type 1 neuron. B: type 2 neuron. C: type 3 neuron. Scale, 100 μ.
We recorded from 62 neurons in the PrV and distinguished three types on the basis of neurobiotin staining and firing modes. The initial membrane potentials were similar, falling into a narrow range (-52 to -58 mV). More than one half of the neurons were spontaneously active, frequently firing action potentials in bursts (type 1; Figures 1A, 2; n = 33). Neurons of another group fired single repetitive action potentials only in response to depolarizing current pulses (type 2; Figures 1B, 4A; n = 23). The action potentials of types 1 and 2 neurons included large, long-lasting afterhyperpolarizations (AHPs). It was not possible to distinguish type 1 from type 2 neurons by their morphological features. Both types 1 and 2 neurons had multipolar somata and dendritic trees extending mainly along one axis (cf. Figure 1A,B). Another group of neurons "tonically" fired action potentials with fast AHPs in response to depolarizing current pulses (type 3; Figures 1C, 5A; n = 6). Type 3 neurons had more expansive, radial dendritic arborizations than types 1 or 2 neurons. In each type, the soma usually was elongated, sometimes triangular in shape, measuring about 10-15 μm (long axis).
3.2 Physiological properties of PrV neurons

Firing patterns of type 1 neurons. In response to depolarizing current injection type 1 neurons started firing with a burst pattern, consisting of doublets, i.e., a superposition of two action potentials on a slower hump (n = 33). Because these neurons were spontaneously active (Figure 2B), we injected a constant current to hold cells at potential (V_h) of -60 mV, -65 mV, or -70 mV for a systematic investigation of their responses to current pulse injections. Figure 2 shows that depolarization to threshold with current pulses elicited one or two bursts of action potentials. An increase in the depolarizing current decreased the latency to the initial burst, as well as the duration of the hump and the amplitude of the AHP. The primary spike burst remained steadfast while the secondary doublets eventually disappeared and the firing of single action potentials increased (Figure 2A), with an increase in current pulse magnitude. Eleven type 1 neurons fired a burst of three spikes ("triplet") at the onset of a depolarizing current pulse, followed by a doublet and/or single spike firing. Only one neuron fired a burst of four action potentials. For all type 1 neurons, the burst duration was <21 ms and the intraburst frequency was between 100 and 250 Hz.
Figure 2. Characteristic firing behavior of type 1 neuron (holding potential, $V_h = -60$ mV).
A: voltage responses evoked by 500 ms current pulses of increasing amplitude (right).
B: spontaneous activity of a type 1 neuron at its "resting" membrane potential ($V_h = 0$). Note that the neuron fired mostly doublet and triplet action potentials (not shown) and that variations in amplitude reflect low sampling rate (500 Hz).
3.2.1 Inward rectification in type 1 neurons

The injection of hyperpolarizing current pulses (500 ms duration) into type 1 neurons evoked voltage responses that exhibited a large depolarizing sag, implying an activation of a depolarizing current such as $I_h$ (Figure 3A). We investigated this possibility by applying Cs$^+$, a selective blocker of $I_h$ (McCormick and Pape 1990). Figure 3B shows that the application of Cs$^+$ (2 mM) eliminated the voltage sag in a type 1 neuron. As evident in the current-voltage relationships (Figure 3C), Cs$^+$ application completely blocked the pronounced rectification that activated near -70 mV, and developed fully by a membrane potential ($V_m$) of -100 mV. A contribution of an inward K$^+$ rectifier ($I_{IR}$; Travagli and Gillis 1994) seemed unlikely in our experiments because the calculated K$^+$ reversal potential was -98 mV. From the activation voltage and Cs$^+$-blockade, we concluded that an $I_h$ was the major current that produced the inward rectification in a voltage range between -70 and -100 mV in type 1 neurons.
Figure 3. Inward rectification, evoked by hyperpolarization, and rebound firing in type 1 neuron ($V_h = -60$ mV). A,B: voltage responses evoked by hyperpolarizing current pulses of increasing amplitude under control conditions (A) and during Cs$^+$ application (B) show blockade of inward rectification and increased latency to rebound firing. C: current-voltage relationships for early and late responses (measured as indicated in A,B) to current pulse injections show blockade of inward rectification by Cs$^+$. D: dependence of latency to first action potential of rebound on amplitude of current pulse amplitude under control conditions and during Cs$^+$ application. Note that Cs$^+$ abolished the voltage-dependence of the latency to rebound firing.
3.2.2 Contribution of inward rectification to spike generation in type 1 neurons

A brief, depolarizing hump remained at the offset of hyperpolarizing current pulses. This "rebound response" led to a burst of action potentials and often a second burst or a single action potential (Figure 3). The latency to the rebound response became progressively shorter with current pulse injections of increasing amplitude (Figure 3D). Extracellular Cs+ application (2 mM) did not significantly change the amplitude or the number of spikes in the rebound (Figure 3B). However, Cs+ eliminated the voltage-dependence of the latency along with the sag (Figure 3C). Evidently, an I_{h}-like tail current did not greatly contribute to the amplitude of the depolarizing rebound response but was an important contributor to the latency of firing emerging from hyperpolarization.

3.2.3 Firing patterns of type 2 neurons

Figure 4 shows the characteristic firing of a type 2 neuron to depolarizing current pulses, generating a tonic pattern of action potentials with slow AHPs. As in the case of the bursting type 1 neuron, an increase in the current pulse injected into a type 2 neuron
Figure 4. Characteristic tonic firing behavior of type 2 neuron ($V_h = -58$ mV) evoked by current pulses of increasing amplitude (right). A: depolarizing voltage responses. B: hyperpolarizing voltage responses show evidence of inward rectification.
produced a decrease in the latency to the first action potential, interspike interval and amplitude of the AHP (Figure 4A).

Similarly, current pulse injections that produced a hyperpolarizing voltage displacement of \(-15\) mV activated a depolarizing sag, resulting in a new steady-state level of the membrane potential (Figure 4B). In all type 2 neurons, larger hyperpolarizing pulses produced larger rebound responses, consisting of a single action potential on top of a slow depolarizing hump. We did not investigate the ionic mechanism of the depolarizing sag or rebound responses in type 2 neurons.

3.2.4 Firing patterns of type 3 neurons

Figure 5 shows the characteristic tonic firing of a type 3 neuron depolarized by current pulse injection \((n = 6)\). A distinguishing feature of the action potential in this type of neuron was a biphasic AHP, consisting of fast and slow components (cf. Figure 5C). Type 3 neurons exhibited little, if any, adaptation during the tonic firing to depolarizing current pulses of 0.5 s duration and no voltage sag in the hyperpolarized range \((e.g., to V_m = -100 \text{ mV})\). We did not observe rebound responses at the offset of current pulses in type 3 neurons (Figure 5B).
Figure 5. Characteristic tonic firing behavior, biphasic afterhyperpolarization, and hyperpolarizing voltage responses of type 3 neuron \((V_h = -58\, \text{mV})\) evoked by current pulses of increasing amplitude (right). A: depolarizing responses. B: hyperpolarizing responses. C: faster time scale shows afterhyperpolarization with fast and slow components, from an action potential marked by the asterisk in A.
3.3 Plateau potentials in type 1 neurons

During the course of these investigations, we occasionally (n=3) found that a depolarizing pulse produced an oscillatory burst of three or four action potentials with decreasing peak amplitudes until the potential plateaued for a variable period (~100 ms); towards the end of the plateau, a similar oscillation of increasing amplitude re-appeared, abruptly ending in complete repolarization (Figure 6B). We also observed a replacement of initial burst responses to injected pulses by such "plateau potentials" and then, a resumption of the regular type 1 firing behavior. In contrast, the plateau potentials were not apparent in the types 2 and 3 neurons. We assumed that the plateau potentials may relate to bursting ability of type 1 neurons, with a special role in PrV function, and proceeded to investigate their ionic mechanism.
Figure 6. Type 1 neurons generate plateau potentials during Ca\textsuperscript{2+}-free perfusion. 
A: time course for plateau potential development ($V_h = -63$ mV). The records to the same current pulse (27 pA) were obtained at the indicated times, before and after starting Ca\textsuperscript{2+}-free perfusion. B: current pulse-evoked plateau potential response in a different neuron under normal ionic conditions (inset is on faster time scale). C: a reduction in latency to the first spike in the burst accompanies the development of the plateau potential during time of Ca\textsuperscript{2+}-free perfusion (as in A). The plot shows the latency as a function of time after initiation of perfusion without Ca.
3.4 Ionic mechanism of plateau potential generation in type 1 neurons

3.4.1 Relationship of plateau potential firing to extracellular Ca$^{2+}$

On perfusion of Ca$^{2+}$-free ACSF to reduce Ca$^{2+}$ influx (n = 4), we observed a dramatic change in the firing pattern of the type 1 neuron evoked by depolarizing current pulses. During perfusion with 2 mM [Ca$^{2+}$], the neuron fired a doublet followed by a single action potential (Figure 6A, Control). During the Ca$^{2+}$-free perfusion, however, we observed a progressive reduction in the latency of the initial action potential, enhanced bursting, and onset of a plateau potential (Figure 6A,C). After ~2 min. of perfusion with the Ca$^{2+}$-free ACSF, the neuron fired triplets followed by a doublet (Figure 6A, 130 s). By 200 s, the neuron fired an initial burst comprised of four spikes and AHPs of decreasing amplitude followed by another burst and an action potential (Figure 6A). The initial burst of the neuron transformed into a plateau at $V_m \approx -20$ mV for ~100 ms (Figure 6A, 370 s), although the plateau duration varied greatly between experiments.

We performed similar experiments using blockade of Ca$^{2+}$ conductance by perfusion of Ca$^{2+}$-free ACSF containing Co$^{2+}$ (1 mM; n
Figure 7 shows the effects of this blockade which produced changes in firing behavior of a type 1 neuron, similar to those of Ca\textsuperscript{2+}-free perfusion but with a much more rapid transition from the normal firing mode to the plateau potential. Thus, in less than 3 min., we observed a well-developed plateau potential, lasting for \(-200 \text{ ms at } V_m = -20 \text{ mV (Figure 7, 160 s), and in less than 4 min., its duration increased to nearly 500 ms (Figure 7, 200 s). In several neurons, application of Co\textsuperscript{2+} (1 mM) in Ca\textsuperscript{2+}-free ACSF for 6-8 minutes resulted in plateau potentials that were longer than the duration of the depolarizing current pulse (not shown). We also conducted a set of experiments using partial substitution of the [Ca\textsuperscript{2+}] (0 mM) in the ACSF with Cd\textsuperscript{2+} (1 mM) with very similar results (n = 6; not shown).
Figure 7. Time course for development of plateau potential under Ca\(^{2+}\)-free and 1 mM Co\(^{2+}\) conditions (V\(_m\) = -60). Note prominent oscillations on plateau during perfusion for 200 s.
3.4.2 Involvement of a persistent Na\textsuperscript{+} conductance

Based on the data obtained during Ca\textsuperscript{2+}-free perfusion without and with Co\textsuperscript{2+} or Cd\textsuperscript{2+}, we hypothesized that the activation of a persistent Na\textsuperscript{+} conductance, such as in Purkinje cells and motoneurons (Llinás and Sugimori 1980a,b; Schwindt and Crill 1980), produced the plateau potentials. As a test, we evoked plateau potentials by applying Co\textsuperscript{2+} in Ca\textsuperscript{2+}-free ACSF and then, tetrodotoxin (TTX) to block the persistent Na\textsuperscript{+} conductance. After evoking a long-duration, plateau potential (Figure 8A, Control), the application of TTX (600 nM) annihilated the plateau potential and the fast action potentials in <1 min. (not shown). We also applied lower concentrations of TTX, bearing in mind that lower concentrations of the hydrophilic toxin would take longer to saturate the tissue. The application of 60 nM TTX caused, after 40 s, a division of the plateau potential into two shorter depolarizations (Figure 8A). Over the subsequent 3 min., there was a progressive reduction in amplitude and duration, and after 6-7 min., an elimination of the plateau potential, leaving behind a burst of two action potentials of slightly reduced amplitude (Figure 8A). With this concentration, then, it was possible to annihilate plateau potentials without markedly affecting
Figure 8. Blockade of persistent Na+ conductance in a type 1 neuron eliminates plateau potential and post-pulse hyperpolarization (PPH). A: time course of TTX application (60 nM) under Ca\textsuperscript{2+}-free and 1 mM Co\textsuperscript{2+} conditions shows splitting of plateau potential into two (40 s), a shortening of its duration (160-240 s) and complete blockade (340 s). After 420 s, TTX application blocked the burst of 3 action potentials (incompletely resolved) present at 340 s. B: changes in temporal sum of PPH ($W_{PPH}$, see Eq. 1 and text) measured in same neuron as a function of time of TTX application.
the neuron's ability to fire action potentials. A reduction in the slope of the rising phase of the fast action potential was apparent after a total blockade of the plateau potential with TTX. The action potentials disappeared 5-6 min. after TTX application. These findings show that small changes in the persistent Na\(^+\) conductance due to TTX application can produce dramatic changes in the firing behavior of the neuron.

3.4.3 Sensitivity of plateau potentials to TTX.

We investigated the sensitivity of the plateau potentials to TTX blockade by performing concentration-response experiments (Figure 9). First, we changed the firing mode of type 1 neurons by perfusion of Ca\(^{2+}\)-free solutions with Co\(^{2+}\) (1 mM) and evoked plateau potentials with depolarizing current pulses (500 ms, duration). We then applied TTX at different concentrations for 6-10 min. Application of TTX for 10 min. allowed observations of full recovery which we considered an important criterion for assessment of its long-lasting effects. For quantification of effects on firing behavior, we used a ratio of slopes of the plateau potential under TTX and control conditions. We obtained the slope from a linear fit of the voltage points between the local minimum after the second
Figure 9. Reduction in slope of plateau due to TTX blockade of persistent Na$^+$ conductance in a type 1 neuron under Ca$^{2+}$-free and 1 mM Co$^{2+}$ conditions. A: superposition of plateau potentials shows the more rapid descent of the plateau, observed at ~10 min of TTX application in 0.6, 1.2, and 1.8 nM concentrations. Solid lines are a linear fit to voltage response between the local minimum after the second spike at the start of the plateau and the local minimum before the plateau terminated in a rapid repolarization. B: concentration-response relationship for TTX effects on the slope of the plateau (as in A). Each point (± SE) represents 8 to 11 measurements taken from each neuron (n = 5) and solid line represents a Langmuir model fit.
action potential in the initial oscillation, and the local minimum just before termination of the plateau potential.

Figure 9A shows that TTX application increased the latency to the first spike and decreased spike amplitude as well as the magnitude and duration of the plateau itself, in a concentration-dependent manner. However, the slope of the plateau decay provided a more sensitive, reproducible indicator of TTX action. Figure 9B shows that the concentration-response relationship is consistent with a Langmuir model, representing the binding of TTX to Na\(^+\) channels.

3.4.4 Extracellular replacement of Na\(^+\) with choline

As additional confirmation for an involvement of persistent Na\(^+\) conductance, we investigated the effects of extracellular Na\(^+\) replacement with choline on plateau potentials (Figure 10). As before, we used depolarizing current pulses to induce the plateaus during blockade of Ca\(^{2+}\) currents with Ca\(^{2+}\)-free ACSF containing 1 mM Co\(^{2+}\). In contrast to TTX, the effects of extracellular Na\(^+\) deficiency were unspecific. Thus, a sequential reduction of the Na\(^+\) concentration caused corresponding reductions in the amplitude of the plateau potentials as well as action potentials. These
Figure 10. Effects of reduced extracellular [Na$^+$] on plateau potentials evoked during perfusion without [Ca$^{2+}$] and with 1 mM Co$^{2+}$. Choline CI was used to replace NaCl in the ACSF. All measurements were made at ~10 min after exchanging the perfusion. Note the systematic reduction in plateau (and spike) amplitude, in contrast to the reduction in duration observed under TTX (cf. Figure 8).
observations are in good agreement with the expectations based on the calculated change in the Na\(^+\) reversal potential due to the change in extracellular [Na\(^+\)]. At low Na\(^+\) concentrations, the duration of the plateau was determined by the injected current pulse (cf. Figure 10, 1/8 and 1/16 [NaCl]). Therefore, the terminal repolarization of the plateau may require a critical extracellular [Na\(^+\)]. In summary, the marked reductions in the amplitude of plateau potentials due to a deficiency in the extracellular [Na\(^+\)] and their complete blockade by TTX provide strong evidence for a Na\(^+\) involvement, likely a persistent Na\(^+\) conductance.

3.4.5 Involvement of Ca\(^{2+}\)-dependent rectification

From our results it seemed likely that activation of Ca\(^{2+}\) currents in type 1 neurons did not cause and, indeed, prevented the formation of plateau potentials. We investigated possible reasons why a blockade of Ca\(^{2+}\) influx supported their generation. While holding the neuron at \(V_m\) near -60 mV, we applied TTX (600 nM), 4-aminopyridine (4-AP; 0.5 mM) and tetraethylammonium (TEA; 10 mM) in order to unmask this possible Ca\(^{2+}\) conductance (n = 5). As shown in Figure 11A, depolarizing current pulses evoked voltage humps during the initial 200-300 ms of the responses. The current-voltage
Figure 11. High-threshold Ca$^{2+}$-activated K$^+$ conductance in type 1 neuron.
A: voltage responses evoked by 500 ms depolarizing current pulses of increasing amplitude (36, 41, 54, 58, 63, 68 and 72 pA) during Na$^+$ and K$^+$ conductance blockade by application of TTX (0.6 mM), TEA (10 mM) and 4-AP (0.5 mM).
B: current-voltage relationships for peak and steady voltage responses (as in A).
C: as in A, but during application of Ni$^{2+}$ (0.6 mM) which completely blocked inward rectification.
relationships for the peak and steady voltage responses (Figure 11B) show that this rectification activated at \(-40\) mV. Extracellular application of Ni\(^{2+}\) (600 mM) completely blocked the rectification (Figure 11C). These data are consistent with the activation of a transient, high-threshold Ca\(^{2+}\) conductance. This rectification may contribute to the initiation of the plateau potential and have some bearing on its limitation.

3.4.6 Involvement of Ca\(^{2+}\)-activated K\(^{+}\) conductance

We considered the possibility that a Ca\(^{2+}\) conductance provided the influx necessary for activation of a K\(^{+}\) conductance and hence, a 'controlling AHP'. For example, a plausible explanation for the plateau potential is that the blockade of Ca\(^{2+}\) entry prevented the activation of a K\(^{+}\) conductance that normally repolarized the neuron. As a check, we applied TEA (1 mM) to see if a K\(^{+}\)-channel blocker also produced a propensity for plateau generation, similar to that caused by a blockade of Ca\(^{2+}\) influx. Figure 12 illustrates the time course of effects produced by extracellular application of TEA (1 mM). The first sign of TEA action on the firing behavior of the neuron depolarized with a current pulse was a conversion of the initial burst of two spikes into a broader hump crowned by fast
Figure 12. Blockade of $K^+$ conductance by TEA application (1 mM) transforms spike burst firing of type 1 neuron into plateau potential generation ($V_h = -57$ mV). The same pulse amplitude was used for the recordings before and after the indicated times after initiation of TEA-application.
spikes (Figure 12, 120 s). This resulted from a blockade of repolarization, also evident from the reduced AHPs of single action potentials. Later, the initial depolarizing hump became broader and the number of spikes increased, exaggerating the initial burst (Figure 12, 160 s). By this time, TEA application delayed repolarization, as reflected in a 20-30% increase in the duration of the second action potential of the burst (not shown). Such bursts slowly transformed into plateau potentials whereas doublets and later, bursts and plateaus, replaced the single action potentials that followed the bursts (Figure 12, 260 s). These effects of TEA are comparable to the changes in the firing pattern of the same types of neurons deprived of external Ca$^{2+}$ (cf. Figures 6 and 12) and support the concept that a Ca$^{2+}$ activated K$^+$ conductance regulates plateau potential expression.

3.5 The post-pulse hyperpolarization (PPH)

An abrupt repolarization terminated the Na$^+$-dependent, plateau potentials in type 1 neurons (Figures 7, 8). The exact reason for the sudden repolarization remains unclear. The undershoot of the initial resting potential resulted in a long-lasting hyperpolarization
after termination of the stimulus pulse (PPH; Figure 8A). Because
the PPH occurred during blockade of Ca\(^{2+}\) influx but not during
blockade of K\(^+\) conductances by TEA, we hypothesized that the
activation of a Na\(^+\)-dependent K\(^+\) conductance (cf. Bader et al. 1985;
Schwindt et al. 1989) produced the PPH. In order to estimate the
effect of the Na\(^+\) conductance blockade on the PPH size, we measured
the temporal sum of the PPH amplitude (W\(_{PPH}\)) which is

\[
W_{AHP} = \int_{t=0}^{\infty} [V_h - V_m(t)] dt, \quad (1)
\]

where \(V_h\) is an initial value and \(V_m(t)\) is membrane potential at a
given time. The integral was taken from the point where the \(V_m\)
repolarized to \(V_h\) (\(t = 0\)), to the end of the recording (\(\infty\)). Indeed, a
reduction of Na\(^+\) influx due to TTX blockade (60 nM) caused a
concealing reduction in the PPH (\(n = 4\)). Figure 8B shows the time
course of the reduction of the normalized \(W_{PPH}\) which corresponded
roughly to the decrease in the plateau potential during TTX
application.

A long-lasting hyperpolarization also occurred, in a voltage-
dependent manner, following a depolarizing current pulse in the
absence of a plateau potential. We used Eq. 1 to quantify the
hyperpolarization at the offset of depolarizing pulses that evoked
sub- and suprathreshold responses under conditions of blockade of Ca\textsuperscript{2+} influx, before and after TTX application (n = 4). For this series of experiments, we only partially blocked Na\textsuperscript{+} conductances with low concentrations (e.g., 5 nM) of TTX. At 15-20 min. of the TTX application, the neuron did not generate a plateau potential but still was able to discharge one or more action potentials.

Figure 13 shows the results of such an experiment in a type 1 neuron where $V_h = \sim -70 \text{ mV}$. In this neuron, a substantial reduction of the PPH was apparent during TTX application when the current pulse amplitude exceeded 25 pA (Figure 13A,B). The application of TTX had little or no effect on the hyperpolarization following subthreshold voltage responses below -60 mV (Figure 13C). The effects of TTX were pronounced with larger current pulses that evoked plateaus, bursts, and single action potentials. Note that we also observed these effects when the voltage responses were still under the threshold for a plateau potential (~-48 mV) in this neuron. These observations provide strong support for a Na\textsuperscript{+} activated K\textsuperscript{+} conductance mechanism in the PPH.
Figure 13. Na\(^{+}\)-activated K\(^{+}\) conductance in a type 1 neuron during perfusion with Ca\(^{2+}\)-free ACSF and Co\(^{2+}\) (1 mM). A: temporal sum of post-pulse hyperpolarization (\(W_{RH}\), see Eq.1 and text) as a function of current pulse amplitude during control conditions and TTX application (5 nM). B: examples of hyperpolarizations shown at high gain in Control (1 in A) and TTX (2 in A) conditions. C: dependence of \(W_{RH}\) on the level of subthreshold depolarization.
4. DISCUSSION

In this first study of the electrophysiology of nucleus principalis trigemini neurons, we distinguished three classes of neurons (types 1, 2, and 3), according to their responses to DC and current-pulse injections. In all neurons, we observed a pattern of repetitive (tonic) firing in response to depolarizing currents, at rates that depended on current strength. This stimulus-response relationship may represent a simple rate code for the intensity of sensory stimuli which, classically, is the basis for quantifying the responses of neurons in receptive fields (cf. thalamo-cortical levels, Mountcastle 1984).

4.1 Depolarizing responses in types 2 and 3 neurons

The simplest transformation of an input current into a spike-count / intensity code was evident in type 3 neurons. A slow depolarization with a ramplike slope preceded a regular firing pattern. Because this slope increased with current magnitude, an orderly inverse relationship resulted between stimulus strength and latency. The responses of type 2 neurons to depolarizing currents
were similar, except that they exhibited slow AHPs and, therefore, somewhat lower firing rates. In type 3 neurons, the biphasic AHPs led to complex depolarizing slopes that preceded repetitive firing of single action potentials and seemed to control the rate and regularity of firing (e.g., Figure 5).

4.2 Hyperpolarizing current pulse injections into PrV neurons

Types 1 and 2 neurons exhibited a rectifying, depolarizing sag in their responses to hyperpolarizing current pulses. In contrast, type 3 neurons responded to such pulse injections with voltage changes that appeared passive, possibly influenced by a radiating dendritic tree. After a hyperpolarizing pulse, both type 1 and 2 neurons responded with a rebound depolarizing hump. Action potentials occurred at shorter latencies on top of the hump after larger hyperpolarizing pulses. The voltage sags and rebounds were reminiscent of an $I_h$-like rectification (McCormick and Pape 1990) that would tend to limit long hyperpolarizations. In type 1 neurons, we found that Cs$^+$ application, a blocker of $I_h$, eliminated the sag as well as the voltage dependence of the latency for the rebound
response. It is likely, then, that $I_h$ contributes to post-inhibitory rebound responses in type 1 neurons.

4.3 Classification of types 1 and 2 neurons

In many aspects, types 1 and 2 neurons were similar. However, type 1 neurons had other outstanding features that invited a detailed examination of their electrical behavior: (1) spontaneous firing; (2) burst firing of two to four spikes in conjunction with plateau potentials; and, (3) a long-lasting, post-excitatory hyperpolarization. As discussed below, these criteria for classification as "type 1" relate to the activation of a persistent $Na^+$ conductance. If it were possible to regulate this conductance, these morphologically indistinguishable neuronal groups could represent functional states of one class. Our results, however, suggest the separate classification of types 1 and 2 neurons.

4.4 Burst firing in type 1 neurons

In these slice preparations, type 1 neurons fired spike bursts spontaneously. In response to depolarizing current injection, a neuron characteristically started with a brief burst discharge,
followed by a tonic pattern of single action potentials. The burst discharge consisted of 2-4 action potentials superimposed on a slow depolarizing hump. The application of TTX blocked the slow depolarization and eliminated the action potentials. A slow, Ca\(^{2+}\)-dependent depolarization was apparent, during blockade of both Na\(^+\) and K\(^+\) currents (cf. Figure 11). Under these conditions, application of Ni\(^{2+}\) annihilated the slow depolarizing response to current pulse injection which had a threshold of \(-40\) mV. This may reflect variations in a Ca\(^{2+}\)-channel protein combination that binds to Ni\(^{2+}\) (cf. Zamponi et al. 1995) or a distribution of Ca\(^{2+}\)-channels in the dendrites, which are located remotely from a likely somatic site of recording. While such a transient Ca\(^{2+}\) current may provide a minor contribution, the spike burst is largely attributable to the activation of persistent and transient Na\(^+\) conductances.

4.5 Is the burst firing pattern of type 1 neurons representative of PrV neurons in vivo?

In the early extracellular investigations of the PrV, Darian-Smith (1960) observed that some neurons always started their response to a stimulus with a spike burst but dismissed this pattern
as evidence for possible cell damage. In the *in vivo* studies, an increase in the intensity of electrical cutaneous (lip) stimulation did not change the interval between the two action potentials in the initial burst. Much stronger stimuli caused these spikes after the burst to appear at shorter latencies; single action potentials occurred repetitively at a greater firing rate. This behavior is very similar to the responsiveness of type 1 neurons injected with depolarizing current pulses of increasing amplitudes. Although a further comparison of the *in vivo* and *in vitro* data may be problematic, we suggest that the striking similarities provide representative evidence for the firing patterns of PrV neurons *in vivo*.

### 4.6 The plateau potentials of type 1 neurons

A distinguishing feature of type 1 neurons was an ability to generate a plateau potential. In many respects, this potential has similarities to the somatic plateau potential of Purkinje cells (Llinás and Sugimori 1980a), including (1) a dependence of its latency on stimulus amplitude; (2) an independence of its duration from the stimulus amplitude; (3) plateau oscillations, usually of
inactivating action potentials; and, (4) a relatively fixed plateau at a membrane potential of $-20$ mV, despite the various stimulus amplitudes. Also, TTX-application blocked both PrV and Purkinje cell plateau potentials. The $\text{Ca}^{2+}$-dependent plateau potentials in cerebellar and spinal neurons (Hounsgaard and Kiehn 1989; Llinás and Sugimori 1980b), and the $\text{Na}^+$-dependent plateau potentials in striatal and hippocampal neurons (Hoehn et al. 1993) are insensitive to high TTX-concentrations. The elimination of plateau potentials without significant alterations to action potentials by low TTX concentrations provides firm evidence that their generation in PrV neurons requires the activation of a persistent $\text{Na}^+$ current.

4.6.1 Influence of cation currents

Occasionally, depolarizing stimuli elicited plateau potentials in neurons under normal extracellular conditions. When we minimized $\text{Ca}^{2+}$ currents with Ca-free, $\text{Co}^{2+}$-application, or blocked $\text{Ca}^{2+}$-activated $\text{K}^+$ currents with TEA application (cf. Galvan and Sedlmeir 1984), such stimuli always evoked plateau potentials. During the presumed, progressive reduction of these currents, there was a gradual prolongation of the plateau (cf. Figures 7 and 12). Therefore, $\text{Ca}^{2+}$-dependent currents may contribute to the generation
and limit the duration of the plateau. Under normal extracellular conditions, an interaction of Ca\(^{2+}\), Ca\(^{2+}\)-activated K\(^+\), and persistent Na\(^+\) currents would produce a depolarizing hump, causing a brief spike burst in response to an excitatory stimulus or, on termination of a hyperpolarizing input. In view of possible modulation of these interactive currents (cf. Schwindt et al. 1992), mechanisms that modulate various aspects of plateau potentials seem likely under physiological conditions (cf. Zheng and Gallagher 1995).

In their investigations of Purkinje cells, Llinás and Sugimori (1980a) observed an increased plateau magnitude after blocking K\(^+\) conductances with Ba\(^{2+}\) replacement of extracellular Ca\(^{2+}\) or intracellular application of high TEA-concentrations. We observed a reduced plateau magnitude in PrV neurons under conditions of reduced extracellular [Na\(^+\)] or partial blockade of Na\(^+\) conductances by TTX. These findings directly support the possibility (Llinás and Sugimori 1980a) that the plateau may represent a balance of Na\(^+\) and K\(^+\) currents.

4.6.2 Repolarization of plateau potential

What terminates the plateau in such an abrupt manner? We have observed that a reduction of Ca\(^{2+}\) influx causes a prolongation
of the plateau (Figures 6, 7). The application of TEA also gradually prolonged the total plateau time. Therefore, one explanation for termination of the plateau involves activation of a $K^+$ conductance by internal $Ca^{2+}$. Another is that the depolarization-initiated $Na^+$ influx activates a similar, repolarizing $K^+$ conductance. Neither a critical intracellular $[Ca^{2+}]$ nor $[Na^+]$, alone, can explain the decreased plateau duration when $Na^+$ influx was gradually reduced by TTX, under conditions where there is very little $Ca^{2+}$ influx (cf. Figure 8). On reduction of the "persistent" $Na^+$ influx, the $K^+$ currents that balance with a persistent $Na^+$ current to limit the plateau potential to $\sim$ -20 mV, are sufficient to repolarize the neuron. These considerations justify a hypothesis that a combination of a voltage-dependent $K^+$ current with a $Ca^{2+}$-dependent, or a $Na^+$-dependent, $K^+$ current repolarizes the plateau.

4.7 Post-pulse hyperpolarization (PPH)

Whatever its exact mechanism, the repolarization of the plateau potential presumably relates to the hyperpolarization which occurred on termination of a depolarizing current pulse (post-pulse hyperpolarization or PPH). The PPH was not likely a consequence of
the sole activation of a voltage-dependent K⁺ conductance. In the first place, the TTX application blocked the PPH in a voltage range expected for the activation of the persistent Na⁺ current. Indeed, blockade of the PPH required only low concentrations of TTX. The voltage range where the TTX-sensitive PPH activated includes membrane depolarizations that were subthreshold for the generation of plateau potentials. We observed that the temporal sum (or area) of the PPH correlated roughly to the magnitude of the stimulus pulse or depolarization. On the other hand, we did not observe PPHs during TEA-application which is consistent with the hypothesis that the activation of a K⁺ conductance and not, electrogenic Na⁺ pumping, produces the PPH in these neurons. In axons, the equivalent of the PPH (hyperpolarizing afterpotential) is affected by changes in the K⁺ reversal potential and extracellular TEA application, in a manner that is predictable for an activated K⁺ conductance, but not affected by inhibitors of electrogenic Na⁺ pumping (Poulter et al. 1995). We conclude that a "persistent" increase in internal [Na⁺] (rather than in voltage) during the depolarization triggered the activation of a K⁺ conductance, producing the PPH in type 1 neurons.
4.8 Functional considerations in type 1 neurons

The early burst of a doublet during a depolarizing input in type 1 neurons implies that the onset of a natural stimulus would receive emphasis. The spike firing within the burst usually was constant over a wide range of input current amplitudes. Hence, the initial rate of firing in the burst cannot provide coding for the stimulus amplitude, unlike the subsequent firing of single action potentials. It seems probable that spike bursts would occur as threshold responses to natural excitatory (and post-inhibitory) inputs whereas the tonic firing pattern would depend on the stimulus duration and the nature (or modality) of afferent fiber inputs. On the one hand, brief excitatory postsynaptic potentials (EPSPs) mediating activity from rapidly adapting mechano-receptors may evoke only spike bursts. On the other, the extended activities of slowly adapting receptors could cause depolarizations of sufficient duration for tonic firing. If rapidly adapting afferents were to provide input to type 1 neurons, the latency to the spike burst may represent the only available code for the stimulus intensity, an uncommon consideration in somatosensory physiology. A more likely scenario is that type 1 neurons have specialized response patterns for the dynamic aspects of mechanical stimuli. The larger EPSPs arising
from the faster vibrissal deflections, for example, would decrease the burst latency and automatically tune system for the higher frequency for vibratory inputs. Since the vibrissal (and other hair follicle) afferents are often directionally sensitive, the relatively strict relationship between the magnitude of hyperpolarization and the latency to a rebound burst response (Figure 3) would tend to emphasize certain aspects of stimulus dynamics.

It had been shown (Schultz, Galbraith et al. 1976) that deflection of the sinus hair of the second type (StII) in the opposite to the optimal direction reduced background activity of both primary afferent and cortical neurons to a zero-level. When the hair was released a rebound response was evoked so that its amplitude in peri-stimulus-time-histogram (PSTH) was 3-5 times bigger (our own estimation based on the Fig. 1C, Fig. 4A and Fig. 4B in the (Schultz, Galbraith et al. 1976) than background level. Although an effect of either change of the deflection angle or the change of the speed of vibrissae release to the neutral position on the rebound response magnitude had not been studied systematically in this work one can get some insight about it analyzing the data presented in the paper (Schultz, Galbraith et al. 1976). It is visible (see Fig. 1C, Fig. 4
A', Fig. 4B', Fig. 4C') in the (Schultz, Galbraith et al. 1976) that fast vibrissae return to the neutral position after higher angle non-optimal deflection evoked responses that were bigger in amplitude (impulses/bin in PSTH) and more sharply tuned in time. The fact that the same dependence can be observed at the two extreme levels of the sensory pathway leads us to assume that similar responses can be recorded in the secondary sensory neurons such as those located in the brainstem trigeminal complex, including the PrV. If this assumption is correct (unfortunately, we could not find a single work where this or similar question was addressed) the ability of the Type I PrV neurons to generate rebound response after transient hyperpolarisation suddenly appears to be an important feature in relation to the sensory information processing. For example, temporary background firing suppression in the primary afferent innervating vibrissae follicle caused by non-optimal hair deflection can result in a reduction of a background excitatory transmitter release and thus in hyperpolarization of the postsynaptic site which in its turn can contribute to the whole neuron hyperpolarization. Then, transient increase of the primary afferent activity can lead to a neuron depolarization from the more hyperpolarized level of its
membrane potential. Such type of depolarization can evoke rebound response in the neuron so that its probability and temporal characteristics would reflect certain parameters of the incoming signal such as duration and depth of background activity reduction as well as the rate of its change. These parameters in their turn are directly connected to the real-world mechanical stimulation of the vibrissae.

Based on the data obtained in the presented work one can hypothesize that the activation of an \( I_h \)-like current, which markedly affects the latency-amplitude relationship (Figure 3), may represent an adaptation for transmission of dynamic change in mechanical stimuli. An empirical examination of these considerations would allow determination of the types of mechanoreceptive afferents that produce depolarization and hyperpolarization in type 1 neurons of the nucleus principalis trigemini.
5. REFERENCES


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6. APPENDIX 1.

6.1 Data acquisition and processing program VMS

In order to perform the experiments presented above, it was necessary to design and write a new data acquisition- and analysis program for the Macintosh Quadra 950 computer and National Instruments data acquisition and DMA (direct memory access) boards. LabView instrumentation development environment was chosen for this purpose.

LabVIEW is a general-purpose programming system that includes libraries of functions and developmental tools designed specifically for data acquisition and instrument control. LabVIEW programs are called Virtual Instruments (VIs) because their appearance on a computer monitor and their operation imitate real-world instruments (e.g. oscilloscope, tape-recorder, chart recorder, amplifier). The main advantage of using the virtual instrumentation approach, compared with conventional programming languages (e.g. C, Pascal), is that every VI is a fully functional unit, similar to a stand-alone instrument, which can be used separately or in combination with other VI's, supplied commercially or written
independently. Thus, one can assemble an entire new instrumentation complex by simply connecting different VI's in an imaginary laboratory. In addition, one can, in future, add new VI's to a set of already working instruments without modification of existing VI's, or change parameters or functionality of the VI's that are already at work.

The VMS data acquisition and processing program used in the present work consists of seven main VI's. These are: 1. A hardware setup; 2. The build protocol; 3. A play back module; 4. The chart recorder; 5. The protocol data acquisition module (DAQ); 6. A waveform generator; 7. A digital signal processing unit (DSP). A user can access each of these VI's by clicking on the corresponding button in the main panel (Fig. A1) appearing on a computer monitor when the VMS program is loaded. The “Stop” button terminates execution of the VMS.

![Figure A 1 Main panel](image-url)
The VI “Hardware Setup” is used to change data acquisition board settings such as the device number of the A-to-D conversion board, and the number of input and output channels. The typical configuration used in the present project includes two input channels for voltage and current recordings expandable to a maximum of 8 and one output channel (maximum 2) for current or voltage command generation. The subpanel “External Gains” (Fig. A2) is used to feed information about external amplifier parameters (voltage gain, current gain and headstage gain) into the VMS program. The subpanel “Continuous DAQ” (Fig. 2A) defines parameters of a chart recorder VI (explained later).

**Figure A 2** Subpanel “Continuous DAQ”
The VI "Build Protocol" is used to create a voltage or current command protocol. Every protocol can be a stimulus or a set of stimuli repeated in time. Three types of stimuli are implemented: 1. A pulse-type stimulus; 2. A chirp-type stimulus (frequency modulated sinewave); 3. Sinewave stimuli. Figure A3 shows an example of the "Build Protocol" VI for a pulse-type stimulus protocol generation.

In the given example the protocol consists of the 10 voltage pulses (Fig. A3., right-hand slide dial) increasing in amplitude by 5 mV (Fig A3., right-hand column of controls). Each stimulus consists of 5 episodes (E-1, E-2, E-3, E-4, E-5; Fig. A3).

**Figure A 3** "Build Protocol" VI
Stimuli can be created visually by marking parts of the waveform with a mouse and simply dragging and placing the corresponding cursor in a specific position on the display (e.g. Fig A3, E-1 cursor is in the position x = 0.1 s and y= 0 mV). X- and Y-scaling on the display can be adjusted automatically or manually. The total number of episodes available for a user is 7 in the visual mode of stimulus construction and unlimited in an alternative text mode. Every protocol can be saved in a file and loaded later for use or modification. Figures A4 and A5 show the “Build Protocol” VI in the modes of the chirp-type and sinewave-type stimulus protocols respectively.

Figure A 4 “Build Protocol” VI in the mode of the chirp-type stimulus generation
Figure A 5  "Build Protocol" VI in the modes of the sinewave-type stimulus generation

The VI "Chart Recorder" is designed to substitute a standard chart recorder (Fig. A6). It is capable of continuously logging data to the disk without interruption of the data acquisition. In order to start data logging a user has to choose a file name for a record of the acquired information. The VI automatically creates a new file every minute, adding the current time to the file name specified by user (e.g. cell1_5-37 PM). The sampling rate for the data acquisition and displayed time period are specified in the VI "Hardware Setup" in the "Continuos DAQ." panel.
The front panel of the “Protocol DAQ” VI is shown in the Figure A7. This VI is used to conduct single-cell recording in discontinuous mode using pulse protocols (created with the “Build Protocol” VI) or arbitrary waveforms (created with the “Waveform generator” VI) or imported from a text file.
A protocol or arbitrary waveform can be loaded by pressing the "Protocol" or "Waveform" buttons. Stimuli can be delivered manually (by pressing button "Next", Fig A7) or automatically with a delay that is specified on the slide dial "Delay" (Fig. A7). Dials in the panels "Input limit" and "Output limit" control on-board amplification for signal acquisition and signal generation respectively. Acquired data are saved in a file defined by the user. The file name is shown in the window "Path to save". The buttons "Record" and "Approach" permit changing the voltage scale on the screen to a predefined value. In addition, both voltage and current
windows can be transferred into an auto-scale mode so that, independently of the input signal magnitude or duration, scaling is maintained at an optimum.

Figure A8 shows the front panel of the “Play back” VI. This VI is designed to provide easy access to the data obtained during experiment. A user can load a data file, like a video-tape into a video player, so that every experimental trial (frame) can be accessed sequentially in both forward and backward playback. To play back all frames from a file the user should press the “Total” button.

![Figure A8 “Play back” VI](image_url)
The button “Print” permits a transfer of both voltage and current screens with file name and location information from the storage media (hard drive) to the printer, creating a hardcopy of the displayed data.

The main VI for data analysis is called “DSP” (fig. A9).

**Figure A 9 “DSP” VI**

All data processing operations in this VI are performed on the data from either voltage or current trace between two cursors. The position of these cursors can be changed and fixed at selected positions with a mouse. As a segment of a trace is chosen, the user
can perform following operations: 1. fit data (button “Fit”) to a function. Only linear and single exponential fits have been implemented to date; 2. define maximum and minimum (button “Max/Min”): cursors are positioned automatically to the maximum and minimum of the chosen trace segment; 3. calculate the average over the trace segment (button “Mean”); 4. take the derivative (button “dX/dt”) using an arbitrary time window (dial “Window”); 5. Filter data (button “Filter”); 6. take the time integral (button “Int(mV*ms)”). An example of a linear fit to a plateau potential is shown, with the front panel of the “Fit” VI, in Figure A10. Figure A11 shows front panel of the “dX/dt” VI with an example of the temporal derivative taken from the voltage trace segment between the cursor markings in the Figure A9.

A user can transfer a chosen data segment of the voltage or current trace directly to the HiQ numerical computation package (National Instrument software) for on- or off-line analysis (fig. A9; button “HiQ”). The data captured into a text file can be imported into any standard spread-sheet or data processing program (fig A9; button “Capture”, option “ASCII”, not shown). Finally any data segment can be captured and stored in binary format as an arbitrary
waveform to be used as current- or voltage-command during experiment (fig. A9; button "Capture", options "Command", "Voltage" or "Current" (not shown).

The VMS program is a new flexible tool designed for whole cell recording and data analysis. The advantage over alternative programs is, that new functions can be added without the risk of introducing errors in already existing modules. The design of experiment control and data analysis, therefore, is ideally suited to expansion in any direction of potential development of the modern neurophysiology.

**Figure A 10** “Fit” VI
Figure A 11 "dX/dt" VI