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An early outward conductance modulates the firing latency and frequency of neostriatal neurons of the rat brain

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Summary. An in vitro slice preparation was used to obtain intracellular recordings of neostriatal neurons. Indirect evidence for the presence of an early outward conductance in neostriatal neurons is presented. With near threshold stimulation neostriatal neurons fired very late during the pulse. The long firing latency was associated with a slow (ramp-like) depolarization. In the presence of TTX the slow depolarization was lost and outward-going rectification dominated the subthreshold response. This finding demonstrated that both, outward- and inward-going conductances play a role during the ramp-like depolarization. Outward-going rectification during depolarizing responses could be further augmented if the depolarizing stimulus was preceded by a conditioning hyperpolarization. A conditioning hyperpolarization prolonged the firing latency and slowed the firing frequency. A conditioning depolarization had opposite effects. After TTX treatment, the response showed a hyperpolarizing "sag" when depolarizing stimulation was preceded by conditioning hyperpolarization. 4-AP (0.5–2.5 mM) blocked the effects of the conditioning hyperpolarization on the firing latency and on the voltage trajectory. 4-AP also disclosed a slow depolarization which could produce neuronal firing very early during the pulse. This depolarization was TTX-sensitive and Co^{++}-insensitive. In contrast to 4-AP, TEA (20 mM) did not produce a reduction in the firing latency but disclosed a membrane oscillatory behavior most probably produced by the interplay of these opposing conductances: the slow inward (probably Na^+) and the transient outward (probably K^+). Repetitive firing during 4-AP treatment was of the "phasic-tonic" type with an initial burst riding on the initial Co^{++}-insensitive slow depolarization and a somehow irregular train of spikes during the remainder of the stimulation. Action potentials during 4-AP treatment were followed by an afterdepolarization which dominated the initial part of the interspike interval.

Key words: Neostriatum – Neostriatal slices – A-current – 4-aminopyridine – TEA

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Introduction

It has been shown (Galarraga et al. 1985; Kita et al. 1985a; Calabresi et al. 1987a) that stimulation of neostriatal neurons with depolarizing currents of near threshold intensity evokes neuronal firing only very late during the pulse. The long initial spike latency is associated with a slowly rising (ramp-like) depolarization that could last hundreds of milliseconds. The contribution of an inward-going voltage-dependent ionic conductance, such as a slow inward sodium or calcium current, has been postulated in this depolarization (Kita et al. 1985a, b; Calabresi et al. 1987a). However, it has been shown in other neurons and systems (Connor 1975; Gustafsson et al. 1982; Galvan and Sedlmeir 1984; Galvan 1982; Segal et al. 1984; Salkoff 1985) that activation of outward currents could also play a role in shaping slowly-rising voltage trajectories toward firing threshold.

Although activation of many outward currents could delay action potential firing and dampen excitability, an early transient outward current has characteristically been critical for delaying responsiveness (Byrne 1980a, b; Galvan 1982; Segal et al. 1984) and regulating the frequency of repetitive tonic firing (Connor and Stevens 1971; Connor 1975; Connor 1985). Since some neurotransmitters affect this type of current (Aghajanian 1985; Rogawski 1985; Nakajima et al. 1986), it follows that altera-

tions in firing latency and discharge pattern produced by neuromodulators could be mediated through changes in this ionic conductance (Strong and Kaczmareck 1987). Thus, the presence of this class of outward current in neostriatal neurons could be of value in understanding the actions of striatal transmitters. Here we show some evidence that the activation of an early conductance which is blocked by 4-aminopyridine (4-AP: 0.5–2.0 mM) but not by tetraethylammonium (TEA: up to 20 mM) slows the firing frequency and prolongs the firing latency of neostriatal neurons. Some of these results have been communicated in preliminary form (Galarraga et al. 1985).

Methods

Experiments were performed in rat brain slices maintained in vitro. The method used to obtain slices is similar to those already reported (Misgeld et al. 1979; Kitai and Kita 1984). Briefly, Wistar rats (250–300 g) were decapitated, had their brains removed quickly and placed in cold (8 ° C) Krebs solution. Coronal slices of 350 μm with the caudate-putamen nucleus were obtained with a vibratome. The slices were incubated in oxygenated Krebs at room temperature for at least 30 min before being transferred to the recording chamber. Slices were maintained in a warm, moist chamber and superfused continuously from below (Kitai and Kita 1984). Normal Krebs solution contained in mM: 120 NaCl, 1.75–5 KCl, 1.25 KH_2PO_4, 1.75 $MgSO_4$, 25 $NaHCO_3$, 2 $CaCl_2$, 11 glucose. Solutions were gassed with 95% O_2 and 5% CO_2. The pH was adjusted to 7.4, and osmolarity to 310 mOsM/l. Total extracellular potassium concentration in each experiment is indicated in the results. Tetrodotoxin, 4-AP and TEA were purchased from Sigma (St. Louis). Intracellular recordings were made with glass microelectrodes (80–120 MΩ) filled with potassium acetate (3–4 M) and pulled with a Brown-Flaming horizontal puller. Data were accepted if, during the collection period, the resting membrane potentials (RMPs) were more negative than −60 mV and action potentials amplitudes were at least 70 mV when measured from the resting potential level. Intracellular potentials were fed into a high input impedance electrometer (WPI) with an active bridge circuit. Potentials were monitored with a digital (Nicollette-206) oscilloscope. Digitalized records chosen for analysis were recorded on diskettes for further enlargement and measurement. Afterwards, these records were drawn on paper using an HP x/y plotter.

Results

Threshold behavior

The presence of subthreshold voltage-dependent ionic conductances can be inferred from the I–V relationship in this range (Bargas et al. 1988). Figure 1A shows this relationship. When only the measurements near the resting membrane potential (RMP) are considered, the data can be fitted by a straight line (marked "a"; same letters were used for the correspondent functions given in the figure legends) whose slope and intercept give the input resistance and the RMP respectively. However, if the complete curve in control conditions is considered ("b": filled squares, corresponding to late measurements of records in Fig. 1B) an inward-going rectification for depolarizing currents is apparent. This rectification is manifested as an apparent increase in input resistance and by the fact that the I–V plot is now better fitted by a quadratic function with a positive square term. After the application of TTX ("c": empty squares, measurements taken from late measurements of records shown in Fig. 1C), the same neuron shows outward-going rectification to the same depolarizing currents. This is manifested by the now negative square term in the fitted quadratic function. This suggests that both inward and outward currents are being activated by the applied subthreshold currents. Otherwise, TTX would only straighten the plot for depolarizing pulses towards the straight line marked "a". Thus, although in normal conditions inward currents dominate subthreshold responses, a large component of these appear to be suppressed by TTX. A similar result was obtained by Calabresi et al. (1987a). This may explain why autoregenerative responses cannot be produced in these neurons after TTX application if outward currents are not blocked at the same time (Kita et al. 1985b; Calabresi et al. 1987b).

A number of K^+ conductances could contribute to the outward-going rectification after TTX. Here we will offer some evidence suggesting that at least part of the outward going rectification is due to an early transient conductance which is more sensitive to 4-AP than to TEA (but see: Galarraga et al. 1989).

Under control conditions and applying long duration current pulses of near threshold strength, neurons fired after a long latency with a characteristic voltage trajectory (Figs. 2A–D, 3B (top), 3C, D, 4A, 5A). When currents of significantly smaller amplitude were applied (Fig. 2A lower voltage trace) only a passive response was evoked. The response reached a steady-state membrane potential (V_m) with a mean time constant of about 6–14 ms (Bargas et al. 1988). With the larger depolarizing currents the membrane voltage did not reach a steady state. Instead, a voltage trajectory consisting of a slowly rising depolarization up to the end of the current pulse or to the spike threshold was observed (Fig. 2A–D, see also 3C, 4A, 5A). The top trace in Fig. 2A shows the firing of the neuron (action potential clipped) after a latency greater than 100 ms. This depolarization has been attributed to slow inward sodium and/or calcium currents in cau-

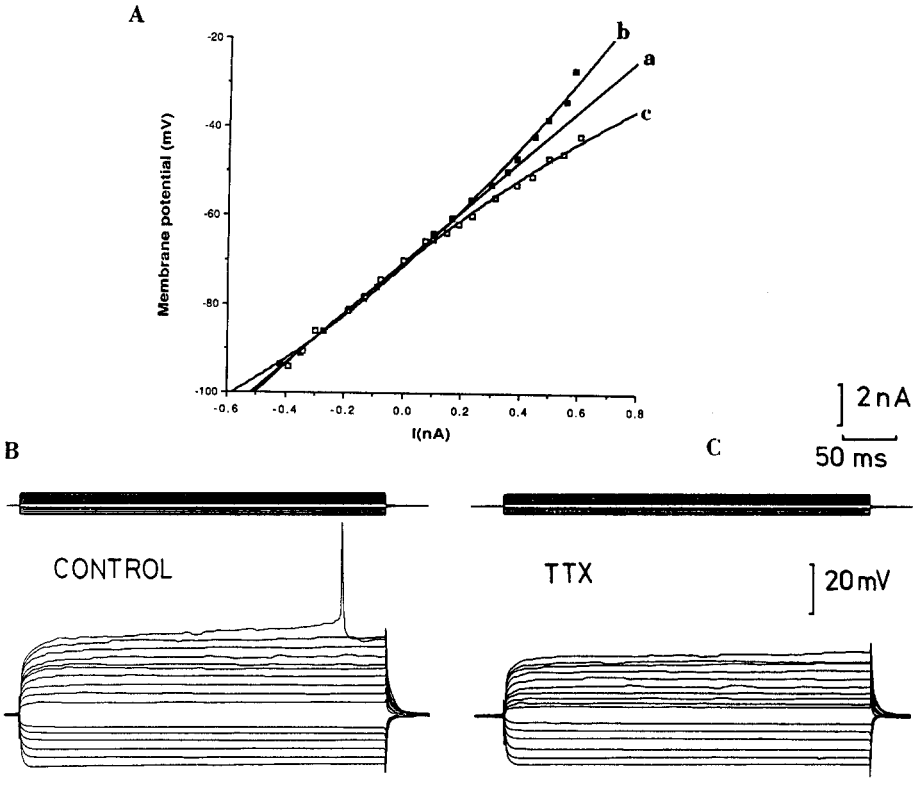

Fig. 1A–C. Effect of TTX on the I–V curve. **A** I–V curve taken before (b: filled squares) and after (c: empty squares) TTX (1 μM). Zero on the abscissa indicates the RMP (–70 mV in this cell with $[K^+]_o$ = 5 mM). V_m is the membrane potential measured at the end of the current pulse. "a" is a straight line fitted by $Y = -70.4 + (56.8)X$. Only a few points near the RMP were taken for this fit. Curve "b" shows inward going rectification for depolarizing pulses and therefore was more conveniently fitted by a quadratic function, $Y = -71.3 + (59.3)X + (17.4)X^2$, instead of a straight line. After TTX the curve ("c") shows outward going rectification: $Y = -71 + (52.2)X - (11.0)X^2$. For all fitted functions: $r \geq 0.998$, Y = membrane potential and X = injected current. Samples of current (top) and voltage (bottom) pulses are shown in **B** (control) and **C** (same cell after TTX)

date-putamen neurons (Galarraga et al. 1985; Kita et al.1985b; Calabresi et al. 1987a). However, in other neurons and excitable cells, outward currents have also been postulated to play a role in determining this type of voltage trajectory (Connor 1975; Gustafsson et al. 1982; Galvan 1982; Galvan and Sedlmeir 1984; Segal et al. 1984; Salkoff 1985). Figure 2B, illustrates an early outward-going deviation from the expected passive response during the slowly-rising depolarization, in spite of inward conductances present in control conditions (see Fig. 1). The slow depolarization (active response) is superimposed on the electrotonic response (dashed line) expected for the intensity of the applied pulse assuming linear properties of the membrane (Bargas et al. 1988). It can be seen that the early part of the active response is less than that expected for electrotonus, suggesting the prevalence of outward currents upon the early part of the response. However, later in the pulse, the response is greater than that expected for electrotonus, indicating the prevalence of inward currents. Therefore, outward and inward currents, acting sequentially or simultaneously, shape the response in control conditions, as if the membrane time constant had been enormously increased. The result is a delayed firing.

The predominance of outward currents early in the response to depolarizing stimuli can also be inferred from Fig. 2D. Here the response to increasing amplitudes of depolarizing (and hyperpolarizing) currents are superimposed. At the beginning of the slow depolarizations, the traces superimpose as if they were being forced downwards (arrow). This is not seen for hyperpolarizing currents. Figure 2C (action potential clipped) shows that the firing latency could be hundreds of milliseconds without evidence of accomodation (cf., Frankenhauser and Vallbo 1965). Similar voltage trajectories can be seen in Figs. 1B, 3C, 4A, 5A which are very similar to the ones reported in previous studies (Connor 1975; Galvan 1982; Gustafsson et al. 1982).

These results suggest that outward-going ionic conductances can be manifested during the early part of a subthreshold response in control conditions. Next, we will provide indirect evidence that an early activated conductance which can be de-inactivated by a previous hyperpolarization may contribute to these outward-going rectification.

Effect of hyperpolarizing prepulses on firing latency and frequency

Transient outward currents reported in other systems are partially inactivated at an RMP of –60 or –70 mV (Gustafsson et al. 1982; Segal et al. 1984; Dekin and

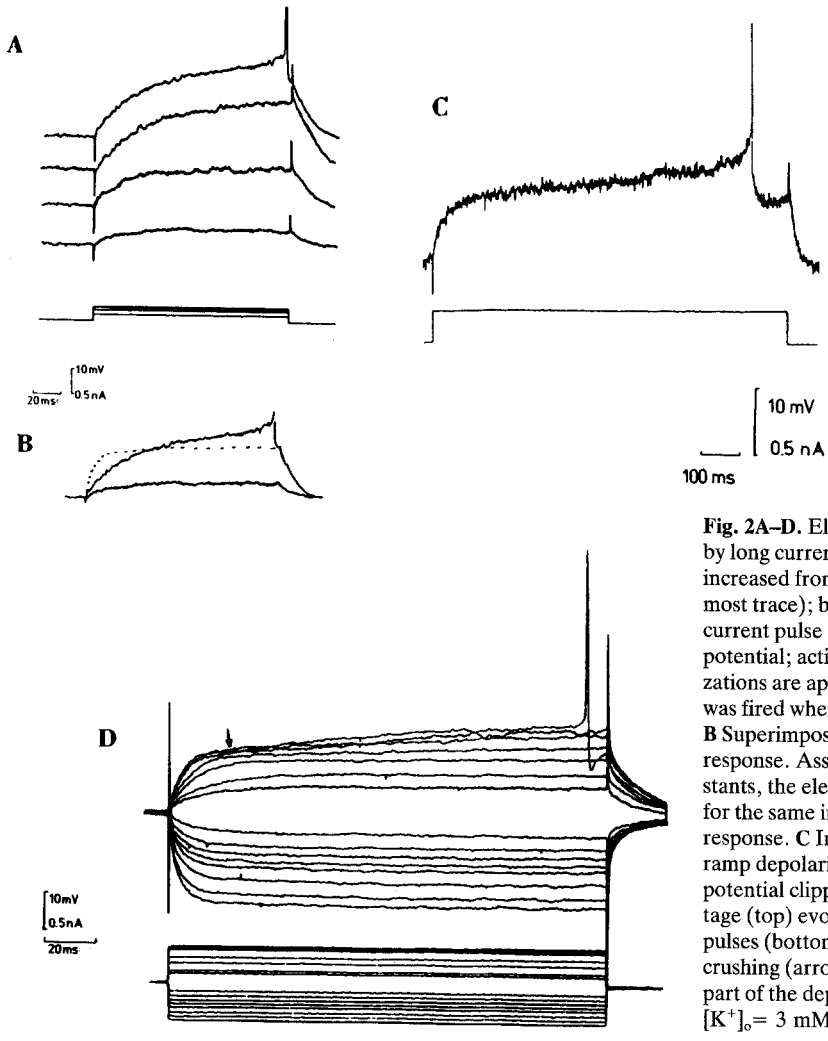

Fig. 2A–D. Electrotonic and subthreshold potentials induced by long current pulses. **A** Above, responses that were increased from a minimum (bottom) to a maximum (uppermost trace); below, current pulses. We assumed that the current pulse of lowest strength only evoked an electrotonic potential; active responses expressed as slowly rising depolarizations are apparent at higher strengths; a spike (truncated) was fired when the slow depolarization reached the firing level. **B** Superimposition of the electrotonic potential with the active response. Assuming linearity of membrane electrical constants, the electrotonic response was normalized (dashed line) for the same intensity of stimulation used for the active response. **C** In another cell it is shown that latency and slow-ramp depolarization can last hundreds of milliseconds (action potential clipped). **D** Superimposed traces of membrane voltage (top) evoked by depolarizing or hyperpolarizing current pulses (bottom). Note the outward going rectification and the crushing (arrow) of the depolarizing responses at the very early part of the depolarizing pulse. **A, C, D** Different neurons. $[K^+]_o$= 3 mM. RMPs ≅ −80 mV

Getting 1984; Zbicz and Weight 1985). The possibility exists that a similar conductance might be partially inactivated in neostriatal cells at these membrane potentials. These potentials are better attained in caudate neurons if the $[K^+]_o$ is near 6 mM (Bargas et al. 1988). Thus, if inactivating outward-going conductances are present in neostriatal neurons, one should be able to increase their contribution during a depolarizing response by means of a conditioning hyperpolarization.

In Fig. 3A, B the effect of a constant hyperpolarizing pre-pulse on the firing characteristics of neostriatal neurons at variable strengths of stimulation can be seen. In Fig. 3C, D the effect of a variable pre-pulse on the firing characteristics of a constant stimulating pulse is shown. Special care to avoid any drifts in the resting potential and to discard records which presented such a drift were taken, since variations of the RMP could alter latency and frequency. Frequency of stimulation was 0.5 Hz. In column A, responses to stimulating currents of increasing intensity are shown. It can be seen that the firing frequency of the train increased and the latency to the first action potential decreased as the stimulus intensity was augmented. The discharge was tonic and without significant adaptation (Calabresi et al. 1987b). In column B, depolarizing currents of equal intensity than in control conditions were preceded by a constant hyperpolarizing conditioning pulse (same RMP). It can be seen that the effect of the pre-pulse was to prolong the latency to the first action potential and to decrease the firing frequency during the pulse. The latency increase was seen in more than 70% of the tested neurons. The reduction in frequency was clearly seen in about 40% of the tested neurons (N = 20).

Figures 3C and D illustrate the behavior of another neuron. In this case, the polarity of the pre-

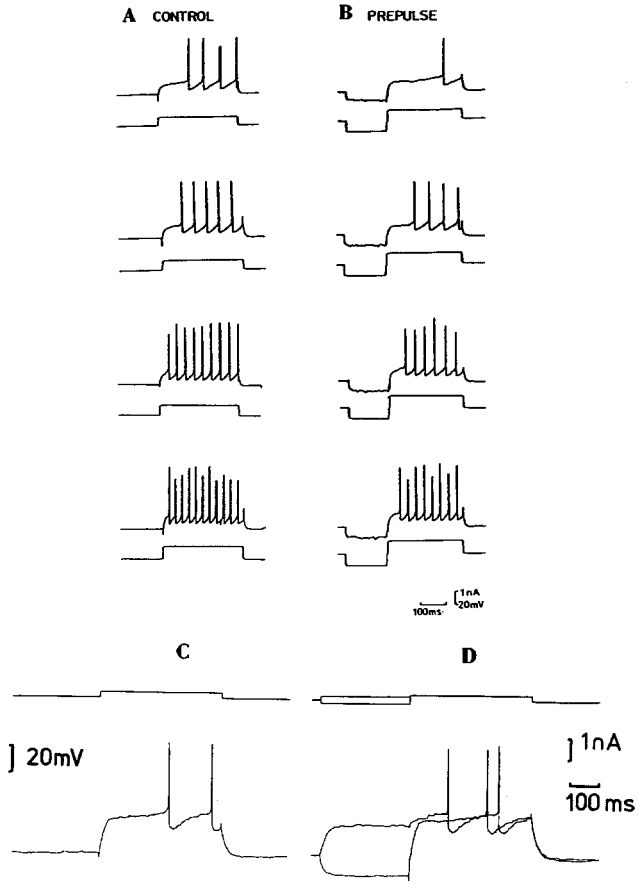

Fig. 3A–D. Effect of a conditioning pulse on firing latency and frequency of discharge. Four sets of recordings are shown in columns **A** (control) and **B** (with a constant conditioning hyperpolarizing pre-pulse). The strength of the stimulating current was the same in the absence or presence of the hyperpolarizing pre-pulse. From top to bottom the strength of the depolarizing current (lower trace) was increased. Firing frequency was proportional to the depolarizing current, and latency for the first action potential decreased as the strength of stimulation increased. The conditioning hyperpolarization prolonged the firing latency and reduced the frequency of discharge. $[K^+]_o \cong 6$ mM, RMP $\cong -70$ mV. **C** Suprathreshold stimulation to show control voltage trajectory toward the first spike in another neuron. **D** Two traces are superimposed. Same neuron as in **C** to show the changes induced in the voltage trajectory toward firing by conditioning hyperpolarizing and depolarizing subthreshold pulses. The depolarizing test-pulse remained constant in **C** and **D**. The conditioning pre-pulse was only changed in polarity in **D**. $[K^+]_o \cong 3$ mM, RMP $\cong -75$ mV

pulse varies and the intensity of the stimulation during the second depolarizing (test) pulse remains constant (0.39 nA in all cases). A control trace without the pre-pulse is seen in C showing the characteristic voltage trajectory toward spike threshold. The latency to the first spike was 245 ms. Two superimposed records of the same neuron with different pre-pulses are shown in D. In one case, the pre-pulse was hyperpolarizing and the latency for firing was 315 ms, in the other case the pre-pulse was depolarizing (subthreshold) and the latency to the first spike was 135 ms. Therefore, there is an increase in latency directly related to the previous transmembrane potential difference. Note that the voltage trajectory toward firing (Fig. 3D) is different when the pre-pulse is either depolarizing or hyperpolarizing. In spite of the fact that both test pulses begin at the same time and that they are of equal stimulation intensity, the pulse preceded by a depolarizing pre-pulse is of higher amplitude than the pulse preceded by a hyperpolarizing pre-pulse. Moreover, the voltage trajectory toward spike threshold evoked by the pulse preceded with a hyperpolarizing pre-pulse has a concave trajectory (see also Fig. 5A). Since this cannot be explained by different membrane time constants (Bargas et al. 1988), we can hypothesize that the voltage trajectory with the highest amplitude has less outward-going rectification than the trajectory with less amplitude. We suggest that the difference between both test pulses may be attributable to the preceding conditioning pulse, perhaps because a hyperpolarizing pre-pulse would de-inactivate part of the outward-going rectification present in this neurons.

The conclusion from these experiments is that there may be an early activated outward conductance that could be partially de-inactivated with a previous hyperpolarization if the RMP of neostriatal neurons is around –60 or –70 mV. Depending on its degree of activation, this conductance could play an important role in regulating latency and firing frequency in control conditions.

4-AP decreased the prolonged firing latency

Since 4-AP is known to preferentially block a transient outward conductance in other systems (see Rudy 1988), the effect of 4-AP was tested in the presence of hyperpolarizing conditioning pulses (Fig. 4). Records from A to D are from the same neuron and were taken at the same resting membrane potential using equal intensities of stimulation. In 4A, an action potential is shown, which was evoked after a typical depolarizing voltage trajectory. The neuron fired after a latency more than 200 ms, which was longer than in the absence of the pre-pulse (not shown but see Fig. 3). After adding 4-AP (500 μM) to the bath, the same protocol produced firing after a short latency (Fig. 4C, compare with 4A). Thus, 4-AP drastically reduced the firing latency. This suggests that activation of a 4-AP sensitive conductance helps to prolong the firing latency of neostriatal neurons.

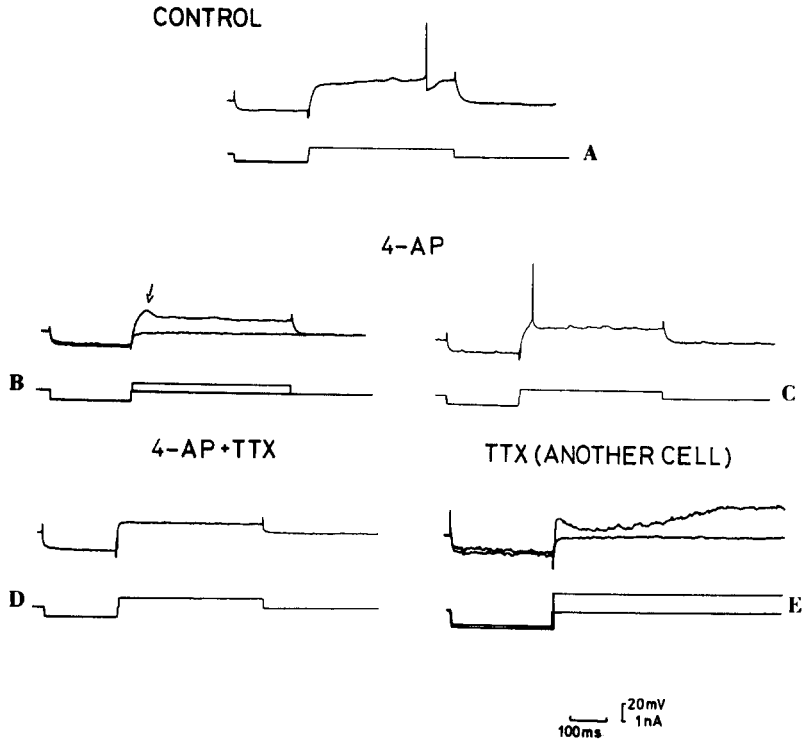

Fig. 4A–E. Effect of 4-AP on the firing latency. In every case a hyperpolarizing conditioning pulse preceded the injection of the depolarizing current. Upper trace: voltage; lower trace: current. Same neuron from **A** to **D**. **A** Control. **B**, **C** 20 min after addition of 4-AP (500 μM). **B** Subthreshold stimulation; two superimposed traces, in the absence (bottom) and presence (top) of the depolarizing pulse. An early "hump" (arrow) is seen in the top trace. **C** Threshold stimulation. **D** 30 min after adding TTX (1 μM) to the 4-AP containing solution. Same strength of stimulating current was used in **A**, **C**, and **D**. RMP (–71 mV) of this cell did not change appreciably throughout the experiment ($[K^+]_o \cong$ 5 mM). **E** 30 min after adding TTX (1 μM) in another cell; two traces superimposed in the absence (bottom) and presence (top) of the depolarizing pulse. Note the early hyperpolarizing response. RMP = –69 mV, $[K^+]_o \cong$ 5 mM

After adding 4-AP to the bath, a slow (± 50 ms), non-regenerative potential was evident at the beginning of the depolarizing pulse (Fig. 4B). This subthreshold depolarizing response (preceded by the hyperpolarizing pre-pulse) is compared with the passive relaxation from the hyperpolarizing pre-pulse alone (two traces superimposed in 4B). The main difference is the early appearance of a slow depolarizing "hump" (arrow) during the applied depolarizing current. This slow "hump" could hardly be a local response of a neostriatal action potential that normally lasts about 1.5 ms (to compare with a local response see Sugimori et al. 1978 Fig. 4D). The action potential in Fig. 4C, seems to arise from this "hump". The "hump" seems to repolarize very fast after it peaks, indicating that other outward currents may participate in the modulation of the slow ramp-like depolarization (see Fig. 6C and accompanying paper: Galarraga et al. 1989). After adding TTX (1 μM) to the bath (Fig. 4D), the "hump" could no longer be evoked, and firing was absent. However, note that a similar evoked "hump" (i.e., evoked after 4-AP) persisted after the addition of Co^{++} to the bath (Fig. 6C). This suggests that this slow "hump" mostly is the voltage manifestation of a Na^+ conductance (Calabresi et al. 1987a).

By comparing Fig. 4B and 4C, it is evident that other sources of outward-going rectification are still present. The amplitude and the time constants measured at the constant depolarizing test pulse are less after TTX (see also Fig. 1), while the amplitude and time constant measured at the constant hyperpolarizing pre-pulse show little (if any) change. These changes cannot be attributed simply to less input resistance because of the time of impalement or rundown, otherwise both the hyperpolarizing and the depolarizing pulses would show similar changes. The same result was obtained in five neurons (with 4-AP concentrations ranging from 0.5 to 2.0 mM). This suggests that the slow depolarizing potential and the action potential are partly dependent on TTX-sensitive ionic conductances.

From the effects of TTX described above, it can be inferred that, a slow Na^+-conductance is being activated at about the same membrane potential as the presumed early 4-AP sensitive outward conductance (Galarraga et al. 1985; Calabresi et al. 1987b). Since the subthreshold activation of these conductances produces opposite currents, it is expected that blockade of one will make the effect of the other more evident. Thus, blockade of the early outward conductance by 4-AP made the presence of a slow TTX-sensitive depolarization more obvious (Fig. 4B). To see whether the opposite was true, that is whether the blockade of the slow inward-going conductance made the activation of the outward-going conductance more evident, the experiment illustrated in Fig. 4E was done. As seen here, after

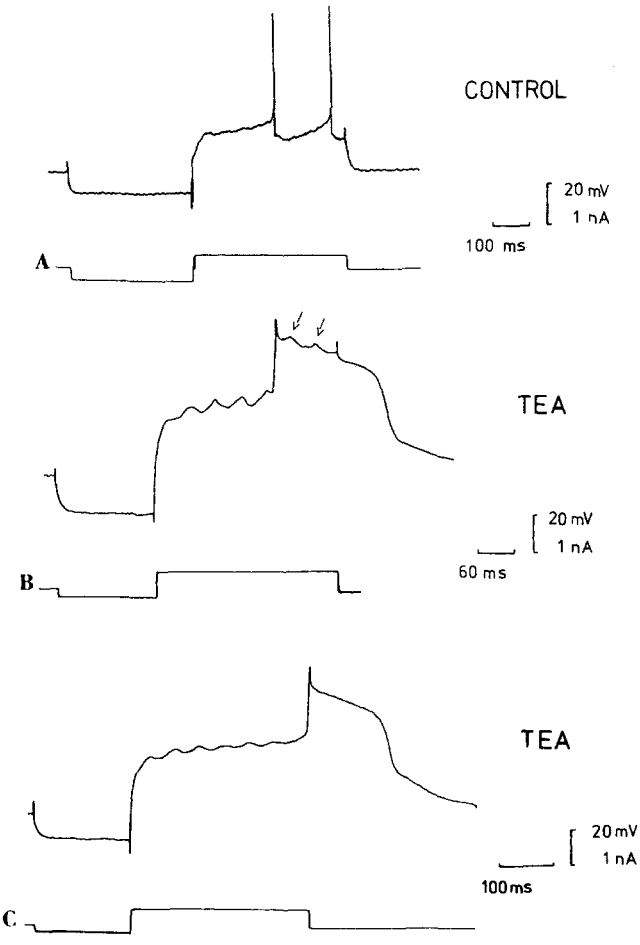

Fig. 5A–C. Effect of TEA on the firing latency. **A** Control. **B, C**, 30 min after adding TEA (20 mM). TEA-Cl substituted NaCl equimolarly. In contrast to 4-AP, TEA did not shorten the firing latency. Note the oscillations of the membrane potential in the presence of TEA. The oscillations were present even during the plateau of the action potential (arrows), but persisted only until the "off" of the depolarizing pulse. RMP = –75 mV. $[K^+]_o \cong 3$ mM

adding TTX only, a depolarizing current pulse applied after a hyperpolarizing conditioning pulse produced a hyperpolarizing "sag" instead of the depolarizing "hump" seen after 4-AP (Fig. 4B). Although variable in amplitude and duration from neuron to neuron, the hyperpolarizing response was a consistent finding, if the depolarizing test pulse was preceded by a hyperpolarizing pre-pulse. This response may be interpreted as an augmentation of the outward-going rectification manifested in many neurons as a concave voltage trajectory if preceded by a conditioning pulse (see: Figs. 3D and 5A). This concave voltage trajectory or "sag" has been shown by many investigators in other neurons and excitable cells (Connor 1975; Galvan 1982; Gustafsson et al. 1982; Galvan and Sedlmeir 1984; Segal et al. 1984; Salkoff 1985). It can be shown that it persists in the presence of divalent calcium blockers (see Fig. 5F of the accompanying paper: Galarraga et al. 1989). However, it is worth to noting here that a hyperpolarizing "sag" is hardly seen when coming from the "off-break" of a hyperpolarizing pulse alone (Jahnsen and Llinás 1984; Yarom et al. 1985). In the neostriatal neuron, a depolarizing pulse has to follow the conditioning hyperpolarization to evoke this kind of response. This could be due to different voltage sensitivity of the early-activated conductance in different neurons (see Rudy 1988) and shows that the voltage manifestation of similar ionic conductances could vary from neuron to neuron. So far, the results suggest that in normal conditions, both inward and outward conductances are activated around the same voltage range when the cell is depolarized.

In summary, 4-AP, a blocker of some early-activated K^+-conductances at millimolar concentrations (Thompson 1982; Gustaffson et al. 1982; Galvan 1982; Galvan and Sedlmeir 1984; Segal et al. 1984; Josephson et al. 1984; Zbicz and Weight 1985; Numann et al. 1987), unmasked a depolarizing potential early in the response and practically abolished the long firing latency which is characteristic of neostriatal neurons. On the other hand, TTX, an inward sodium current blocker, unmasked a hyperpolarizing response which appeared to resist the induced depolarization. As 4-AP and TTX blocked opposite conductances (outward and inward respectively), they produced opposite effects.

TEA did not block the prolonged firing latency

As shown in Fig. 5, the firing latency observed after a hyperpolarizing conditioning pre-pulse, was still present after TEA. TEA's effect on neostriatal neurons can be seen as a prolongation of the action potential (Fig. 5B, C). Even though both TEA and 4-AP are K^+ conductance blockers, they appear to have very different effects on neostriatal neurons (Kita et al. 1985b, c; Calabresi et al. 1987b). The present result confirms the findings of these authors. Furthermore, in these conditions another phenomenon was present in several occasions during the voltage trajectory that preceded firing: comparing the traces in 5A and B, it is seen that TEA, together with the hyperpolarizing pre-pulse, induced the appearance of oscillations of the membrane potential during the slow depolarization (together with an increase in input resistance, see: Bargas et al. 1988). These oscillations followed a regular frequency and could even be observed on top (arrows) of the slow

Ca-potential (Kita et al. 1985b; Calabresi et al. 1987b; Cherubini and Lanfumey 1987) generated in the presence of TEA. This is not rare because in cardiac fibres the first phase of repolarization is attributed to a fast, 4-AP sensitive, outward current (Kenyon and Gibbons 1979). The oscillations disappeared briskly at the braking of the stimulating pulse (Fig. 5B). The oscillations of the membrane potential can be seen gradually attenuating during a lengthened firing latency (Fig. 5C). In the absence of the conditioning hyperpolarizing pre-pulse, TEA rarely induces the appearance of these oscillations suggesting that de-inactivation of the early outward conductance favors the appearance of oscillations of the membrane potential. This suggestion is in line with the known oscillatory properties of the I_A current (Connor 1985; Strong and Kaczmareck 1987).

Effect of 4-AP on the firing pattern

Normally, neostriatal neurons fire tonically for the duration of the stimulating current, even if this lasts more than one sec (not shown). In most cases the tonic discharge shows little adaptation (Calabresi et al. 1987b). The experiment presented in Fig. 6 shows that 4-AP changed the firing pattern from "tonic" (Fig. 6A) to one that could be classified as "phasic-tonic" (Fig. 6B). During the "phasic" component, the action potentials showed marked frequency adaptation, while no adaptation was observed in the "tonic" component (Fig. 6B). In addition, after 4-AP, neurons fired at irregular frequencies even during the "tonic" part of the discharge (Fig. 6B) having lost the regular firing observed before the 4-AP (Fig. 6A). Finally, after 4-AP, an after-depolarizing potential was observed together with an increase in the spike duration (compare 6A with 6B) (Storm 1987).

It has been reported that in neostriatal neurons (see accompanying paper: Galarraga et al. 1989) and in motoneurons (Barrett and Barrett 1976) calcium blockade first increases firing frequency and then prevents sustained repetitive discharge. Here, this effect of the blockade of the Ca^{++} channels with Co^{++} (3 mM) was examined in the presence of 4-AP. In this condition, neostriatal neurons did not fire repetitively anymore (Galarraga et al. 1989). They fired only one action potential followed by a slow depolarizing potential (Fig. 6C, arrow). Moreover, subthreshold currents, after 4-AP and Co^{++}, still evoked the slow "hump" at the beginning of the depolarization as was previously shown in 4-AP without Co^{++} (see Fig. 4B). Thus, in contrast with TTX, Co^{++} did not abolish the slow "hump" which

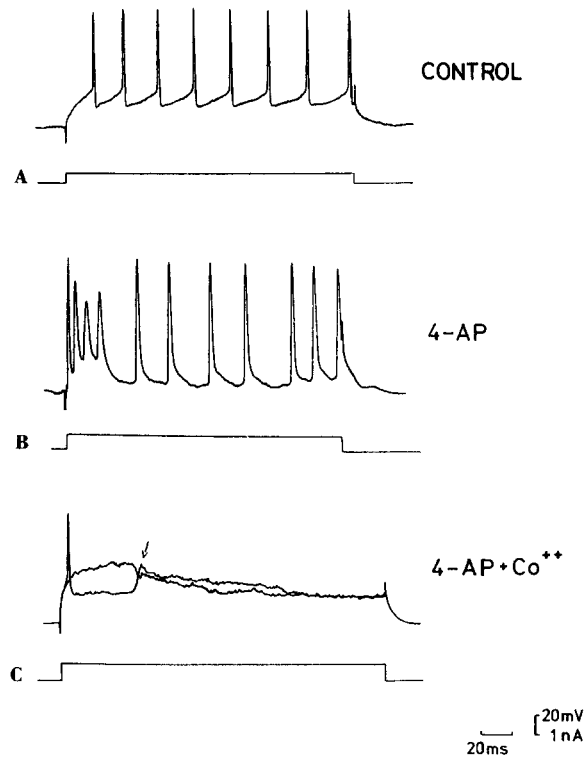

Fig. 6A–C. Effects of 4-AP on the firing discharge. **A** Firing discharge evoked by a long depolarizing pulse. **B** Firing evoked 30 min after adding 4-AP (1 mM). Note the high frequency firing at the beginning of the pulse and the irregularity of the interspike intervals. **C** After 30 min of addition of Co^{++} to the 4-AP-containing medium. Here two responses evoked by a depolarizing current of threshold strength are superimposed. One trace shows an action potential followed by a slow depolarizing potential (arrow); the other trace shows a subthreshold depolarizing potential. After adding Co^{++}, no repetitive firing could be evoked

appears in the presence of 4-AP at subthreshold stimuli (Fig. 6C). Note that the "hump" underlies the initial phasic burst that characterizes the firing pattern in the presence of 4-AP (compare 6B and 6C). A difference with the "hump" in Fig. 4B would be the slower rate of repolarization in the presence of Co^{++}. This could mean that Co^{++} may be blocking a part of the outward-going conductance which helps repolarize the "hump" (Galarraga et al. 1989). Furthermore, slow depolarizations can also be evoked after Mn^{++} or low Ca^{++}-saline (see Figs. 8D and 9G in: Galarraga et al. 1989). In conclusion, the slow potential facilitated by 4-AP is blocked by TTX and is not blocked by divalent cations. This suggests that it is Na^+ dependent. Note in Fig. 6C that the slow depolarization evoked by subthreshold stimulation and the slow depolarizing potential that followed the spike repolarized following the same time course, which suggests that both may be attributed to the same conductance (Calabresi et al. 1987a).

Discussion

The present results indicate that the voltage trajectory preceding the firing of neostriatal neurons in response to near threshold depolarizing currents, cannot be solely explained by voltage-dependent slow inward currents but that outward currents may also be involved. Several types of K^+ conductances could be present in neurons: namely, the delayed rectifier; at least one of the Ca-activated K^+ conductances; an early K^+ conductance; and an M-type conductance (see: Rudy 1988 for references). The aim of this work was to disclose the voltage manifestation of one of this components; the early activated potassium conductance. The effects of this early outward conductance were demonstrated by inducing changes in the firing latency (Figs. 3 and 4) and in the firing pattern (Figs. 3 and 6) of neostriatal neurons with a conditioning hyperpolarization.

Our results show that the effects of a conditioning pulse on the firing latency and frequency of neostriatal neurons were the expected ones for an early-activated and transient outward-going conductance. It was seen that a conditioning hyperpolarization increased the latency (i.e.: utilization time) to the first spike (Fig. 3B, D), and that in many occasions, the hyperpolarizing pre-pulse also decreased the firing frequency (Fig. 3B; see also: Connor 1982) of neostriatal neurons. These pre-pulse effects would be expected if the conditioning hyperpolarization increased the contribution of an outward-going rectification during the depolarizing response. This could occur by the removal of inactivation of such a conductance with hyperpolarization (Connor and Stevens 1971; Segal et al. 1984). This interpretation is supported by the fact that a conditioning depolarization had opposite effects (Fig. 3C, D).

The slowing of the firing frequency as well as the shaping of the interspike interval by an early-activated outward conductance have been discussed elsewhere (Connor 1982, 1985). It has also been emphasized that this type of conductance explains, in part, the "tonic" type of discharge in neurons (Connor 1982, 1985). In line with this, 4-AP disrupted the tonic firing pattern in neostriatal neurons and converted it in a kind of phasic-tonic pattern albeit quite irregular in its tonic component (Fig. 6B). It is worth noting that blockade of other K^+-conductances does not affect firing pattern in the same way (Galarraga et al. 1989). For instance, a probable blockade of the Ca^{++}-dependent component of the outward conductance first increased and after decreased the firing pattern (Galarraga et al. 1989). It was also shown here that Co^{++} may reduce the rate of repolarization of the "hump" that appears in the presence of 4-AP (Fig. 6C). This slow depolarization seems to underlie the phasic component or burst which appears at the beginning of the phasic-tonic train facilitated by 4-AP (Fig. 6B).

In the neostriatal neurons tested, the effects of the hyperpolarizing conditioning stimulation were abolished by 4-AP. Thus, when 4-AP was used the neurons fired with a short latency, losing their characteristic firing delay and voltage trajectory toward the first action potential (Fig. 4). This was evident in the presence of the same stimulation protocol as that used in control conditions (Fig. 4C). As mentioned, in the presence of 4-AP threshold or suprathreshold stimulation produced the action potentials on the top of a slow depolarizing potential or "hump" (Figs. 4BC, 6BC). This "hump" disappeared in the presence of TTX but persisted in the presence of Co^{++}. Therefore, it is probably dependent on Na^+. Since this "hump" was slower than expected for a local response of the fast sodium spike and since a slow sodium conductance has been postulated as playing a role in the slow voltage trajectory toward spike threshold (Calabresi et al. 1987a), we tend to think that this "hump" evoked in the presence of 4-AP (Figs. 4B, 6C) reflects the voltage manifestation of a mostly slow Na^+-conductance acting in the subthreshold region. Thus, inward and outward subthreshold conductances may activate at a similar voltage range during depolarizing responses near the spike threshold. However, to our knowledge, nobody has explored the possibility of a Ca^{++}-conductance acting at subthreshold voltage levels, so that this contribution cannot be ruled out.

In contrast to 4-AP, the effect of a conditioning hyperpolarization on the latency was relatively resistant to TEA (Fig. 5). 4-AP sensitivity together with TEA resistance points towards a particular kind of early-activated conductances; the so-called I_A-type of potassium conductances that are reported to be present in many excitable cells (Rogawski 1985) with some degree of variation (Dolly 1988; Rudy 1988). A conductance of this type was suspected in an earlier study because of the characteristic voltage trajectory (see Figs. 2, 3C, 5A) during threshold depolarizing responses (Cherubini and Lanfumey 1987). Indeed, our records showing the voltage trajectory toward firing are similar to the ones reported by previous studies in which an I_A type of current has been confirmed by voltage-clamp (Connor 1975; Gustafsson et al. 1982; Galvan 1982; Galvan and Sedlmeir 1984; Segal et al. 1984; Salkoff 1985). Moreover, a parallel voltage-clamp study has confirmed the existence of an I_A-like current in cultured striatal neurons (Surmeier et al. 1988). Therefore, it is likely that future studies on the firing pattern and on its

underlying responsible currents will further confirm and characterize this conductance. In this respect, other investigators have suspected the presence of an early-activated outward conductance using intracellular current injection methods with or without associated voltage-clamp techniques (Jahnsen and Llinás 1984; Dekin and Getting 1984; Yarom et al. 1985). In some of these cases a voltage manifestation of the early outward-going rectification was a delay in the off-break of an induced hyperpolarizing pulse. A similar delay is not conspicuous in most neostriatal neurons. Aside from the variability on voltage-sensitivity, threshold, etc. which has been demonstrated between excitable cells (Dolly 1988; Rudy 1988), this showed us that a unique protocol of stimulation is not enough to reveal the voltage manifestation of a given "ubiquitous" conductance in different neurons.

In conclusion, in physiological conditions the firing latency of neostriatal neurons seems to be determined mainly by the interplay of at least two opposed conductances. The firing latency would depend on how fast the slowly rising depolarization reaches threshold level (Fig. 2). A slow TTX-sensitive, Co^{++}-resistant conductance may be an important inward-going component during the slowly rising depolarization (Galarraga et al. 1985; Calabresi et al. 1987a). This component would bring the neuron toward threshold level. At the beginning of the response, an early activated, 4-AP sensitive, TEA-resistant component seems to be a main source for the outward-going rectification. This outward component would tend to slow the process of reaching firing threshold. If somehow the outward component is reduced, the contribution of the inward component increases and manifests itself as a "hump" with a faster rise (Figs. 4B and 6C). Alternatively, if the inward component is reduced, the contribution of the outward component increases and manifests itself as a hyperpolarizing concave trajectory that retards the process (Fig. 4E). A similar arrangement of membrane voltage events was reported in a mainly voltage-clamp work of cultured hippocampal neurons where it was shown that the outward current modulating the early part of the voltage response was due to an I_A type of current and that later parts of this response were due to TEA and Cd^{++} sensitive components (see: Fig. 22 of Segal and Barker 1984). Therefore, the voltage trajectory before firing, and probably between spikes, would be evenly shaped by the participation of these conductances acting at similar voltage range. In neostriatal neurons too, the participation of other conductances at later times during the response is likely to occur (Galarraga et al. 1989). Indeed, when TEA was added the latency was not affected but the voltage trajectory toward firing did not remain smooth (Fig. 5BC). Oscillatory properties of the system of opposed conductances were revealed.

Recently, it has been proposed that the A-current would also shape the action potential configuration by participating in the repolarization (Belluzzi et. 1985; Storm 1987; Belluzzi and Sacchi 1988). As low concentration of 4-AP broadenend the spike and favored the appearance of an afterdepolarization in neostriatal neurons (Fig. 6B), it is likely that this type of conductance influences firing behavior in this way too.

The results presented here would suggest that, if an early type of potassium current is present in neostriatal neurons, any transmitter that affects it (Strong 1984; Aghajanian 1985; Nakajima et al. 1986) would also modify the firing pattern, the latency, the threshold and therefore, the firing characteristics of neostriatal neurons.

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References

Aghajanian GK (1985) Modulation of a transient outward current in serotonergic neurones by α_1-adrenoceptors. Nature (Lond) 315: 501–503

Bargas J, Galarraga E, Aceves J (1988) Electrotonic properties of neostriatal neurons are modulated by extracellular potassium. Exp Brain Res 72: 390–398

Barrett EF, Barrett JN (1976) Separation of two voltage-sensitive potassium currents and demonstration of a tetrodotoxin-resistant calcium current in frog motoneurons. J Physiol (Lond) 255: 737–774

Belluzzi O, Sacchi O (1988) The interactions between potassium and sodium currents in generating action potentials in the rat sympathetic neurone. J Physiol (Lond) 397: 127–147

Belluzzi O, Sacchi O, Wanke E (1985) A fast transient outward current in the rat sympathetic neurone studied under voltage-clamp conditions. J Physiol (Lond) 358: 91–108

Byrne JH (1980a) Analysis of ionic conductance mechanisms in motor cells mediating inking behavior in Aplysia californica. J Neurophysiol 43: 630–650

Byrne JH (1980b) Quantitative aspects of ionic conductance mechanisms contributing to firing pattern of motor cells mediating inking behavior in Aplysia californica. J Neurophysiol 43: 651–668

Calabresi P, Mercuri N, Stanzione P, Stefani A, Bernardi G (1987a) Intracellular studies on the dopamaine-induced firing inhibition of neostriatal neurons in vitro: evidence for D1 receptor involvement. Neuroscience 20: 757–771

Calabresi P, Misgeld U, Dodt HU (1987b) Intrinsic membrane properties of neostriatal neurons can account for their low level of spontaneous activity. Neuroscience 20: 293–303

Cherubini E, Lanfumey L (1987) An inward calcium current underlying regenerative calcium spikes in rat striatal neurons in vitro enhanced by BAY K 8644. Neuroscience 24: 997–1005

Connor JA (1975) Neural repetitive firing: a comparative study of membrane properties of crustacean walking leg axons. J Neurophysiol 38: 922–932

Connor JA (1982) Mechanisms of pacemaker discharge in invertebrate neurons. In: Carpenter DO (ed) Cellular pacemakers. J Wiley and Sons, New York

Connor JA (1985) Neural pacemakers and rhythmicity. Ann Rev Physiol 47: 17-28

Connor JA, Stevens CF (1971) Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma. J Physiol (Lond) 213: 31–53

Dekin MS, Getting PA (1984) Firing pattern of neurons in the nucleus tractus solitarius: modulation by membrane hyperpolarization. Brain Res 324: 180–184

Dolly JO (1988) Potassium channels – what can the protein chemistry contribute? TINS 11: 186–188

Frankenhaeuser B, Vallbo AB (1965) Accomodation in myelinated nerve fibers of Xenopus laevis as computed on the basis of voltage clamp data. Acta Physiol Scand 63: 1–20

Galarraga E, Bargas J, Aceves J (1985) Slow sodium and I_A currents in neostriatal neurons. Soc Neurosci Abstr 11: 202

Galarraga E, Bargas J, Sierra A, Aceves J (1989) The role of calcium in the repetitive firing of neostriatal neurons. Exp Brain Res 75: 157–168

Galvan M (1982) A transient outward current in rat sympathetic neurones. Neurosci Lett 31: 295–300

Galvan M, Sedlmeir C (1984) Outward currents in voltage-clamped rat sympathetic neurones. J Physiol (Lond) 356: 115–133

Gustafsson B, Galvan M, Grafe P, Wigström H (1982) A transient outward current in a mammalian central neuron blocked by 4-aminopyridine. Nature (Lond) 299: 252–254

Jahnsen H, Llinás R (1984) Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurons in vitro. J Physiol (Lond) 349: 227–247

Josephson IR, Sanchez-Chapula J, Brown AM (1984) Early outward current in rat single ventricular cells. Circ Res 54: 157–162

Kenyon JL, Gibbons WR (1979) 4-Aminopyridine and the early outward current of sheep cardiac purkinje fibers. J Gen Physiol 73: 139–157

Kita H, Kita T, Kitai ST (1985a) Active membrane properties of rat neostriatal neurons in an in vitro slice preparation. Exp Brain Res 60: 54–62

Kita H, Kita T, Kitai ST (1985b) Regenerative potentials in rat neostriatal neurons in an in vitro slice preparation. Exp Brain Res 60: 63–70

Kita T, Kita H, Kitai ST (1985c) Effects of 4-Aminopyridine (4-AP) on rat neostriatal neurons in an in vitro slice preparations. Brain Res 361: 10–18

Kitai ST, Kita H (1984) Electrophysiological study of the neostriatum in brain slice preparation. In: Dingledine R (ed) Brain slices, Plenum Press, New York, pp 285–296

Misgeld U, Okada Y, Hassler R (1979) Locally evoked potentials in slices of rat neostriatum: a tool for the investigation of intrinsic excitatory processes. Exp Brain Res 34: 575–590

Nakajima Y, Nakajima S, Leonard RJ, Yamaguchi K (1986) Acetylcholine raises excitability by inhibiting the fast transient potassium current in cultured hippocampal neurons. Proc Natl Acad Sci USA 83: 3022–3026

Numann RE, Wadman WJ, Wong RKS (1987) Outward currents of single hippocampal cells obtained from the adult guinea-pig. J Physiol (Lond) 393: 331–353

Rogawski MA (1985) The A-current: how ubiquitous a feature os excitable cells is it? TINS 8: 214–219

Rudy B (1988) Diversity and ubiquity of K channels. Neuroscience 25: 729–749

Salkoff L (1985) Development of ion channels in the flight muscles of *Drosophila*. J Physiol (Paris) 80: 275–282

Segal M, Barker JL (1984) Rat hippocampal neurons in culture: potassium conductances. J Neurophysiol 51: 1409–1433

Segal M, Rogawski MA, Barker JL (1984) A transient potassium conductance regulates the excitability of cultured hippocampal and spinal neurons. J Neurosci 4: 604–609

Storm JF (1987) Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. J Physiol (Lond) 385: 733–759

Strong JA (1984) Modulation of potassium current kinetics in bag cell neurons of Aplysia by an activator of adenylate cyclase. J Neurosci 4: 2772–2783

Strong JA, Kaczmareck LK (1987) Potassium currents that regulate action potentials and repetitive firing. In: Kaczmarek LK, Levitan IB (eds) Neuromodulation. Oxford University Press, Oxford, pp 119–137

Sugimori M, Preston RJ, Kitai ST (1978) Response properties and electrical constants of caudate nucleus neurons in the cat. J Neurophysiol 41: 1662–1675

Surmeier DJ, Bargas J, Kitai ST (1988) Voltage-clamp analysis of a transient potassium current in rat neostriatal neurons. Brain Res 473: 187–192

Thompson S (1982) Aminopyridine block of transient potassium current. J Gen Physiol 80: 1–18

Yarom Y, Sugimori M, Llinás R (1985) Ionic currents and firing patterns of mammalian vagal motoneurons in vitro. Neuroscience 16: 719–737

Zbicz KL, Weight FF (1985) Transient voltage and calcium-dependent outward currents in hippocampal CA3 pyramidal neurons. J Neurophysiol 53: 1038–1058

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