ERK Integrates PKA and PKC Signaling in Superficial Dorsal Horn Neurons II: Modulation of Neuronal Excitability

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Running Head: ERK Modulation of Dorsal Horn Membrane Properties

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Abstract:
Protein kinases belonging to the PKA, PKC and ERK families have been identified as key players in modulating nociception at the level of the spinal cord dorsal horn, yet little is known about the effects of these kinases on membrane properties of the dorsal horn neurons. PKA, PKC and ERK exert inhibitory effects on transient potassium currents (A-type currents or $I_A$) in mouse superficial dorsal horn neurons (Hu et al. 2003). Here we aimed to determine the effects of these kinases on action potential firing and membrane properties of these neurons to evaluate the impact of the modulation of $I_A$ (and other conductances) in these neurons. We find that activating PKC and PKA have dramatic effects on action potential firing reflecting an increase in the excitability of superficial dorsal horn neurons. In addition, we find that inhibitors of both PKC and ERK signaling decrease the excitability of dorsal horn neurons, suggesting that these kinases exert a tonic excitation of these cells. Consistent with our findings that these kinases inhibit A-type currents, we found that PKA, PKC and ERK act to shorten the first spike latency following depolarization induced by current injection. In addition, activation of these kinases increases spike frequency and action potential amplitude of dorsal horn neurons. Interestingly, we find that the effects of PKA and PKC activators are blocked by inhibitors of ERK signaling, suggesting that PKA and PKC may exert their actions by activation of ERKs.

Keywords: pain, phosphorylation, Kv4, central sensitization, MAPK, action potential
INTRODUCTION:

The sensation of pain, like other sensory modalities, is subject to modification by experience. In particular, pain sensitivity can be dramatically increased following an injury or in response to prolonged activation of nociceptive primary afferent neurons. A large body of literature suggests that this nociceptive sensitization can occur at the level of the spinal cord dorsal horn, and further that this so called “central sensitization” is induced by the activation of a number of protein kinases including protein kinase A (PKA), protein kinase C (PKC), and extracellular signal regulated kinases (ERKs) (Ji and Woolf 2001; Karim et al. 2001).

This and the accompanying study are concerned with understanding the changes in neuronal excitability in the dorsal horn that occur in response to activation of PKA, PKC, or ERKs. We have shown that activation of these protein kinases leads to an inhibition of transient outward potassium currents (known as A-type currents or $I_A$) in mouse superficial dorsal horn neurons from mice(Hu et al. 2003). Previous studies in other brain regions have revealed that modulation of A-type currents can alter both first spike latency and spike frequency (McCormick and Huguenard 1992; Yang et al. 2001). In the present study, we examine the modulation of neuronal excitability by these kinases, with a particular eye to evaluating the impact of kinase-mediated inhibition of $I_A$ on neuronal firing properties. We report that activation of PKA, PKC, and ERK lead to a decrease in first spike latency, and an increase in spike frequency in response to direct current injection. Furthermore, the effects of PKA and PKC activation appear to require activation of ERK, as blocking ERK activation prevents the modulation of firing properties induced by activators of PKA or PKC. This mirrors nicely the interaction of these kinases in the modulation of $I_A$(Hu et al. 2003). We suggest that ERK phosphorylation of channels underlying $I_A$ lead to at least the alteration in first spike
latency we have observed here, and further that the effects of PKA and PKC are due to their ability to activate ERK signaling.

MATERIALS AND METHODS

Cell culture

Primary cultures of spinal cord superficial dorsal horn neurons were prepared from 5- to 10-day-old ICR mice using a procedure similar to a previously described method (Hugel and Schlichter 2000). Briefly, after decapitation a laminectomy was performed and the spinal cord was carefully removed. Spinal cord superficial dorsal horn was isolated by cutting through lamina III with a surgical blade, and cut into 1-2 mm fragments. The tissue fragments were incubated for 45 min at 37°C in Hanks' balanced salt solution (HBSS, Invitrogen Life Technologies, Carlsbad, CA) (in mM: 137 NaCl, 5.4 KCl, 0.4 KH$_2$PO$_4$, 1 CaCl$_2$, 0.5 MgCl$_2$, 0.4 MgSO$_4$, 4.2 NaHCO$_3$, 0.3 Na$_2$HPO$_4$, 5.6 Glucose) containing papain (15 U/ml, Worthington Biochemical Corporation, Lakewood, NJ), rinsed 3 times with HBSS, and then replaced with culture medium containing Neurobasal medium (Gibco), fetal calf serum (5%, Gibco), heat-inactivated horse serum (5%, Gibco), L-glutamax-1(2 mM, Gibco), B-27 (2%, Gibco), glucose (20 mM, Sigma). The fragments were mechanically dissociated by gently triturating with a fire-polished Pasteur pipette. The resulting cell suspension was plated onto 12 cm poly-d-lysine and collagen-coated coverslips, and cultured for 6 to 8 days (for action potential recordings) or 3 days (for Na$^+$ and Ca$^{2+}$ current recordings) in humidified air with 5% CO$_2$ at 37°C.

Electrophysiological recording

Whole-cell current clamp recordings were made by standard procedures at room temperature using either a Patch Clamp PC-501A amplifier (Warner Instrument
Corporation, Holliston, MA) and CLAMPEX 8.0 software (Axon Instruments, Union City, CA), or an EPC-10 amplifier and Pulse software (HEKA Elektronik, Lambrecht, Germany). Electrodes were pulled from filamented borosilicate glass and fire-polished. The electrode solution contained (in mM): 140 potassium methylsulfate, 2 MgCl$_2$, 1 EGTA, 10 HEPES, 3 Na$_2$ATP, 0.3 Na$_2$GTP, pH adjusted to 7.4 with KOH. Pipette resistances were 4-7 MΩ. Most neurons had series resistances around 10-14 MΩ (ranged from 8 to 20 MΩ). The bath solution was HBSS. Recordings of Na$^+$ currents and Ca$^{2+}$ currents were performed under voltage clamp configurations, and the membrane voltage was held at −80 mV. All neurons included in this study had resting membrane potentials < −50 mV and had stable input resistance. For Na$^+$ current recordings, the extracellular solution contained (in mM) 95 NaCl, 5 KCl, 10 CsCl, 20 TEA-Cl, 1 MgCl$_2$, 1 CoCl$_2$, 1 CaCl$_2$, 5 HEPES and 10 Glucose. The intracellular pipette solution contained (in mM) 120 mM CsCl, 20 TEA-Cl, 2 MgCl$_2$, 1 EGTA, 10 HEPES, 3 Na$_2$ATP, 0.3 Na$_2$GTP. The same intracellular solutions were used for Ca$^{2+}$ current recordings, and the extracellular solution was the same except that 1 µM TTX was added, CoCl$_2$ was omitted, and 2-3 mM BaCl$_2$ was used instead of 1 mM CaCl$_2$.

**Drug application.**

4-aminopyridine (4-AP) was purchased from Sigma (St. Louis, MO), and dissolved in water as stock solution. PMA, PD098,059, U0126, 8-Br-cAMP, PKI-(6-22) amide, Forskolin (FSK), IBMX, and Ro31-8220 were purchased from Biomol (Plymouth Meeting, PA) and prepared as concentrated stock solutions in DMSO. All of these were diluted to final concentration in extracellular solution. Coverslips were placed in a small laminar flow perfusion chamber and continuously perfused with extracellular solution or various pharmacological manipulations at approximately 2 ml/min.
Data analysis

Off-line evaluation was done using Clampfit (Axon Instruments), Pulsefit (HEKA Elektronik) and Origin 5.0 software (Microcal, Northampton, MA). Data are expressed as original traces or as mean ± s.e.m. Treatment effects were statistically analyzed by paired or independent Student's t-test. Error probabilities of $P<0.05$ were considered statistically significant.
Results:

In our conditions, neurons were grown in culture for less than 3 days were not able to fire action potentials in response to current injection. This is likely due to a low density of Na⁺ channels (Wolff et al. 1998). After 3 days in culture, most neurons have longer neurites, and a small percentage of these cells can generate single spikes with relatively high thresholds. These action potentials are TTX sensitive (data not shown). After 6 days in culture, most neurons were able to generate multiple spikes in response to current injection from a holding potential of –70 mV. Among the 78 neurons tested after 6 days in culture, the population can be divided into the following four categories based on their firing properties (Fig.1): repetitive (49%), delayed firing (29%), phasic (those neurons showing spike frequency adaptation, 18%) and single spike (4%, not included in present study). These firing properties resemble those described for rat and hamster neurons in slices (Grudt and Perl 2002; Hochman et al. 1997). Under voltage clamp, all neurons we recorded displayed A-type currents. In many cell types, A-type currents are more sensitive to 4-AP blockade than sustained K currents (Hille 1992). Therefore, in an effort to evaluate the impact of A-type currents on neuronal excitability, we first tested the effect of 4-AP on action potential firing and membrane properties. Multiple action potentials were generated by a step of depolarizing current injection at holding potential of –70 mV (maintained by adjusting current injection in this and the remaining experiments). In response to bath application of 1 mM 4-AP for 1-2 min, first spike latency (the time from initiating current injection to the first action potential) was decreased by 71.1±6.5%, spike frequency (defined as the inverse of the interval between the first two spikes) was increased by 51.5±11.5%, action potential amplitude was increased by 13.5±2.9%, action potential threshold was reduced from –39 ±1.2mV to –46 ± 2.0mV, resting membrane potential was increased from –58 ±1.6 mV to –52
±1.5 mV and input resistance was slightly increased by 9.3±3.5%. The effects of 4-AP were reversible. Under voltage clamp, 4-AP reduced A-type currents by 59.2±1.8% (Fig.2). While not conclusive, these data suggest that A-type currents may regulate action potential firing in superficial dorsal horn neurons.

**PKC effects on excitability of neurons in superficial dorsal horn**

We have demonstrated that PKC activation modulates A-type K+ channels (Hu et al. 2003). To determine whether PKC modulates neuronal excitability, we investigated the effects of PMA, a PKC activator, on action potentials evoked by direct current injection. 5 μM PMA reduced first spike latency by 58.8±11.3%, increased spike frequency by 35.4±4.7%, and decreased action potential threshold from –39.5±1.8 mV to –42.8±1.2 mV. PMA also elicited a small but reproducible increase in action potential amplitude (5.2±0.9%), but did not significantly change resting membrane potential or input resistance (Fig.3). These results show that PMA increases superficial dorsal horn neuron excitability. To confirm that the effects of PMA on action potentials were a specific consequence of PKC activation, we examined the effects of a PKC inhibitor on action potentials. In response to addition of 1 μM Ro31-8220, a PKC inhibitor, to the perfusion system for 5 min, first spike latency was increased by 138±45% and spike frequency was decreased by 32.0±5.8%. Ro31-8220 also produced a small but significant decrease in action potential amplitude (4.3±1.1%) and a slight increase in action potential threshold (from –41±2.6 mV to –39±2.5 mV), but had no effect on resting membrane potential or input resistance (Fig.3). Thus, the effect of Ro31-8220 was the opposite effect of PMA on neuronal excitability for each property that was modulated by PMA. We further tested whether PKC inhibition blocks the effects of PMA. When Ro31-8220 (1 μM) was applied prior to and during the application of PMA, no effects of PMA
were observed (Fig.3). These data support our hypothesis that PKC activation potentiates neuronal excitability, and further suggest that there is tonic PKC modulation of neuronal excitability in cultured spinal cord dorsal horn neurons.

**PKA activation enhances neuronal excitability**

Activators of PKA have been found to decrease A-type currents in the brain and spinal cord (Hoffman and Johnston 1998; Hu et al. 2003). To ascertain the effects of PKA activation on neuronal excitability in superficial dorsal horn neurons, we tested the effects of Forskolin (FSK), an adenylyl cyclase activator, on action potential properties. After application of 50 µM FSK with IBMX, an inhibitor of phosphodiesterases, first spike latency was decreased by 56.9±9.6% and spike frequency was increased by 38.5±9.6%, action potential amplitude was increased by 10.5±2.1%, action potential threshold was reduced from –38±1.8 mV to –43±2.4 mV and resting membrane potential was increased from –57±5.0 mV to –52±6.3 mV with no a significant change in input resistance (Fig.4). When 50 µM PKI, a specific inhibitor of PKA, was included in the patch pipette, FSK did not produce a significant change in action potentials or membrane properties, suggesting that FSK+IBMX increased excitability by activating PKA (Fig.5). To further establish the role of PKA, we tested the effects of 8-Br-cAMP, a membrane permeable analog of cAMP, on action potential properties. As can be seen from figure 4, application of 8-Br-cAMP (1 mM) had effects similar to those of FSK. These results suggest that PKA activation enhances excitability of neurons in the superficial dorsal horn.
Inhibition of ERK signaling decreases neuronal excitability

Data from our lab indicate that ERK activation in the dorsal horn has an important role in inflammatory pain plasticity (Karim et al. 2001), and that ERK inhibition enhances A-type currents in dorsal horn neurons (Hu et al. 2003). To investigate whether ERK activation modulates neuronal excitability in the superficial dorsal horn, we applied PD098,059 and U0126, specific inhibitors of MEK (the upstream activator of ERK), to our cultures. After application of 20 µM PD098,059 for 4-5 min, first spike latency was increased by 35.3±5.6%, spike frequency was decreased by 35.8±5.9%, action potential amplitude was reduced by 10.2±2.5%. PD098,059 had no significant effects on resting membrane potential, action potential threshold or input resistance (Fig.6). Similarly, U0126 produced inhibitory effects on dorsal horn neurons. 500 nM U0126 prolonged first spike latency, reduced spike frequency and decreased action potential amplitude (Fig.6). These data indicate that decreasing ERK activity attenuates excitability of neurons in mouse superficial dorsal horn.

ERK inhibition prevents PKC and PKA excitatory effects in superficial dorsal horn neurons

The results discussed above show that ERK, PKC and PKA have similar effects on excitability of superficial dorsal horn neurons. To determine whether these kinases share a single pathway, we perfused 20 µM PD098,059 to neurons for 5 min, which caused a prolongation of first spike latency and a decrease in spike frequency that reached a steady state. Under these conditions, neither PMA nor FSK significantly altered any action potential or passive membrane properties (Fig.7). Thus, inhibition of ERK abolishes PKC- and PKA-mediated increases in neuronal excitability.
ERK inhibition has no effect on Na\(^+\) or Ca\(^{2+}\) currents, but reduce sustained K\(^+\) currents in dorsal horn neurons

The effects of PMA and FSK on ion channels have been studied extensively. However, little is known about effects of MEK inhibitors on sodium, calcium and sustained potassium channels. We tested whether changes in these currents could contribute to the inhibitory effects of PD098,059 on action potentials. Under voltage clamp, neurons were held at –80 mV, and six steps of depolarization from –50 mV to +50 mV evoked fast activating, fast inactivating voltage dependent Na\(^+\) currents. These currents were completely blocked by 500 nM TTX (data not shown). Application of 20 µM PD098,059 had no effect on Na\(^+\) currents (Fig.8). We then tested the effects of PD098,059 on voltage-gated Ca\(^{2+}\) currents. Ba\(^{2+}\) was used as the charge carrier. Application of 20 µM PD098,059 had no effect on Ba\(^{2+}\) currents (Fig.8). To test effect of PD098,059 on sustained K\(^+\) currents, we applied a prepulse protocol to –20 mV to inactivate the A-type K\(^+\) current. Sustained currents were evoked by a depolarizing step to 40 mV from a –80 mV holding potential after the prepulse. PD098,059 decreased sustained currents by 16% (Fig.8).

Discussion

The accompanying study reports that PKA, PKC and ERK activation lead to a decrease in A-type K\(^+\) currents in superficial dorsal horn neurons. In this study, we show that PKC, PKA and ERK increase excitability of neurons in spinal cord superficial dorsal horn. The changes in action potential firing properties induced by these kinases are consistent with A-type current inhibition. Thus, activation of PKC or PKA decreased first spike latency, increased spike frequency and increased action potential amplitude. In contrast, inhibition of PKC or ERK prolonged first spike latency, reduced spike frequency and
decreased action potential amplitude, while PKA inhibitors had no effect. In addition, inhibition of ERK prevented PKC- and PKA-mediated modulation of action potentials.

A-type channels are believed to be important in controlling the rate of action potential generation, delaying the onset of firing and lengthening interspike interval. Downregulation of A currents decreases first spike latency and increases spike frequency in other brain regions (Yang et al. 2001). Firing rate and first spike latency are two major parameters that determine the timing of neurotransmitter release. An important consequence of the A current is to delay first spike firing in response to small depolarizations. Data presented in these studies show that activation of PKA, PKC, or ERK in superficial dorsal horn neurons leads to an inhibition of A currents and a consequent decrease in first spike latency. Thus, a small, normally subthreshold EPSP could generate an action potential when these kinases are activated. In this way, inflammation, which leads to enhanced ERK activation in superficial dorsal horn neurons (Karim et al. 2001), could increase signal-to-noise ratio for synapses onto dorsal horn neurons. This property could account for a component of central sensitization seen following inflammation. Whether such changes actually occur in spinal cord dorsal horn neurons in the context of inflammation is an open question.

In addition to A-type K⁺ channels, many other ion channels also contribute to regulation of action potentials. Na⁺ currents, Ca²⁺ currents and sustained K⁺ currents are known to affect the initiation, duration and repolarization of action potentials. PKC and PKA activation decrease the peak amplitude of TTX sensitive Na⁺ currents and increase Na⁺ current inactivation in other brain areas (Cantrell et al. 1996; Cantrell et al. 1997; Cantrell et al. 2002; Franceschetti et al. 2000). Such changes induced by PKC and PKA in dorsal horn neurons would not lead to the observed increase in neuronal excitability. In
dendrites of hippocampal CA1 pyramidal neurons, activation of PKC increases the amplitude of action potentials by modulating A type K⁺ currents and not through modulation of Na⁺ channels (Yuan et al. 2002). Our data demonstrate that MEK inhibitors have no effect on Na⁺ or Ca²⁺ currents, but these drugs do decrease sustained K⁺ currents. The decrease in sustained K⁺ currents caused by PD098,059 would not result in a decrease in neuronal excitability. The excitatory effects of PKC and PKA on action potentials in dorsal horn neurons require ERK, and these data suggest that their effects are likely not caused by modulation of Na⁺ channels and Ca²⁺ channels. Previous studies have also shown that activators of PKA and PKC modulate other ion channels, such as persistent Na⁺ channels and the hyperpolarization-activated channels (Boland and Jackson 1999; Franceschetti et al. 2000; Maccaferri and McBain 1996; Matthias et al. 2002), and these effects may also contribute PKC- and PKA-mediated increases in neuronal excitability. Future studies will endeavor to explore the effects of PKC and PKA on these channels.

The present studies were performed utilizing superficial dorsal horn neurons from mouse grown for several days in cell culture. While we have shown that the electrophysiological properties of these neurons are similar to those reported for superficial dorsal horn neurons in acute slice preparations, some caution must always be used when utilizing cell culture systems. For example, it is possible that some of the cells from which data were collected are from deeper laminae, making their utility for dissecting pain mechanisms suspect. Furthermore, it is important to consider that some of the neurons recorded from in the present study may be GABAergic or glycinergic inhibitory interneurons. Increases in excitability in these cells may have profoundly different effects on pain perception in an animal than would similar changes in excitability of glutamatergic excitatory neurons. Finally, the neurons utilized are prepared from very
young mice, and it is possible that such changes are not seen in more mature animals, where the role of the kinases under investigation have been shown to be behaviorally important in pain models. These are all important questions that are difficult to address with an in vitro culture system. Studies recording from visually identified neurons in laminae I-II of acute spinal cord slices will be done to confirm these findings in an identified population of cells from older animals.

In conclusion, our data suggest that A-type currents significantly regulate neuronal excitability in dorsal horn neurons, and that A-type potassium currents are likely important in the modulatory effects of PKA, PKC and ERK in these cells. These results may have implications in pain plasticity.

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References Cited:


Figure Legends:

Figure 1. Based on their firing properties in response to a depolarizing current injection, neurons in mouse spinal cord superficial dorsal horn can be divided into four groups: repetitive, delayed firing, phasic (cells showing spike frequency adaptation) and single spike. The current injection protocols are shown below the voltage traces, and the percentage of cells demonstrating this firing profile are given in parentheses above the traces. All neurons are manually clamped at -70 mV by current injection.

Figure 2. 4-AP modulates action potential firing and membrane properties of spinal dorsal horn neurons. A. Representative examples of action potentials (generated by the current injection protocol shown below the voltage traces) and A-type currents recorded from the same cell before and after 1.5 min bath application of 4-AP (1 mM). A currents shown in the insets were obtained by subtraction of the sustained current (outward current remaining after a prepulse to -10 mV) from the total current induced by a depolarizing step to 40 mV from -80 mV. B. Summary of changes in action potential firing and membrane properties induced by 4-AP. Values are mean ± s.e.m.; n=6. *p<0.05, paired t-test, compared to pre-drug control.

Figure 3. Activation and inhibition of PKC modulates action potential firing and membrane properties of spinal dorsal horn neurons. A. Representative examples of action potentials (generated by the current injection protocols shown below the voltage traces) recorded before and after 5 min bath application of PMA (5 µM) and during washout. B. Representative examples of action potentials recorded before, after 5 min bath application of Ro32-8220 (1µM) and after application of PMA + Ro32-8220. C-E. Summary of changes in action potential firing and membrane properties induced by PMA
(C), Ro32-8220 (D) and PMA in the presence of Ro32-8220 (E). Values are mean ± s.e.m.; n=6 neurons each. *p<0.05, paired t-test.

**Figure 4.** PKA activation modulates action potential firing and membrane properties of spinal dorsal horn neurons. **A.** Representative examples of action potentials (generated by the current injection protocol shown below the voltage traces) recorded before and after 5 min bath application of 50 µM forskolin (FSK) with IBMX (50 µM) and during washout. **B, C.** Summary of changes in action potential firing and membrane properties induced by FSK+IBMX (B) or bath application of 1 mM 8-br-cAMP (C). Values are mean ± s.e.m.; n=5 - 7 neurons each . *p<0.05, paired t-test, compared to pre-drug control.

**Figure 5.** PKA inhibition blocks FSK-induced modulation of action potential firing and membrane properties of dorsal horn neurons. **A.** Representative examples of action potentials (generated by the current injection protocols shown below the voltage traces) recorded under control conditions with PKI-(6-22)amide (PKI, 50 µM) in the recording pipet, and after 5 min bath application of forskolin (FSK) with IBMX. **B.** Summary of changes in action potential firing and membrane properties induced by FSK/IBMX when PKI is included in the recording pipet. Values are mean ± s.e.m.; n=7 neurons. *p<0.05, paired t-test, compared to pre-drug control (PKI).

**Figure 6.** Inhibition of ERK signaling modulates action potential firing and membrane properties of dorsal horn neurons. **A, C.** Representative examples of action potentials (generated by the current injection protocols shown below the voltage traces) recorded before and after 5 min bath application of 20 µM PD098,059 or 500 nM U0126, and
during washout. **B, D.** Summary of changes in action potential firing and membrane properties induced by PD098,059 (**B**), or U0126 (**D**). Values are mean ± s.e.m.; n=7 neurons each. *p<0.05, paired t-test, compared to pre-drug control.

**Figure 7.** Inhibition of ERK signaling blocks FSK- and PMA-mediated modulation of action potential firing and membrane properties in dorsal horn neurons. **A, C.** Representative examples of action potentials (generated by the current injection protocols shown below the voltage traces) recorded before and after 5 min bath application of 20 µM PD098,059, and in the presence of PMA+PD098,059 or FSK+PD098,059. **B, D.** Summary of changes in action potential firing and membrane properties induced by PMA (**B**) and FSK (**D**) in the presence of PD098,059. Values are mean ± s.e.m.; n=6 neurons each. *p<0.05, paired t-test, compared to PD098,059.

**Figure 8.** The MEK inhibitor PD098,059 has no effect on Na⁺ or Ca²⁺ channels but decreases sustained K⁺ currents. **A.** Representative examples of Na⁺ currents (induced by a depolarizing step as shown below the current traces) recorded before and after PD098,059. **B.** The current-voltage plot of the peak amplitude of Na⁺ current versus test voltage recorded before and during application of PD098,059 (20 µM). **C.** Representative examples of voltage-gated Ca²⁺ channel currents (induced by a depolarizing step as shown below the current traces) recorded before and after application of PD098,059 (20 µM). Ba²⁺ was used as the charge carrier. **D.** Summary of percentage changes in Na⁺ and Ba²⁺ currents induced by PD098,059. Values are mean ± s.e.m.; n=5-8 neurons each. PD098,059 had no significant effect on Na⁺ or Ba²⁺ currents (t-test, compared to control condition in which currents were recorded for the same time with no drug addition). **E.** Representative examples of sustained currents
(induced by a prepulse protocol shown below the current traces, interval between prepulse and test pulse is 3 ms) recorded before and after application of 20 \( \mu \text{M} \) PD098,059. F. Mean current-voltage plot of the peak amplitude of the sustained current versus test voltage recorded before and during application of 20 \( \mu \text{M} \) PD098,059. \( n=6 \) neurons.
Repetitive (49%)

Delayed Firing (29%)

Phasic (18%)

Single Spike (4%)
Hu and Gereau, Figure 2

A

Control

4AP

B

Pre Drug

4-AP

First spike latency (ms)

Spike frequency (Hz)

Action potential threshold (mV)

Resting potential (mV)

Action potential amplitude (mV)

Input resistance (GΩ)
Figure 3

(A) Control, PMA, Washout

(B) Control, Ro32-8220, Ro+PMA

(C) Pre Drug, PMA

(D) Pre Drug, Ro32-8220

(E) Ro32-8220, Ro+PMA
Hu and Gereau, Figure 5

A

PKI

PKI+FSK+IBMX

First spike latency (ms)

20 pA

200 ms

20 mV

-20 pA

-20 pA

B

First spike latency (ms)

Spike frequency (Hz)

Action potential threshold (mV)

Action potential amplitude (mV)

Resting potential (mV)

Input resistance (GΩ)

PKI

PKI+FSK+IBMX
Hu and Gereau, Figure 6

A) Control, PD98059, Washout

B) First spike latency (ms), Action potential threshold (mV), Action potential amplitude (mV), Resting potential (mV), Spike frequency (Hz), Input resistance (GΩ)

C) Control, U0126, Washout

D) First spike latency (ms), Action potential threshold (mV), Action potential amplitude (mV), Resting potential (mV), Spike frequency (Hz), Input resistance (GΩ)