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Cocaine sensitization inhibits the hyperpolarization-activated cation current $I_h$ and reduces cell size in dopamine neurons of the ventral tegmental area.

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1Department of Physiology, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico; and 2Laboratorio de Neurociencias y Comportamiento, Departamento de Ciencias Básicas, Facultad de Salud, Universidad Industrial de Santander, Bucaramanga, Colombia

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Arencibia-Albite F, Vázquez R, Velásquez-Martinez MC, Jiménez-Rivera CA. Cocaine sensitization inhibits the hyperpolarization-activated cation current $I_h$ and reduces cell size in dopamine neurons of the ventral tegmental area. J Neurophysiol 107: 2271–2282, 2012. First published January 18, 2012; doi:10.1152/jn.00818.2011.—The progressive augmentation of motor activity that results from repeated cocaine administration is termed behavioral sensitization. This phenomenon is thought to be a critical component in compulsive drug taking and relapse. Still, the cellular mechanisms that underlie sensitization remain elusive. Cocaine abuse, nonetheless, is known to evoke neuroplastic adaptations in dopamine (DA) neurotransmission originating from the midbrain’s ventral tegmental area (VTA). Here, we report that concomitant with the development of locomotor sensitization to cocaine the hyperpolarization-activated cation current ($I_h$) amplitude is depressed by ~40% in VTA DA cells. Such effect did not result from a negative shift in $I_h$ voltage dependence. Nonstationary fluctuation analysis indicates that this inhibition was caused by an ~45% reduction in the number of h-channels with no change in their unitary properties. The cocaine-induced $I_h$ depression was accompanied by a reduction in cell capacitance of similar magnitude (~33%), leaving h-current density unaltered. Two implications follow from these data. First, $I_h$ inhibition may contribute to cocaine addiction by increasing bursting probability in DA cells and this effect could be intensified by the decrease in cell capacitance. Second, the cocaine-induced diminution of DA cell capacitance may also lead to reward tolerance promoting drug-seeking behaviors.

DRUGS OF ABUSE are known to evoke striking adaptations in dopamine (DA) neurons from the midbrain’s ventral tegmental area (VTA) (Stuber et al. 2010; Wolf et al. 2004). Prolonged cannabis treatment, for example, activates VTA cannabinoïd CB1 receptors to induce transient depression of glutamate synapses onto DA cells through activation of N-methyl-D-aspartate (NMDA) receptors and subsequent endocytosis of GluR2 subunits from 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors (Liu et al. 2010). Repeated morphine administration, in contrast, results in a significant reduction of VTA DA cell size (Sklair-Tavron et al. 1996; Spiga et al. 2003). Meanwhile, chronic amphetamine treatment leads to a more responsive excitatory drive mediated by AMPA receptors (Giorgetti et al. 2001) and increases the DA cell’s dendrite length (Mueller et al. 2006). Similarly, single (Ungless et al. 2001) or recurrent (Borgland et al. 2004) in vivo cocaine administration causes a robust AMPA receptor-mediated synaptic potentiation in VTA DA cells that lasts for several days. Plasticity changes such as these are without doubt significant, but their connection to the anomalous behaviors that emerge under addiction is currently unclear. Resolving this issue demands experimental approaches that explicitly correlate drug-induced neuronal adaptations with behavioral alterations.

Frequent cocaine exposure results in a progressive enhancement of locomotor activity that persists even when drug use has been interrupted for periods as long as a year (Robinson and Berridge 1993; Valjent et al. 2010). This enhancement of the motor response is termed behavioral sensitization, and its underlying mechanism remains for the most part unknown. Drug sensitization, nonetheless, is thought to be a major contributor to compulsive drug seeking and relapse (Vanderschuren and Kalivas 2000; Vezina and Leyton 2009). Indeed, it is possible that the inability to reverse the effects of sensitization is what impedes the addict from extinguishing or controlling the urge to consume drugs (Ciccocioppo et al. 2001; Vorel et al. 2001). As a result, animal models of the sensitized response provide a simple readout in which drug-evoked neuronal alterations can be correlated with drug-related behavioral changes (Valjent et al. 2010).

Experimental evidence indicates that one of the main roles of VTA dopaminergic neurons is to encode reward-related information (Schultz 2007). DA cells appear to accomplish such a task by shifting from a low-frequency spontaneous tonic discharge to a transient high-frequency afferent-driven burst of spikes (Grace et al. 2007; Schultz 2007). In monkey DA cells, this transition from tonic firing to bursting codes differences between actual and expected rewards (Fiorillo et al. 2003). The significance of burst activity is that it results in a greater DA release in contrast to low-frequency spontaneous firing (Floresco et al. 2003; Grace 1991). Consequently, it is possible that the augmented DA neurotransmission induced by drug sensitization results at least from an increase in burst activity (Cooper 2002; Kitai et al. 1999; Overton and Clark 1997; Zhang et al. 2009).

The cellular mechanisms that underlie sensitization may in part reflect drug-evoked alterations in the ionic currents that characterize DA neuron firing. One prominent conductance in DA cells is the inward-rectifying hyperpolarization-activated cation current ($I_h$) (Cooper 2002; Kitai et al. 1999; Overton and Clark 1997; Zhang et al. 2009).

The exact role of $I_h$ in DA cell physiology is not clear, but it has been suggested to affect bursting activity (Arencibia-Albite et al. 2007; Inyushin et al. 2010), as it does in pyramidal neurons (Berger et al. 2003; Magee 1998; van Welie et al. 2006). In other brain regions $I_h$ is known to participate in the genesis of pacemaker-
like activity (Chan et al. 2004; Luthi and McCormick 1998), but in DA cells \( I_h \) is not necessary for the expression of spontaneous firing (Kuznetsova et al. 2010; Mercuri et al. 1995). Recently it was shown that in VTA DA cells of rat and mice repeated exposure to ethanol leads to a significant reduction in \( I_h \) current (Hopf et al. 2007; Okamoto et al. 2006). Therefore, we asked whether a similar inhibition is observed in VTA DA cells after the development of cocaine sensitization. This work reports that 24 h after the end of a 7-day intermittent cocaine treatment, \( I_h \) amplitude is significantly reduced. Interestingly, this effect was accompanied by a dramatic decrease in DA cell capacitance.

**METHODS**

**Animals.** Procedures involving experimental animals were performed according to the U.S. Public Health Service publication Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at the University of Puerto Rico Medical Sciences Campus. Behavioral and electrophysiological experiments were performed with male Sprague-Dawley rats (35–51 days postnatal). Animals were housed two per cage and maintained at constant temperature and humidity with a 12:12-h light-dark cycle. Water and food were provided ad libitum.

**Sensitization protocol.** Locomotor activity (i.e., horizontal + stereotyped motions) was measured with a Versamex automated animal activity cage system (AccuScan Instruments, Columbus, OH). The cages are made from clear plastic (42 cm × 42 cm × 30 cm), with 16 evenly spaced (2.5 cm) infrared beams set at a height of 2 cm from its floor. All beams were connected to a detector that counts stereotyped locomotion as the repeated interruption of the same beam, while horizontal locomotion is counted as the sequential breaking of different beams. The collected data were displayed and stored in a PC with the Versamex software Versadat. Before the beginning of all experiments (day 0) the animals were adapted to the activity cages and injection procedures. On experimental day 1 animals were habituated for 15 min, after which animals were treated with either 15 mg/kg intraperitoneal (ip) cocaine (Sigma, St. Louis, MO) or isovolumetric saline (0.9%) injections. Immediately after the injections, locomotion activity was assessed for an hour, once per day for 7 days.

**Electrophysiology.** Midbrain horizontal slices (220 μm) containing the VTA were prepared from male Sprague-Dawley rats (35–51 days postnatal) as previously described (Arencibia-Albité et al. 2007). Whole cell voltage- and current-clamp recordings were obtained from postnatal VTA were prepared from male Sprague-Dawley rats (35–51 days postnatal) as previously described (Arencibia-Albité et al. 2007). Whole cell voltage- and current-clamp recordings were obtained from putative DA neurons by the presence of the h-current with differential interference contrast (DIC) (BX51WI Olympus). Whole cell current to allow a more efficient implementation of our experiments. Nonstationary fluctuations analysis (NSFA) is often performed on cell-attached currents because it offers several advantages: less background noise, prevention of cell dialysis, and the ability to localize the membrane distribution of the ion channel under study (Barrow and Wu 2009; Kole et al. 2006). NSFA of whole cell currents, nonetheless, yields estimates of single-channel properties of accuracy and precision similar to those obtained with the cell-attached technique (Barrow and Wu 2009). Therefore, we used NSFA on whole cell currents to allow a more efficient implementation of our experiments.

**Drugs.** The pharmacological agents used in this study were cocaine hydrochloride, apamin, 2D7288, tetrodotoxin (TTX), tetraethylammonium chloride (TEA), and 4-aminopyridine. All chemicals were purchased from Tocris (Ballwin, MO) or Sigma and were made fresh on the day of experimentation.

**Statistics.** Statistical analysis was performed with Prism (GraphPad Software, San Diego, CA), and averaged data are presented as means ± SE. Behavioral sensitization experiments were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test or unpaired \( t \)-tests as appropriate. Electrophysiological experiments were analyzed with unpaired \( t \)-tests. For all tests a two-tailed \( P < 0.05 \) was considered significant.

**Nonstationary noise analysis.** Nonstationary fluctuations analysis (NSFA) is often performed on cell-attached currents because it offers several advantages: less background noise, prevention of cell dialysis, and the ability to localize the membrane distribution of the ion channel under study (Barrow and Wu 2009; Kole et al. 2006). NSFA of whole cell currents, nonetheless, yields estimates of single-channel properties of accuracy and precision similar to those obtained with the cell-attached technique (Barrow and Wu 2009). Therefore, we used NSFA on whole cell currents to allow a more efficient implementation of our experiments.

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**Whole cell I\( _h \) nonstationary fluctuations were evoked by 80–100 consecutive hyperpolarizing steps of identical amplitude: \( V_{\text{hold}} = -60 \text{ mV}, V_{\text{step}} = -120 \text{ mV}, \text{duration 1 s, frequency 0.1 Hz.} \) Command potentials more negative than \(-120 \text{ mV} \) were not used since in our case this frequently resulted in dielectric breakdown of the whole cell patch. To correct for channel rundown (Alvarez et al. 2002; Barrow and Wu 2009; Conti et al. 1980) \( I_h \) variance (\( \sigma_h^2 \)) was computed as follows:

\[
\sigma_h^2 = \frac{n}{k} \sum \left[ z(t) - Z(t) \right]^2
\]

with

\[
z(t) = \frac{1}{2} [y_n(t) - y_{n+1}(t)]
\]

where \( n \) is the number of evaluated current traces, \( y_n(t) \) is the current value of the \( n \)-th trace at time \( t \), and \( Z(t) \) is the average taken over all \( z \). Mean h-current was obtained by computing the current average at each sampled time point across the entire set of current traces. Scatterplots of variance vs. mean h-current \( <I_h> \) were fitted with the following quadratic equation:

\[
\sigma_h^2 = \frac{1}{N} \left( I_h - \frac{1}{N} \right)^2 + B
\]

As customary, \( I_h \) is unitary h-current, \( N \) is number of h-channels present at the cell membrane, and \( B \) is a constant that represents the variance of the background noise. From these coefficients the open.
probability ($P_o$) and single-channel conductance ($\gamma_h$) were estimated according to the next expressions:

$$P_o = \frac{<I_h>_{max}}{I_h N}$$

$$\gamma_h = \frac{I_h}{(V_m - E_h)}$$

Here $<I_h>_{max}$ is the maximal amplitude of the mean current and $E_h$ is the $I_h$ reversal potential (see Fig. 3 for details on $E_h$ estimation).  

**Cell capacitance estimation.** To resolve capacitance changes due to fusion of synaptic vesicles the method employed should allow for measurements on the order of femtofarads. The approach relies on using a phase-lock amplifier to measure currents in and out of phase with a sinusoidal applied voltage (Neher and Marty 1982; Zhang et al. 2010). However, when determination of cell capacitance is done with the sole purpose of estimating cell size, a standard methodology is typically used (Gentet et al. 2000; Hopf et al. 2007).

We determined DA cell capacitance in the $I_h$ experiments with a voltage step from $-60$ to $-70$ mV. First the series resistance ($R_S$) was computed from the peak value of the capacitive transient ($I_{peak}$)

$$R_S = \frac{\Delta V}{I_{peak}}$$

where $\Delta V$ is the amplitude of the voltage step. Similarly, the input resistance ($R_N$) was determined with the instantaneous membrane current ($I_m$) value at $\sim50$ ms from the disappearance of the capacitive transient

$$R_N = \frac{\Delta V}{I_m} - R_S$$

Finally, the first 40 ms of the capacitive transient was fitted with a sum of two exponential functions and the time constant ($\tau_{final}$) of the fastest term was used to estimate membrane capacitance ($C_m$)

$$C_m = \tau_{final} \left( \frac{1}{R_S} + \frac{1}{R_N} \right)$$

An alternative approach for estimating $C_m$ is to numerically integrate the membrane current transient. However, this method turns out to be error prone because of changes in the depth of the bath or in the shape of the meniscus around the patch electrode (Gentet et al. 2000).

**RESULTS**

The present investigation reports effects of cocaine locomotor sensitization as induced by a single daily injection (15 mg/kg ip) for 7 consecutive days. After 24 h from the final injection, one or two cells were recorded per rat with whole cell recordings in midbrain slices. All tested cells expressed the ZD7288-sensitive $I_h$ current (data not shown), and nearly 50% of them showed slow spontaneous activity (0.5–3 Hz). These characteristics are typical electrophysiological properties of midbrain DA neurons (Grace and Bunney 1983; Ungless et al. 2004).

**Repeated exposure to cocaine leads to development of locomotor sensitization.** We first evaluated the effects of repeated cocaine injections (15 mg/kg/ip) on locomotor activity. Male rats were first habituated to the recording cage for 15 min. Immediately after the completion of this period they were injected with cocaine or saline vehicle (0.9%), and motor activity was captured at 5-min intervals for 60 min (Fig. 1A). On day 1, acute cocaine significantly increases locomotor activity at all sampled intervals after the injection [Fig. 1A; saline day 1 vs. cocaine day 1, 1-way ANOVA: $F(15,128) = 5.28, P < 10^{-7}$; post hoc comparison: Newman-Keuls multiple comparison test]. When rats at days 1 and 7 of saline are compared, no significant difference is observed [Fig. 1A; 1-way ANOVA: $F(15,128) = 0.725, P > 0.05$]. In contrast, making the same comparison for cocaine rats showed the opposite result [Fig. 1A; 1-way ANOVA: $F(15,128) = 2.84, P < 0.001$; post hoc comparison: Newman-Keuls multiple comparison test]. In line with these results, when total activity was also analyzed, cocaine animals showed significantly greater numbers on day 7 with respect to day 1 (Fig. 1B). Therefore, the animals used in this study were successfully sensitized.

**Cocaine sensitization results in significant reduction of $I_h$ amplitude in VTA DA cells.** As mentioned in the introduction, recent studies indicate that repeated ethanol administration
inhibits h-current functionality in VTA DA cells. The possibility that a similar phenomenon arises with cocaine sensitization has not been explored. Consequently, we investigated whether $I_h$ function is affected by repeated cocaine exposure. Here we found that cocaine sensitization significantly decreases $I_h$ amplitude (Fig. 2). On average, when cells were voltage clamped at $-130$ mV, cocaine sensitization resulted in an $\sim 40\%$ decrease in h-current amplitude [Fig. 2A; saline $478.5 \pm 50$ pA ($n = 11$) vs. cocaine $285.5 \pm 38$ pA ($n = 14$), $P < 0.01$]. Moreover, $I_h$ inward rectification was visibly attenuated after repeated cocaine treatment (Fig. 2B). Consistent with these findings, numerical integration of the $I_h$ time course reveals that charge transfer was also significantly reduced. For example, at $-130$ mV charge transfer was $381.2 \pm 44$ pC ($n = 11$) in saline animals in contrast to only $213.5 \pm 31$ pC ($n = 14$) in cocaine animals (Fig. 2C, $P < 0.01$). As seen in Fig. 2A, $I_h$ amplitude was computed as the difference between the steady-state current ($I_{ss}$) and the instantaneous current ($I_{ins}$): $I_h = I_{ss} - I_{ins}$. Hence, $I_h$ amplitude inhibition may occur in various different ways. Results indicate that in our recordings $I_{ins}$ remained unchanged (Fig. 2D), but the magnitude of $I_{ss}$ was clearly diminished (Fig. 2E). Note that changes in $I_{ins}$ amplitude are not significant, as confirmed from charge transfer analysis of $I_{ins}$ at $-130$ mV [Fig. 2F; saline $246.2 \pm 19$ pC ($n = 11$) vs. cocaine $204.4 \pm 28$ pC ($n = 14$), $P > 0.05$]. In effect, the $R_N$ computed from the reciprocal of the slope $I_{ins}$ current vs. voltage relationship was also not affected [Fig. 2G; saline $274.8 \pm 25$ M$\Omega$ ($n = 11$) vs. cocaine $331.7 \pm 30$ M$\Omega$].

Fig. 2. Cocaine sensitization leads to the reduction of the hyperpolarization-activated cation current ($I_h$) in ventral tegmental area (VTA) dopamine (DA) neurons. A$_1$: representative voltage-clamp traces showing the effects of cocaine sensitization onto $I_h$ of VTA DA neurons. As shown, $I_h$ amplitude is defined as the difference between the steady-state current ($I_{ss}$) and the instantaneous current ($I_{ins}$). A$_2$: summary bar graph of the example presented in A$_1$ showing that cocaine sensitization reduces $I_h$ amplitude. B: $I_h$ current amplitudes increase with membrane hyperpolarization; however, this behavior is clearly decreased after cocaine sensitization. C: analysis of $I_{ins}$ electric charge transfer when cells were voltage clamped at $-130$ mV (duration: 1 s) also reveals that cocaine sensitization significantly reduces $I_h$ amplitude. D: $I_{ins}$ was not altered in cocaine animals relative to saline animals. E: $I_{ss}$ amplitude was decreased in cocaine animals, in contrast to saline animals. F: analysis of $I_{ins}$ electric charge transfer when cells were voltage clamped at $-130$ mV (duration: 1 s) also shows that cocaine sensitization does not alter $I_{ins}$. G: summary bar graph showing that cocaine sensitization does not alter input resistance ($R_N$) at $-60$ mV. **$P < 0.01$, saline vs. cocaine.
Therefore, the $I_h$ reduction observed during cocaine sensitization was only the result of diminished $I_{sk}$ amplitude. The spontaneous firing frequency, action potential waveform, and $I_{sk}$ amplitude of VTA DA neurons were not altered by the development of cocaine sensitization (data not shown).

Cocaine sensitization decreases $I_h$ conductance in VTA DA neurons without any effect on its voltage- and time dependence. $I_h$ inhibition may result from a hyperpolarizing shift in its voltage dependence. To test this possibility, we constructed activation curves for cocaine and saline animals. The $I_h$ reversal potential ($E_h$) was estimated from tail current analysis. Figure 3A illustrates an example of the double-pulse protocol used and details the ion channel blockers used to avoid contamination with unwanted conductances. The $h$-current for saline animals reversed at $–39 ± 2.9$ mV ($n = 4$), while that of cocaine animals reversed at $–39.8 ± 1.6$ mV ($n = 3, P > 0.05; \text{Fig. } 3, \text{B–D})$.

With these values the $I_h$ current-voltage curves (see Fig. 2B) were converted to conductance ($G_h$) by application of Ohm’s law, i.e., $G_h = I_h/(V_m - E_h)$. The activation curves were obtained by normalizing conductance values with respect to the maximal conductance at $–130$ mV ($G_h/G_{h,max}$). Then the activation curve of each cell was fitted with the following Boltzmann equation to estimate the potential of half-activation ($V_{1/2}$) and the slope factor ($k$):

$$G_h/G_{h,max} = \frac{1}{1 + \exp\left(\frac{V_m - V_{1/2}}{k}\right)}$$

Finally, activation time constants were determined by fitting a single exponential function to the $I_h$ current trace between 100 and 500 ms.
Figure 4A shows that cocaine sensitization significantly \((P < 0.05)\) decreases \(I_h\) maximal conductance \((G_{h,max}; \text{V}_{\text{hold}} = -130 \text{ mV})\) from \(5.35 \pm 0.50 \text{nS} (n = 11)\) in saline-treated animals to \(3.14 \pm 0.42 \text{nS} (n = 14)\) in cocaine-treated rats. In line with this result, Fig. 4B illustrates that the tendency of the \(h\)-conductance to rise with membrane hyperpolarization was also severely attenuated after sensitization. However, \(I_h\) inhibition was not caused by a negative shift of its voltage dependence or a decrease in the current’s voltage sensitivity since \(V_{1/2}\) and \(k\) were, respectively, not altered after a 7-day cocaine treatment [Fig. 4, C–E; \(V_{1/2}\): saline \(-88.5 \pm 1.5 \text{ mV} (n = 11)\) vs. cocaine \(-91.3 \pm 1.5 \text{ mV} (n = 14), P > 0.05\); \(k\): saline \(9.6 \pm 0.7 \text{ mV} (n = 11)\) vs. cocaine \(9.5 \pm 0.4 \text{ mV} (n = 14), P > 0.05\)]. Consistent with this finding, \(I_h\) activation kinetics was also not affected (Fig. 4, F and G).

For example, at \(-130 \text{ mV}\) the activation time constants between saline and cocaine animals were very similar: saline \(172 \pm 24 \text{ ms} (n = 11)\) vs. cocaine \(204 \pm 22 \text{ ms} (n = 14)\) \((P > 0.05; \text{Fig. 4F})\). Altogether, these data indicate that the development of cocaine sensitization leads to a voltage-independent inhibition of the \(h\)-conductance in VTA DA cells.

Cocaine sensitization decreases number of \(I_h\) channels in VTA DA neurons with no effect in single-channel properties. To determine the biophysical mechanism that explains the decrease in \(h\)-conductance observed with cocaine sensitization, we performed NSFA. Figure 5, A and B, show examples of the noise analysis procedures performed on cells from saline and cocaine animals, respectively. As seen in the figure, scatterplots of variance vs. mean \(I_h\) were well fitted with parabolic...
functions. Using the coefficients from these fits, we estimated the number of channels present at the cell membrane (N), the unitary current (i_h), the single-channel conductance (γ), and the open probability (P_o). Figure 5 shows that cocaine sensitization leads to a significant ~45% decrease in the population size of h-channels. On average, saline animals (n = 5) had 3,666 ± 333 channels while cocaine animals (n = 5) only expressed 2,019 ± 341, which is statistically significant (P < 0.01).

Nevertheless, i_h and γ were not affected (Fig. 5, D and E; i_h; saline 116 ± 8 fA (n = 5) vs. cocaine 132 ± 12 fA (n = 5), P > 0.05; γ; saline 1.43 ± 0.09 pS (n = 5) vs. cocaine 1.62 ± 0.15 pS (n = 5), P > 0.05). Furthermore, in accordance with the finding that i_h inhibition was not caused by a negative shift in its voltage dependence (see activation curves in Fig. 4C), the P_o of h-channels remained unchanged after sensitization [Fig. 5F; P_o; saline 0.87 ± 0.05 (n = 5) vs. cocaine 0.85 ± 0.07 (n = 5), P > 0.05]. It follows then that the development of cocaine sensitization leads to a reduction in i_h amplitude that is the result of a decrease in the number of channels with no change in γ and P_o.

Coocaine sensitization reduces cell membrane capacitance of VTA DA neurons but i_h current density remains unaltered. Acute cocaine application is known to block monoamine reuptake. This in turns causes the internalization of DA transporters (Kahlig et al. 2004) and metabotropic receptors (Thompson et al. 2010). As a result, it is possible that VTA DA cell size is altered by sensitization, since evidently the membrane-trafficking machinery has been perturbed by repeated cocaine exposure. To address the question of the effects of cocaine sensitization on neuron size we compared the cell capacitance of saline and cocaine animals. The method employed for capacitance determination was dependent on curve fitting procedures and not on numerical integration of the capacitive transients (see METHODS). Figure 6A shows examples illustrating the analysis of cell capacitance, and Fig. 6B contrasts the average C_m for saline and cocaine animals. In summary, a significant difference was observed between groups, with the capacitance of cocaine-treated animals being ~33% smaller than that of saline-injected rats [saline 61.8 ± 5.6 pF (n = 11) vs. cocaine 41.3 ± 4.2 pF (n = 14), P < 0.01]. We next looked for differences in i_h current density, i.e., the ratio of maximal i_h current (V_0old = −130 mV) to cell capacitance. Surprisingly, repeated cocaine administration did not alter i_h current density [saline 7.75 ± 0.47 pA/pF (n = 11) vs. cocaine 7.73 ± 1.32 pA/pF (n = 14), P > 0.05]. Therefore, the development of cocaine sensitization reduces VTA DA cell size with no apparent effect on i_h current density.

**DISCUSSION**

This study reports that concomitant with the development of locomotor sensitization to cocaine, i_h amplitude is significantly reduced in VTA DA cells. This inhibition was not caused by a negative shift in the current’s voltage dependence since i_h activation curves of saline and cocaine animals were similar. Nonstationary noise analysis demonstrated that this effect re-

**Fig. 5.** Repeated cocaine exposure decreases the number of i_h channels in VTA DA neurons with no change in single-channel properties. A and B: examples of nonstationary noise analysis of i_h current for saline and cocaine animals, respectively. Gray curves represent parabolic fits to the data points. C: noise analysis reveals that the number of i_h channels is significantly decreased after repeated cocaine injections. D–F: noise analysis shows that i_h unitary current (i_h), single-channel conductance (γ), and open probability (P_o) are not altered after repeated cocaine injections. **P < 0.01, saline vs. cocaine.
sulted from a reduced number of h-channels with no change in single-channel conductance or open probability. Unexpectedly, this diminution in the channels’ population size was accompanied by a dramatic decrease in DA cell capacitance.

Recent studies had linked Ih plasticity in VTA DA cells to the underlying neuroadaptations that may account for drug sensitization. For instance, repeated ethanol exposure leads to a significant reduction in Ih current amplitude with no change in its gating kinetics as evidenced from activation curve analysis (Hopf et al. 2007; Okamoto et al. 2006). Likewise, our study shows that cocaine sensitization also induces a similar pattern of h-conductance inhibition. Our data, however, go further in the characterization of this inhibitory action by confirming that Ih current amplitude depression is caused by a reduced number of h-channels at the cell membrane with no change in single-channel properties. As a result, it is possible that the ethanol-induced Ih downregulation could also be explained by a diminished number of h-channels. On the other hand, the present study disagrees with Okamoto et al. (2006) in that repeated ethanol exposure leads to a decrease in Ih current density while in cocaine sensitization this measurement appears to remain unaltered. Such difference follows from the finding that cocaine sensitization also induces a similar pattern of h-conductance inhibition. Moreover, our data further in the characterization of this inhibitory action by confirming that Ih current amplitude depression is caused by a reduced number of h-channels at the cell membrane with no change in single-channel properties.

A prevailing view in addiction research is that the enhanced DA release observed under cocaine sensitization results in part from an increase in burst activity of VTA DA cells (Cooper 2002; Kitai et al. 1999; Overton and Clark 1997; Zhang et al. 2009). In midbrain slice preparations bath application of NMDA typically elevates the firing frequency of DA cells with no further effects in the spiking pattern (Hopf et al. 2007; Johnson and Seutin 1997). In ethanol sensitization, however, NMDA superfusion facilitates the transition from low-frequency firing to bursting (Hopf et al. 2007). The mechanism of this unusual behavior appears to follow from a decrease in SK conductance function. This inference is natural since repeated ethanol treatment leads to SK current attenuation (Hopf et al. 2007) and apamin blockade of SK channels in the presence of NMDA also induces bursts of spikes (Johnson and Seutin 1997; Seutin et al. 1993; Waroux et al. 2005). In the present investigation we found that cocaine sensitization did not modulate spontaneous activity or the action potential waveform of VTA DA cells, and in particular the SK conductance was unaltered by repeated cocaine injections. Consequently, if cocaine sensitization increases burst spiking probability in VTA DA cells then it appears that the mechanisms that explain such alteration are at least independent of SK modulation.

The present work reports that repeated cocaine administration did not alter the autonomous activity or the magnitude of the SK conductance of VTA DA cells, suggesting that biophysical properties had been scaled to accommodate for the observed decreased in capacitance and hence in cell size. Such a hypothesis is physiologically plausible, and its underlying

Fig. 6. Repeated cocaine exposure reduces the membrane capacitance of VTA DA neurons; however, the Ih current density remains unaltered. A: examples illustrating cell membrane capacitance (Cm) analysis for saline and cocaine animals. The first 40 ms of the capacitive transients were fitted with a sum of 2 exponential functions (not shown). The time constant (τfast) of the fastest exponential term was used to estimate Cm according to the following expression: Cm = τfast[1/Rs + 1/RN], where Rs is the series resistance and RN is the input resistance. B: Cm is significantly decreased after repeated cocaine injections. C: Ih current density is not affected after repeated cocaine injections. **P < 0.01, saline vs. cocaine.
mechanisms could be classified as a homeostatic plasticity response (Azdad et al. 2009; MacLean et al. 2003; Turrigiano 1999). Fundamentally this type of plasticity refers to the set of adaptations that adjust the intrinsic cell properties in a direction that preserves neuronal activity within normal ranges (Turrigiano 1999). A remarkable example of homeostatic plasticity can be appreciated when lobster stomatogastric ganglion (STG) neurons are isolated in cell cultures (Turrigiano et al. 1994). Acute-isolation STG cells abolish their in vivo rhythmic bursting. On the third or fourth day in isolation, however, STG neurons regain burst activity by increasing Ca^{2+} inward current densities, decreasing outward current densities and leaving the input conductance unaffected. Consequently, interpreting our observations of no change in spontaneous activity and is_SK as a homeostatic response implies that some of the cell’s active and passive qualities had changed with the purpose of keeping DA cell physiology functional. As a result, it is possible that the cocaine-induced I_h inhibition is also a homeostatic mechanism, suggesting that such neuroadaptation is not an event that leads to sensitization. This conjecture, nevertheless, needs to be further analyzed since previous studies indicate that I_h dysfunction is critical to various neurological disorders. Neuropathic pain, for example, appears to result from an enhanced I_h functionality (Chaplan et al. 2003), whereas the increase in cell excitability of layer III pyramidal neurons of the entorhinal cortex, seen after a single seizure episode, seems to be the result of a decreased I_h function (Shah et al. 2004).

Here we used NSFA to explore how repeated cocaine injections impact the normal biophysics of the h-conductance. When using this type of statistical analysis there are always doubts about its accuracy. A main source of errors in noise analysis arises when the filtering time constant is >10% of the current’s activation time constant (Alvarez et al. 2002; Kole et al. 2006). In I_h experiments whole cell current traces were low-pass filtered at a cutoff frequency (f_c) of 1 kHz, and the mean activation time constant at −120 mV was 247 ± 22 ms (n = 25). Thus, in our case, the filter time constant was 1/(2πf_c) = 1/(2π × 1 kHz) ≈ 159 µs, which is clearly much less than the upper bound for the low-pass filter time constant (25 ms ≈ 10% of 247 ms). Simulation studies also indicate that if the macroscopic current amplitude is greater than 10 pA then the errors involved in the estimation of the unitary current, the number of channels, and the open probability tend to be smaller than 20% (Kole et al. 2006). Altogether this information implies that our recordings have a satisfactory precision to accurately estimate the single-channel properties of I_h channels in VTA DA cells.

The ion channels underlying I_h are appropriately called hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in reference to their characteristic dual control of gating mechanics (Altomare et al. 2001; Biel et al. 2009; Craven and Zagotta 2006). The molecular complex that constitutes the HCN channel consists of four subunits, with each subunit existing in four distinct isoforms: HCN1−4 (Biel et al. 2009; Santoro et al. 1998). A distinctive property of HCN channels is their small single-channel conductance. In their native state, for example, HCN channels express a conductance of ~1 pS in sinoatrial I_h (DiFrancesco 1986; DiFrancesco and Mangoni 1994), hippocampal I_h (Kole et al. 2006), and retinal photoreceptor I_h (Barrow and Wu 2009). Here we add to this group by reporting a comparable small conductance value in VTA DA cells that on average appears to be close to 1.5 pS (see Fig. 5E). Furthermore, to our knowledge, this study is the first estimate of unitary properties for HCN channels in VTA DA cells.

Drugs of abuse are known to evoke striking changes in the neuronal morphology of cells from the brain’s reward circuit. Chronic exposure to morphine, for example, leads to a significant reduction in the cell size of VTA DA neurons (Chu et al. 2007; Russo et al. 2007; Sklar-Tavron et al. 1996). In contrast, prolonged cocaine use increases dendritic branching and spine densities in cells from the nucleus accumbens (NAcc) (Norholm et al. 2003; Robinson et al. 2001). Similarly, repeated amphetamine injections increase dendrite length of VTA DA cells (Mueller et al. 2006), while a single exposure to cocaine increases spine densities in a subset of DA cells in this same nucleus (Sarti et al. 2007). An inconsistency with these data is that in some cases sound electrophysiological evidence does not support these findings. For instance, in repeated cocaine exposures NAcc cell surface area should increase because of the improved dendritic arborization and augmented spine densities. Nonetheless, cell capacitance measurements in NAcc neurons of cocaine-treated animals show no alterations in this parameter (Zhang et al. 1998). Our results also show that cocaine sensitization leads to a significant reduction in cell capacitance and hence in cell size, an outcome that is at odds with previous evidence (Sarti et al. 2007). Thus further experimentation will be needed to properly resolve these types of discrepancies. The diminution in cell size, nevertheless, might be important in the neurobiology of addiction. A recent study has reported that the reduced size of VTA DA neurons seen during chronic morphine administration is linked to development of tolerance toward the drug’s rewarding effects, which may result in escalation of drug taking (Russo et al. 2007). Hence, it is possible that the cocaine-induced decrease in VTA DA cell size may also result in drug tolerance and thus promote drug-seeking behaviors. Further experiments should test this hypothesis.

Abstinence from repeated cocaine administration is known to upregulate the expression of brain-derived neurotrophic factor (BDNF) within the mesolimbic circuit (Grimm et al. 2003; Mao et al. 2010; McGinty et al. 2010). BDNF signaling, through the activation of its high-affinity receptor tyrosine kinase receptor B (TrkB), modulates different cellular processes spanning from neuroprotection and cognition to behavioral and molecular responses to psychoactive drugs (Fumagalli et al. 2009; Russo et al. 2009). In chronic morphine exposure, the reduction in VTA DA cell size persists after withdrawal and has been correlated with a decreased number of BDNF-containing dopaminergic neurons (Chu et al. 2007). Such interconnection may be direct since peripheral electrical stimulation (100 Hz) restores cell size to near normal values by increasing the number of BDNF-containing cells (Chu et al. 2007). If so, it may be possible that repeated cocaine exposure depresses BDNF activity within the VTA, accounting for the decrease in cell capacitance seen 24 h into withdrawal. This hypothesis, however, seems unlikely since Grimm et al. (2003) showed that VTA’s BDNF expression remains unaltered exactly 1 day after withdrawal and is not changed by repeated cocaine treatment (Pierce et al. 1999). Furthermore, extended withdrawal increases BDNF expression in this nucleus (Grimm et al. 2003; Pu et al. 2006), and heterozygous BDNF knockout
mice are known to show less cocaine-induced locomotor activity in addition to reduced cocaine-condition place preference (Hall et al. 2003). Taken together, these findings suggest that the normal BDNF activity that takes place during repeated cocaine injections cannot prevent the cocaine-evoked decrease in DA cell size, but it is possible that prolonged withdrawal could reestablish standard neuronal geometry since extended abstinence upregulates BDNF signaling. This speculation needs to be tested.

In most central nervous system neurons $I_h$ channel density is elevated at distal dendrites in contrast to perisomatic regions (Berger et al. 2001, 2003; van Welie et al. 2006). This non-uniform distribution allows the $h$-conductance to shunt synaptic potentials away from the soma, dampening the temporal summation of these events (Kole et al. 2006; Magee 1998; Stuart and Spruston 1998; Williams and Stuart 2000, 2003). In effect, $I_h$ inhibition is known to improve temporal summation (Carr et al. 2007). Similarly, in midbrain DA cells $I_h$ also appears to have a density that increases toward the distal dendrites as suggested by voltage-clamp data (Arencibia-Albite et al. 2007; Cathala and Paupardin-Tritsch 1999; Watts et al. 1996). Accordingly, the $I_h$ of VTA DA cells may also reduce the likelihood of burst firing since this spiking mode is afferent driven (Cooper 2002; Floresco et al. 2003; Lodge and Grace 2006) and hence dependent on temporal summation. Thus it is possible that the $I_h$ inhibition seen under the development of behavioral sensitization could contribute to cocaine addiction by increasing bursting probability in VTA DA cells. Additionally, this effect might be further amplified by the cocaine-induced decrease in cell capacitance since this may lead to synaptic potentials of greater amplitude. As a final remark, such enhancement of bursting could trigger $D_1$ receptor-mediated long-term potentiation (O’Donnell 2003) at excitatory synapses of NAcc neurons, encoding in this way anticipated reward cues to eventually drive cocaine-seeking behaviors (Berridge 2007; Carter et al. 2009; Kalivas et al. 2005; Salamone et al. 2007).

In conclusion, two cellular adaptations appear to be shared among different drugs of abuse. These are the inhibition of the $h$-current and cell size downregulation in VTA DA neurons. In the case of repeated cocaine administration these alterations appear to arise during the development of behavioral sensitization. Thus understanding the underlying causes of the sensitized response may help to elucidate the neurobiology of addiction.

REFERENCES


