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ORIGINAL INVESTIGATION

Effects of T-type calcium channel blockers on cocaine-induced hyperlocomotion and thalamocortical GABAergic abnormalities in mice

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Abstract
Rationale Repetitive cocaine exposure has been shown to induce GABAergic thalamic alterations. Given the key role of T-type (Ca_V3) calcium channels in thalamocortical physiology, the direct involvement of these calcium channels in cocaine-mediated effects needs to be further explored.
Objective The objective of this study was to investigate the effect of T-type calcium channel blockers on acute and repetitive cocaine administration that mediates thalamocortical alterations in mice using three different T-type blockers: 2-octanol, nickel, and mibefradil.
Methods During in vitro experiments, whole-cell patch-clamp recordings were conducted in ventrobasal (VB) thalamic neurons from mice treated with acute repetitive cocaine administration (3×15 mg/kg, i.p., 1 h apart), under bath application of mibefradil (10 μM), 2-octanol (50 μM), or nickel (200 μM). After systemic administration of T-type calcium channel blockers, we evaluated locomotor activity and also recorded GABAergic neurotransmission onto VB neurons in vitro.
Results Bath-applied mibefradil, 2-octanol, or nickel significantly reduced both GABAergic neurotransmission and T-type currents of VB neurons in cocaine-treated mice. In vivo i.p. pre-administration of either mibefradil (20 mg/kg and 5 mg/kg) or 2-octanol (0.5 mg/kg and 0.07 mg/kg) significantly reduced GABAergic mini frequencies onto VB neurons. Moreover, both mibefradil and 2-octanol were able to decrease cocaine-induced hyperlocomotion.
Conclusion The results shown in this study strongly suggest that T-type calcium channels play a key role in cocaine-mediated GABAergic thalamocortical alterations, and further propose T-type channel blockers as potential targets for future pharmacological strategies aimed at treating cocaine's deleterious effects on physiology and behavior.

Keywords Cocaine · Thalamocortical · Ventrobasal nucleus · Locomotor activity · T-type calcium channels · Mibefradil · 2-octanol · Nickel · GABA

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Introduction

It has been widely demonstrated that chronic abuse of psychostimulants is associated with major neurological and psychiatric conditions (Devlin and Henry 2008). In particular, cocaine use may cause cerebral hypoxia (Devlin and Henry 2008), seizures (Hanson et al. 1999), and delirium sometimes associated with hallucinations (Devlin and Henry 2008). This clinical profile is consistent with cocaine-mediated abnormal thalamocortical processing

(Behrendt 2006). Repetitive administration of cocaine has been recently shown to alter the intrinsic properties of thalamocortical neurons and GABAergic transmission in mice, which resulted in an enhancement of EEG low frequencies (Urbano et al. 2009), similar to what had been previously described as the "thalamocortical dysrhythmia syndrome" (Jeanmonod et al. 2003; Llinás et al. 1999, 2005). This is especially important considering that dysrhythmic thalamocortical dynamics have been associated to several neurological and neuropsychiatric conditions characterized by abnormal coherence between low and high frequencies at a thalamocortical level, altering both thalamic and cortical GABAergic inhibitory networks (e.g., in vitro cortical "edge-effect"; Llinás et al. 2005). Positive and negative neuropsychiatric symptoms have been associated with the existence of asymmetric activation of cortical GABAergic interneurons during awake states (Llinás et al. 2005).

GABAergic networks control thalamocortical processing responsible for both motor execution (Albin et al. 1989; Gerfen et al. 1990) and sensory processing (Steriade and Llinás 1988), and can be disrupted by psychostimulants. In rodents, somatosensory information from "barrel" cortex is relayed to neostriatal areas (Mercier et al. 1990; Wright et al. 1999), proposed to serve as a sensory guidance center during movement execution. In humans, cocaine abusers show GABAergic disruptions in the striatum, thalamus, and parietal cortex (Volkow et al. 1998). In a visuospatial attention task, using fMRI, cocaine abusers showed lower thalamic activation, larger occipital and prefrontal cortex activation, and larger deactivation of parietal regions than controls (Tomasi et al. 2007). These fMRI results suggested the existence of an over-inhibition of thalamic nuclei under the influence of cocaine, as observed in mice after repetitive cocaine administration (Urbano et al. 2009).

Interactions between high-voltage activated (HVA, generally designated as L, N, P/Q, or R-type) and low-voltage activated (LVA, generally designated as T-type) calcium channels play a central role in determining intrinsic neuronal properties (Llinás 1988; Catterall 1998; Perez-Reyes 2003) and in mediating calcium-dependent synaptic transmission (Katz and Miledi 1965). In thalamocortical circuits, P/Q-type HVA calcium channels that were described to be located on the dendrites of ventrobasal (VB) neurons support the characteristic 35–45-Hz gamma-band oscillation characteristic of cognitive brain states (Pedroarena and Llinás 1997; Llinás et al. 2007; Jones 2007), and mediate both glutamatergic and GABAergic synaptic transmission (Iwasaki et al. 2000; Ali and Nelson 2006). N-, L- and R-type HVA are involved in GABAergic release from striatal (Pin and Bockaert 1990) and reticular thalamic neurons (Jocksovic et al. 2010). On the other hand, the functions of T-type LVA channels in thalamocortical circuits have been associated with pacemaker activity, sleep cycles, and seizure susceptibility (reviewed in Llinás 1988; Perez-Reyes 2003). The expression of the three members of the T-type calcium channel family has been described in multiple brain regions in rodents (Talley et al. 1999). In motor thalamocortical loops, ventral anterior/ventral medial thalamic nuclei express T-type channels composed of $Ca_V3.1$ subunits, while in striatum and accumbens, both $Ca_V3.2$ and $Ca_V3.3$ subunits are expressed. Substantia nigra expresses both $Ca_V3.1$ and $Ca_V3.2$ subunits, while the neocortex presents the three subunits throughout the different cortical layers. On the other hand, somatosensory thalamocortical loops express $Ca_V3.1$ subunits in VB versus the expression of both $Ca_V3.2$ and $Ca_V3.3$ in reticular GABAergic neurons (Talley et al. 1999). Abrupt changes in HVA/LVA thalamic ratios can induce dysrhythmic states (Zhang et al. 2002, 2009; Llinás et al. 2007). Likewise, repetitive cocaine administration also induced abnormally higher LVA/HVA calcium current ratios (Urbano et al. 2009).

Based on the fact that T-type channels are widely expressed on both basal ganglia and somatosensory thalamocortical nuclei (Talley et al. 1999), and that somatosensory areas project to motor-related neostriatal areas (Jones et al. 1977; Mercier et al. 1990; Wright et al. 1999; Llinás et al. 2002), we decided to study the role of T-type channels in both cocaine-mediated thalamocortical alterations and cocaine-induced hyperlocomotion. We used three different T-type blockers: *2-octanol* (similar to 1-octanol but with a higher blocking efficiency for T-type currents, Sinton et al. 1989; reviewed in Llinás 1988; Perez-Reyes 2003); *nickel*, a divalent ion that preferentially blocks T-type over other HVA calcium channels (reviewed in Llinás 1988; Perez-Reyes 2003); and *mibefradil*, which has been previously described as a more potent T-type than P- or L-type calcium channel blocker (McDonough and Bean 1998; reviewed in Perez-Reyes 2003). T-type calcium channel blockers were tested in vitro (i.e., bath- applied) and in vivo (i.e., i.p.-injected prior to acute repetitive cocaine administration). We further investigated whether co-administration of mibefradil or 2-octanol could modulate hyperlocomotion induced by cocaine. Our results suggest a direct involvement of T-type calcium channels in cocaine-induced thalamocortical alterations.

Materials and methods

Animals Periadolescent mice (male C57BL/6, postnatal 26–45 days old) from the Central Animal Facility at Universidad de Buenos Aires (UBA) were utilized for this study. Principles of animal care were followed in accordance with "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National

Research Council, 2003) and approved by Universidad de Buenos Aires authorities using OLAW and ARENA directives (NIH, Bethesda, U.S.A.).

Drug administration Repetitive cocaine hydrochloride administration (Sigma-Aldrich, St Louis, MO, U.S.A.) was performed intraperitoneally (i.p., 3×15 mg/kg, 1 h apart). Control groups received three similarly timed saline injections. Since mibefradil takes longer to reach a maximum effect (~2 h; Welker et al. 1998) than 2-octanol (~30 min; Sinton et al. 1989), the schedule for the in vivo administration of the T-type calcium channel blockers consisted in the pre-administration of mibefradil (dissolved in sterile saline) 90 min prior to the first saline/cocaine injection, while 2-octanol (mixed with Tween-80+saline and prepared just before each injection) was injected twice 30 min before the first and third cocaine injections. Control groups received the same volume of saline (for mibefradil groups) or Tween 80+ saline (for 2-octanol groups). We used the same two schedules for both behavioral and patch-clamp studies.

Thalamic slices Coronal slices, including the somatosensory cortex (180–250 μM) were obtained as previously described (Urbano et al. 2009). Slices were allowed to recover at 35°C for at least 30 min in a psychostimulant-free ACSF solution.

Whole-cell patch-clamp recordings Recordings were performed at room temperature (20–24°C). Patch electrodes were made from borosilicate glass (3–7 MΩ) filled with a voltage-clamp high Cs^+/QX314 solution to record GABA-A receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs; Urbano et al. 2009). Picrotoxin (50 μM) was added at the end of a recording session to confirm that the miniature currents were mediated by GABA-A receptors. Inhibitory miniature amplitudes and frequencies (represented here as inter-event intervals) were analyzed using the Mini Analysis software (Version 6.0.7; Synaptosoft, Chapel Hill, NC, USA, www.synaptosoft.com). The threshold for mini detection was set at fivefold the RMS baseline noise. Voltage dependent calcium currents were determined using a high-Cs^+ pipette solution in the presence of both excitatory and inhibitory synaptic receptor blockers. Voltage ramps were used to activate calcium currents of VB neurons. Signals were recorded using a MultiClamp 700 amplifier in combination with the PCLAMP 10.0 software (Molecular Devices, CA, USA). Data were filtered at 4 kHz, digitized, and stored for off-line analysis.

Behavioral studies Mice were housed in plastic cages for at least 1 week prior to the initiation of locomotor experiments. Mice were moved to an experimental room 24 h before drug treatments. Food and water was provided ad libitum in a temperature-controlled room, with a 14/10-h light/dark cycle. Mice locomotor activity (total distance, in cm) was recorded using a CCD camera (Sony, U.S.A.) on custom-designed open field boxes located in a sound-attenuated room. For acquisition and analysis, we used Ethovision XT 5.1 software (Noldus, The Netherlands). Each box consisted of an open-field plastic compartment (19×40×40 cm). For mibefradil experiments, animals were injected and left in their home cage for 60 min, then placed in open field box for a 30-min period in which they remained undisturbed until the first cocaine/saline injection. For 2-octanol experiments, animals were placed in open field boxes for 30 min and later injected with 2-octanol or Tween 80+saline. The first 2-octanol injection was delivered just 30 min before the first cocaine/saline injection.

The total distance traveled (in cm) was quantified in 5-min bins for a total of 30 min before the first cocaine injection, and for a total of 180 min after (i.e., 60 min were recorded after each of the three cocaine injections). Behavioral recordings were made simultaneously in four open-field arenas using Ethovison XT multiple arena features from 9AM to 4PM of the light period of the photocycle (similar to our initial set of patch-clamp experiments, Urbano et al. 2009). Injection time and arenas were fully counterbalanced among subjects and experimental groups.

Pharmacological reagents Drugs were purchased from either Sigma-Aldrich (St. Louis, MO, USA) or Tocris (Ellisville, MO, USA).

Statistical analysis Sigmaplot 10.0 (Systat Software, CA, USA) and GraphPad Prism 4.0 for Windows (GraphPad Software, CA, USA) software were used for statistical comparisons. Statistics were performed using either one-way or two-way ANOVA, with either least significant difference (LSD) or Bonferroni post hoc test comparisons, for either calcium LVA/HVA current ratios (calcium current ratios), GABAergic minis or locomotor activity comparisons, respectively. Differences were considered significant if $P<0.05$. Population statistics are presented here as mean±standard error of the mean (SEM).

Results

T-type channels blockers were able to reduce the over-activation of T-type calcium channels and GABAergic neurotransmission onto VB neurons induced by cocaine

We initially determined the in vitro effects of bath-applied T-type channel blockers on previously reported cocaine-

mediated thalamic alterations (Urbano et al. 2009). Whole-cell patch-clamp recordings were conducted in VB thalamic neurons from cocaine-treated mice (cocaine hydrochloride; 3×15 mg/kg, i.p., 1 h apart). The recordings demonstrated, invariably, higher-frequency, enhanced amplitudes of GABAergic minis (Fig. 1a) and increments in T-type calcium current ratios (Fig. 1b) in VB neurons of cocaine- compared to saline-injected mice (Fig. 1a). Following an initial 30-min recording period, the effect of bath-applied T-type channel blockers on cocaine-induced enhancement of GABAergic minis (in the presence of 3 μM tetrodotoxin (TTX)) was analyzed for another 30-min period. Three different T-type blockers were used: the aliphatic alcohol, *2-octanol* (50 μM), *nickel* ($NiCl_2$, 200 μM), and *mibefradil* (10 μM). One-way ANOVA comparison indicated that all GABAergic mini interval curves were significantly different ($F_{[4,203]}=189$, $P<0.001$). Indeed, *2-octanol* (shown to present a higher blocking efficiency for T-type currents than 1-octanol, Sinton et al. 1989) was able to reduce cocaine-induced changes in both VB T-type currents and GABAergic mini intervals (Fig. 1b and d, *2-octanol* vs. saline, cocaine; Bonferroni post hoc test; $P<0.001$). *Nickel*, a divalent ion that preferentially blocks T-types over other calcium channels (Perez-Reyes 2003), also reduced LVA T-type, as well as HVA currents and GABAergic mini intervals (Fig. 1, *nickel* vs. saline, cocaine; Bonferroni post hoc test; $P<0.001$). In addition, *nickel*, but neither *2-octanol* nor *mibefradil* significantly reduced GABAergic mini amplitudes, suggesting a partial block of HVA channels by this ion (Fig. 1c, *nickel* vs. all other groups; Bonferroni post hoc test, $P<0.001$). *Mibefradil* has been previously described as a 10–30 or 100–1,000 times more potent T-type than L-type or P/Q-type calcium channel blocker, respectively (McDonough and Bean 1998; Perez-Reyes 2003). The administration of *mibefradil* was also able to reduce T-type calcium currents in VB neurons

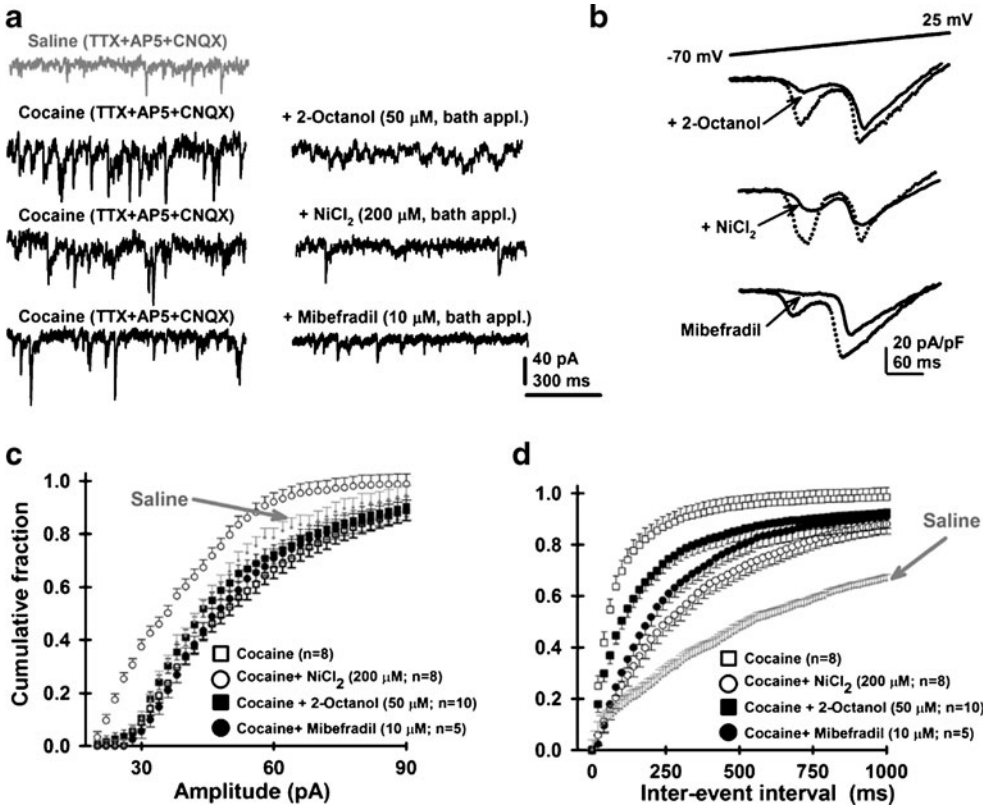

Fig. 1 Cocaine-induced enhancement of GABA-A-mediated synaptic transmission onto VB neurons was reduced by bath application of T-type calcium channel blockers. **a** Raw current traces showing mIPSCs (miniature inhibitory postsynaptic currents) of VB neurons from mice previously injected with saline (*upper left*, *gray traces*) or cocaine before (*left traces*) and after bath application (*right traces*) of 2-octanol, $NiCl_2$, or mibefradil. A holding potential of −70 mV was used in all conditions. **b** Blocking effect of 2-octanol, $NiCl_2$, and mibefradil (*solid lines*) on VB calcium currents generated using 300-ms-long voltage ramps (from −70 mV to 25 mV) recorded from cocaine-injected mice (*black dotted lines*). **c** and **d** Average cumulative probability plots of mIPSCs amplitudes (C) and inter-event intervals (D) for saline (*gray dots*; $n=10$ VB neurons), cocaine (*empty squares*; $n=8$ VB neurons), cocaine±$NiCl_2$ (200 μM, *empty circles*; $n=8$ VB neurons), cocaine±2-octanol (50 μM, *filled squares*, $n=10$ VB neurons) and cocaine±mibefradil (*filled circles*, $n=5$ VB neurons). One-way ANOVA comparisons indicated that all GABAergic mini intervals curves were significantly different ([$F_{[4,203]}=189$, $P<0.001$], Bonferroni post hoc test, $P<0.001$). Also, nickel reduced GABAergic mini amplitudes (nickel vs. all other groups; Bonferroni post hoc test, $P<0.001$)

(Fig. 1b) and cocaine-induced GABAergic mini intervals (Fig. 1d, *mibefradil* vs. saline, cocaine; Bonferroni post hoc test; $P<0.001$). None of the T-type blockers tested in this study affected GABAergic mini intervals in saline-injected mice (data not shown, $n=4$).

Following this initial recordings, we implemented a series of experiments in vivo. GABAergic minis of VB neurons were recorded in mice administered with the following combination sets: set (1) 2-octanol (0.5 mg/kg, 30 min before the first and third cocaine injections)+saline, set (2) mibefradil (20 mg/kg, 90 min before the first injection)+saline, set (3) 2-octanol (0.5 mg/kg, 30 min before the first and third injections)+cocaine, and set (4) mibefradil (20 mg/kg, 90 min before the first injection)+cocaine. Compared to saline injection alone (sets 1 and 2), both 2-octanol and mibefradil pre-administration did not affect basal GABAergic mini frequency (Fig. 2a). However, a drastic reduction in frequency was observed when both T-type blockers were combined with a protocol of repetitive cocaine administration (Fig. 2a) (sets 3 and 4). On average, GABAergic mini inter-event intervals were significantly larger (i.e., lower frequencies) for the combinations 2-octanol+cocaine (set 3) and mibefradil+cocaine (set 4) compared to cocaine alone (Fig. 2b, one-way ANOVA, $F_{[2,160]}=32$; $P<0.001$). By contrast, no significantly different intervals were observed (one-way ANOVA, $F_{[2,160]}=0.8$; $P>0.05$) between 2-octanol+saline or mibefradil+saline goups and those receiving saline alone (Fig. 2b).

These results indicate that T-type calcium channels are over-activated following cocaine repetitive administration at the resting membrane potential. Moreover, the fact that recordings were done in the presence of TTX suggests that T-type calcium channels located on the presynaptic terminals of reticular neurons may be targeted by downstream effectors of cocaine (i.e., 5-HT, dopamine (DA) and/or norepinephrine (NE)).

Co-administration of T-type calcium channel blockers decreased cocaine-induced hyperlocomotion

We continued to study the effects of mibefradil and 2-octanol on locomotor activity induced by repetitive administration of cocaine in vivo. Each T-type channel blocker was tested for behavioral effects at two doses: mibefradil

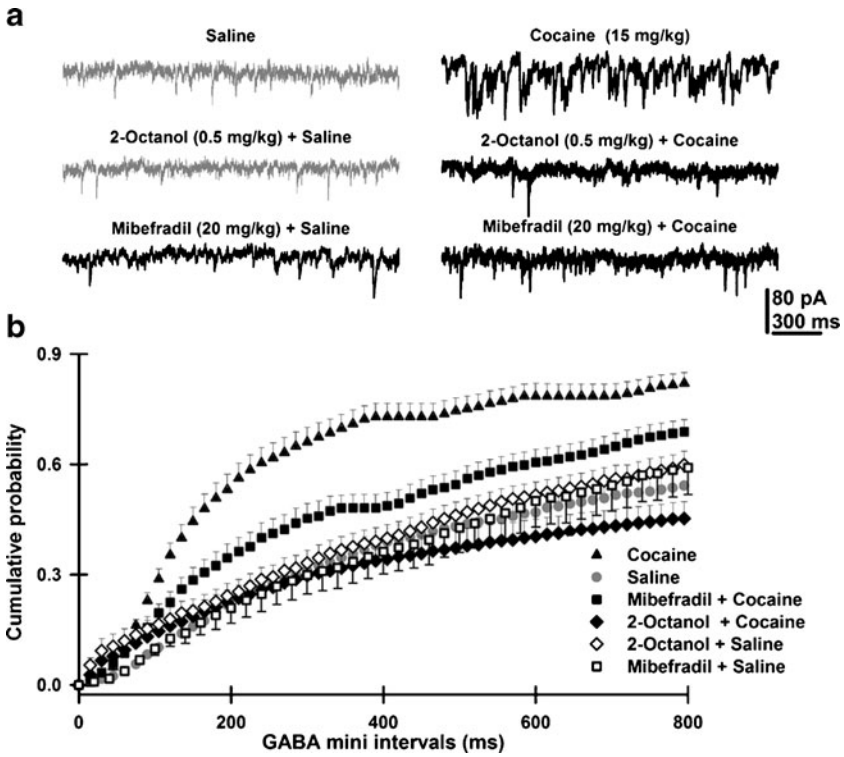

Fig. 2 Co-administration of T-type calcium channel blockers prevented cocaine-mediated enhancement of GABAergic transmission onto VB neurons in vivo. **a** Representative GABAergic minis currents in VB neurons recorded from mice injected with saline (three injections, 1 h apart), 2-octanol (i.p., 0.5 mg/kg)±saline, mibefradil (20 mg/kg)±saline, cocaine (3×15 mg/kg, 1 h apart), 2-octanol (0.5 mg/kg)±cocaine and mibefradil (20 mg/kg)±cocaine. **b** Average cumulative probability plots of GABAergic minis inter-event intervals recorded in VB neurons from mice injected with saline ($n=15$ VB neurons), 2-octanol±saline (0.5 mg/kg; 30 min prior to the first/third saline injections, $n=10$ VB neurons), cocaine ($n=12$ VB neurons), 2-octanol±cocaine (0.5 mg/kg; 30 min prior to the first/third cocaine injections; $n=15$ VB neurons), mibefradil±saline (20 mg/kg; 90 min prior to the first saline injection, $n=8$ VB neurons) and mibefradil± cocaine (20 mg/kg; 90 min prior first cocaine injection, $n=9$ VB neurons)

(i.p.), at 20 and 5 mg/kg; and 2-octanol (i.p.) at 0.5 and 0.07 mg/kg. The average distance traveled by mice increased after each cocaine injection, while it remained unaffected after saline injections (Figs. 3 and 4). Mibefradil co-administration reduced cocaine-induced hyperlocomotion at both 20 mg/kg and 5 mg/kg doses (Fig. 3a and b, respectively). Following 20 mg/kg mibefradil (Fig. 3a, seven to eight mice per group), two-way ANOVA (treatment × time) showed a clear treatment effect ($F_{[3,574]}$= 93.96, P<0.001) and a time effect ($F_{[35,574]}$=8.18, P<0.001). LSD test a posteriori indicated that the group receiving cocaine and the one that received cocaine+ mibefradil were significantly different (P<0.001), as were the two groups that received cocaine, compared to the control group. For 5 mg/kg mibefradil (Fig. 3b, six to ten mice per group), two-way ANOVA (treatment × time) revealed a treatment effect ($F_{[3,1006]}$=8.11, P<0.001) and a time effect ($F_{[35,1006]}$=253.13, P<0.001). LSD test a posteriori indicated that all groups were significantly different (P<0.001), except for the saline and mibefradil groups.

In addition, we found that 2-octanol co-administration reduced cocaine-induced hyperlocomotion (Fig. 4). In this case, a 0.5 mg/kg dose (Fig. 4a, 5–7 mice per group) resulted in both a pharmacological effect ($F_{[3,718]}$=101, P<0.001) and a time effect ($F_{[35,718]}$=4.6, P<0.001) as demonstrated by two-way ANOVA (treatment × time). LSD test a posteriori indicated that all groups were significantly different (P<0.001). Likewise, for 0.07-mg/kg treated mice (Fig. 4b, six mice per group), two-way ANOVA (treatment × time) indicated both a treatment ($F_{[3,707]}$=220, P<0.001) and a time effect ($F_{[35,220]}$=6.3, P<0.001). LSD test a posteriori showed that all groups were significantly different (P< 0.001), except for the saline and 2-octanol groups.

These experiments also showed that the high dose of 2-octanol, at 0.5 mg/kg, reduces basal locomotor activity compared to only saline administration.

Discussion

Our results suggest that T-type calcium channels were over-activated following repetitive cocaine administration. Both in vitro and in vivo, administration of the T-type blockers mibefradil and 2-octanol reduced cocaine-mediated enhancement of thalamic GABAergic transmission. Moreover, cocaine-induced hyperlocomotion in mice was significantly reduced by high and low doses of mibefradil and 2-octanol.

A number of alterations in GABAergic transmission associated with repeated exposure to cocaine have been

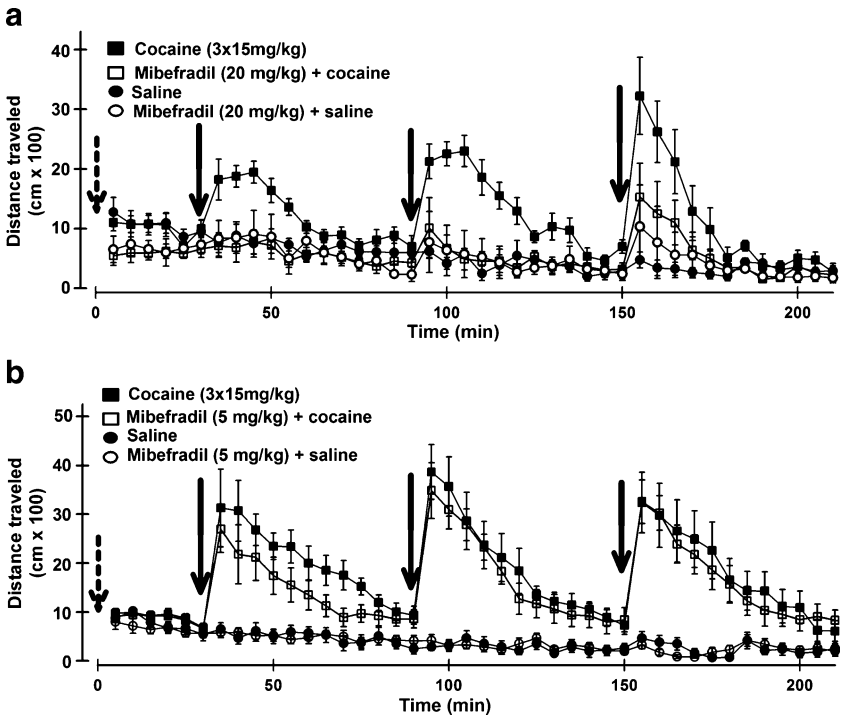

Fig. 3 Mibefradil reduced cocaine-induced hyperlocomotion. **a** Plot showing the average distance traveled (in cm ×100) by mice i.p. -injected with saline (three injections, 1 h apart), cocaine (3×15 mg/kg, 1 h apart), saline pretreated with mibefradil (20 mg/kg, 90 min prior to the first saline injection), and cocaine pretreated with mibefradil (20 mg/kg, 90 min prior first cocaine injection). **b** Same as **A** for 5-mg/kg mibefradil pretreatment with saline and cocaine. *Solid black arrows* represent the time points in which cocaine/saline injections were administered. *Discontinuous black arrow* represents initial mibefradil injection 90 min prior to the first cocaine/saline injections. Each point corresponds to locomotor activity recorded from five consecutive minutes

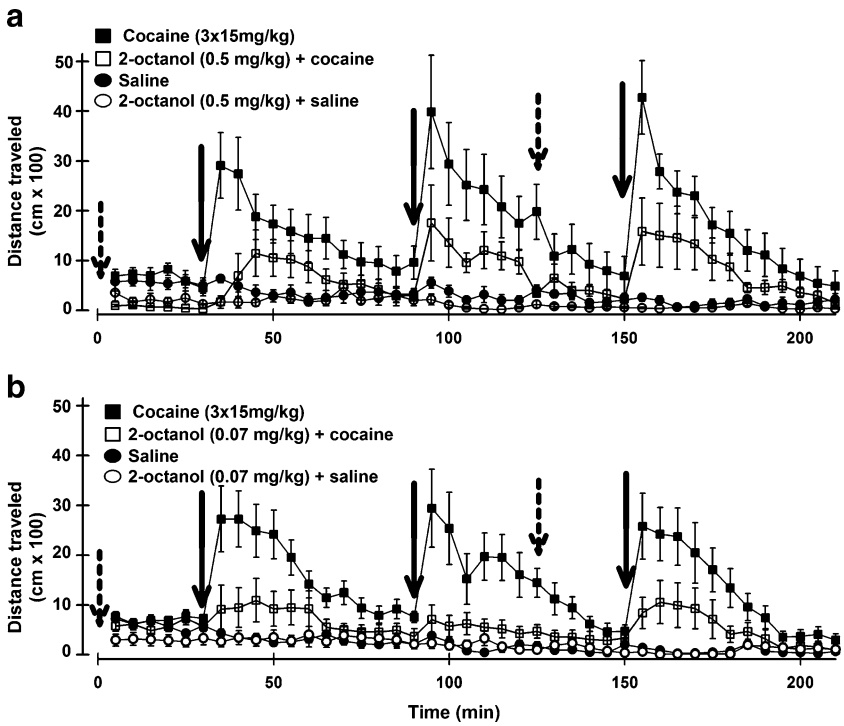

Fig. 4 2-octanol reduced cocaine-induced hyperlocomotion. **a** Plot showing the average distance traveled (in cm ×100) by mice i.p.-injected with saline (three injections, 1 h apart), cocaine (3×15 mg/kg, 1 h apart), saline pretreated with 2-octanol (0.5 mg/kg, 30 min prior first saline injection), and cocaine pretreated with 2-octanol (0.5 mg/kg, 30 min prior first cocaine injection). **b** Same as **A** for 0.07 mg/kg 2-octanol pretreatment of saline and cocaine. *Solid black arrows* represent the moments when cocaine/saline injections were administered. *Discontinuous black arrows* represent 2-octanol injections 30 min prior to the first and third cocaine/saline injections. Each point corresponds to the locomotor activity recorded in five consecutive minutes

identified. Chronic cocaine addicts generally show enhanced sensitivity to benzodiazepines in striatum, thalamus, and parietal cortex (Volkow et al. 1998). In animal studies, cocaine withdrawal increases both GABA-A receptor and GAD proteins in hypothalamus (Ma et al. 2008). A previous study from our group described an enhancement in GABAergic transmission onto the VB thalamic nucleus from mice after repetitive cocaine administration (Urbano et al. 2009). Although no synprint sequence has been reported for T-type channels (Catterall 1998; Perez-Reyes 2003), T-type channels have being described to be coupled to synaptic transmission in rat chromaffin cells (Carabelli et al. 2007a, b) and a pheochromocytoma cell line (Harkins et al. 2003). Therefore, we suggest that T-type channels mediating enhancement of reticular GABAergic release might be twofold: (1) through presynaptic membrane depolarization, and/or (2) through cocaine-mediated intracellular stimuli, triggering a "low-threshold" neurotransmitter release at resting membrane potentials.

Abnormal enhancement at low-frequencies during EEG recordings has been described for cocaine-injected mice (Urbano et al. 2009). Moreover, higher LVA/HVA calcium current ratios, changes in *h*-current density and voltage dependence of T-type currents were observed in thalamic VB neurons from cocaine-injected mice (Urbano et al. 2009). Based on these facts, it was proposed that cocaine abnormally switches the *tonic-firing* to the *burst-firing* mode in the thalamocortical system. The resulting unbalanced LVA/HVA calcium currents were previously proposed to be involved in multiple psychiatric and neurological diseases, grouped recently as "thalamocortical dysrhythmia syndrome" (Jeanmonod et al. 2003; Llinás et al. 1999, 2005). Although reversible after 24-h washout, repetitive cocaine administration induced thalamocortical abnormalities similar to those previously observed in mice lacking P/Q-type calcium channels, and referred to as the "thalamocortical dysrhythmia" animal model (Llinás et al. 2007).

The T-type calcium channel blockers mibefradil and 2-octanol have been extensively used both in vivo and in vitro (reviewed in Llinás 1988; Perez-Reyes 2003). Mibefradil is a non dihydropyridine calcium channel blocker that has 10–30-fold selectivity for T- over L-type channels, with a slightly higher affinity for the cloned $Ca_V3.1$ and Ca_V 3.2 than Ca_V 3.3 subunits (Martin et al. 2000). On the other hand, bath-applied derivatives of octanol (an aliphatic alcohol) have been described to block T-type channels present in inferior olivary (Llinás 1988), hippocampal (Takahashi et al. 1989), thalamic relay (Llinás et al. 2007), and reticular neurons (Joksovic et al. 2010). In vivo, administration of octanol derivatives was reported to reduce harmaline-induced tremor (0.05–0.3 mg/kg, i.p.; Sinton et al. 1989) and EEG low-frequency power spectrum in mice lacking P/Q-type channel, without affecting the wildtype animal (0.2 mg/kg; Llinás et al. 2007). In our hands, bath-applied 2-octanol (more potent than the isomer 1-octanol; Sinton et al. 1989) abolished T-type currents in VB neurons, while it reduced GABAergic mini frequencies. Both mibefradil and nickel showed similar effects. They both reduced GABAergic mini frequencies either in vitro, bath-applied, or in vivo co-administered with cocaine. Our

results further suggest the direct involvement of T-type currents on GABAergic thalamic neurotransmission, and by extension on cocaine-mediated thalamocortical alterations.

The fact that T-type calcium channels seem to be over-activated at resting membrane potentials (e.g., in the presence of TTX to prevent action potential generation) following repetitive cocaine administration, suggests that T-type calcium channels located on both presynaptic terminals of reticular neurons and postsynaptic VB neurons might be the target of downstream effectors of cocaine (e.g., 5-HT, DA and/or NE). Expression of $5HT_{2A/2C}$ serotonergic receptors has been extensively described in thalamocortical nuclei (Cornea-Hebert et al. 1999; Marek et al. 2001). Moreover, 5-HT is known to increase T-channel currents present in spinal motoneurons (Berger and Takahashi 1990) and shifts the I-V curve of T-channels currents ~5 mV in hippocampal interneurons (Fraser and MacVicar 1991). By contrast, although DA receptors present on reticular neurons were described to inhibit GABAergic neurotransmission onto VB neurons (Floran et al. 2004; Khan et al. 1998), neither NE nor DA has been described as acting on T-type calcium channels (Fisher and Johnston 1990). Du et al (2006) previously reported different effects for cocaine and methylphenidate (a psychostimulant that inhibits DAT and NET, but not SERT; Kuczenski and Segal 1997) on $[Ca^{2+}]$ transients in cortical networks associated to serotonergic, but neither dopaminergic nor noradrenergic system. Additionally, recent results from our group showed that repetitive administration of methylphenidate (methylphenidate hydrochloride; 3× 15 mg/kg, i.p., 1 h apart) failed to affect T-type calcium channels, but induced an increment in GABAergic mini frequencies that was significantly lower than that triggered by cocaine (data not shown). Therefore, a partial involvement of serotonin in cocaine-mediated thalamocortical alterations can be suggested.

In summary, serotonergic effects on thalamocortical neurons could result in the hyperpolarization of thalamic cortical projecting neurons, leading to thalamocortical recurrent bursting activity. This could occur by different mechanisms: (1) by increasing the bursting activity of reticular neurons (Llinás and Geijo-Barrientos 1988; McCormick and Wang 1991; Huguenard and Prince 1992; Zhang et al. 2009) that would result in an increased T-type channel activity at the resting potential that would trigger presynaptic membrane depolarization and increase GABAergic transmission onto specific thalamic neurons, and (2) by hyperpolarizing postsynaptic specific VB neurons (Monckton and McCormick 2002) that would, in turn, increase their bursting activity (Jahnsen and Llinás 1984a, b; McCormick and Feeser 1990). Prolonged enhancement of thalamocortical burst-firing mode of thamalocortical neurons during awake states has been suggested to induce ineffective sensory processing (McCormick and Feeser 1990) due to abnormal cortical interactions between low and high-frequency thalamic afferents (Llinás et al. 2005).

Cocaine-induced psychomotor activation has been associated to a transient increment in DA levels in the caudate and accumbens nuclei of basal ganglia (Kuczenski and Segal 1992; Oleson et al. 2009; Budygin 2007). Likewise, reduction in striatal dopamine levels, as in Parkinson's disease, result in reduced movement (Hornykiewicz 1966; Albin et al. 1989). Dopaminergic afferents from substantia nigra pars compacta (SNc; Gerfen et al. 1990; Albin et al. 1989) modulate GABAergic output from medium spiny stellate neurons (which constitute more than 90% of the total striatal neuronal population) to other basal ganglia and thalamocortical nuclei (Albin et al. 1989; Alexander and Crutcher 1990; Gerfen et al. 1990). T-type calcium channels are expressed in striatum, SNc, ventral-anterior, ventral-lateral, and mediodorsal (MD) thalamic nuclei and prefrontal/motor cortices (Talley et al. 1999; Perez-Reyes 2003). In agreement with this localization, T-type calcium channels in SNc neurons have been shown to modulate DA release, as measured by microdialysis in vivo (Bergquist and Nissbrandt 2003) and in an in vitro voltammetric study (Chen et al. 2006). Direct actions of mibefradil (10 μM) on somatic dopamine release have been described in neurons from substantia nigra in culture (Kim et al. 2008). T-type channels would thus be expected to modulate striatal DA levels, altering basal ganglia GABAergic efferents that project to the thalamus which in turn might result in less movement. In the present study, both mibefradil and 2-octanol reduced locomotor activity when administered prior to cocaine. Moreover, we cannot rule out that T-type channels involvement in somatosensory circuits and basal ganglia might be ocurring in parallel. Indeed, the effects of T-type channel blockers in vitro could be explained through their involvement in thalamic synaptic transmission; while the in vivo effects on hyperlocomotion could be related to their association with basal ganglia dopamine release.

Apart from T-type channels, HVA calcium channels are also known to mediate both glutamatergic and GABAergic synaptic transmissions (Iwasaki et al. 2000; Ali and Nelson 2006). Likewise, psychostimulant-induced motor activity has been described to be modulated by HVA blockers. L-type calcium channel blockers decrease cocaine self-administration (Kuzmin et al. 1996) and motor activity (Mills et al. 1998). L-type blockers have been described to reduce by ~8% the normal cocaine-mediated increments in extracellular dopamine and serotonin levels in the nucleus accumbens (Mills et al. 1998). N-, R-, and P/Q-type channels have also been involved in basal ganglia dopaminergic release (Phillips and Stamford 2000; Bergquist and Nissbrandt 2003; Chen et al. 2006). The same way, mice

lacking N-type calcium channels present less monoamine levels in basal ganglia (Nakagawasai et al. 2010). Thus, HVA calcium channels were shown to affect motor activity through their involvement in presynaptic release of monoamines in basal ganglia.

Results shown here strongly suggest that T-type LVA calcium channels are involved in cocaine-mediated GABAergic thalamocortical alterations. Although psychostimulant-induced motor activity is mediated by a variety of high-voltage activated calcium channels, T-type blockers should be considered as potential and effective pharmacological tools to prevent and/or arrest, the acute, as well as the chronic effect of cocaine on thalamocortical networks.

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The experiments included in this work comply with the current laws of Argentina. Authors have full control of all primary data and agree to allow the journal to review their data, if requested.

Conflict of interest Authors also report no conflict of interest, financial or otherwise, related directly or indirectly to this work.

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