Calcium influx through L-type channels generates protein kinase M to induce
burst firing of dopamine cells in the rat ventral tegmental area
Yudan Liu\textsuperscript{1}, Jules Dore\textsuperscript{1}, Xihua Chen\textsuperscript{1,2}

From the \textsuperscript{1} Division of Basic Medical Sciences, \textsuperscript{2} Discipline of Psychiatry, Faculty of Medicine,
Memorial University of Newfoundland, St. John’s, NL A1B 3V6, Canada

Running title: Protein kinase M and burst firing

Address correspondence to: Xihua Chen, Division of Basic Medical Sciences, Memorial University of
Newfoundland, St. John’s, NL A1B 3V6, Canada, Tel. (709) 777-6410, Fax. (709) 777-7010, Email
xihuac@mun.ca

Enhanced activity of the dopaminergic system originating in the ventral tegmental area
is implicated in addictive and psychiatric disorders. Burst firing increases dopamine
levels at the synapse to signal novelty and salience. We have previously reported a
calcium-dependent burst firing of dopamine cells mediated by L-type channels following
cholinergic stimulation, this paper describes a cellular mechanism resulting in burst firing
following L-type channel activation. Calcium influx through L-type channels following
FPL64176 or (S)-(−)-Bay K8644 induced burst firing independent of dopamine, glutamate or
calcium from the internal stores. Burst firing induced as such was completely blocked by the
substrate site protein kinase C (PKC) inhibitor chelerythrine but not by the diacylglycerol site
inhibitor calphostin C. Western blotting
analysis showed that FPL64176 and (S)-(−)-Bay K8644 increased the cleavage of PKC to
generate protein kinase M (PKM) and the specific calpain inhibitor MDL28170 blocked
this increase. Prevention of PKM production by inhibiting calpain or depleting PKC blocked
burst firing induction whereas direct loading of purified PKM into cells induced burst firing.
Activation of the NMDA type glutamate or
cholinergic receptors known to induce burst
firing increased PKM expression. These results
indicate that calcium influx through L-type
channels activates a calcium-dependent
protease that cleaves PKC to generate
constitutively active and labile PKM resulting
in burst firing of dopamine cells, a pathway
that is involved in glutamatergic or cholinergic
modulation of the central dopamine system.

Dopaminergic (DA) projections from the
ventral tegmental area (VTA) constitute the
mesolimbocortical system that underlies drug
abuse and schizophrenia, primarily as the result of
increased DA transmission (1-3). The strength of
DA transmission is regulated by the interplay
between spike-dependent DA release, DA
reuptake and autoreceptor-mediated negative
feedback. Essentially, more intense spiking
releases more DA at the terminal, which in turn
activates the negative feedback mechanism to shut
the system off. For enduring DA transmission at
greater intensity, it would require a higher
accumulation of DA at the synapse without
triggering the negative feedback machinery. Burst
firing is one such mode of discharge that has been
shown to enhance DA transmission (4-7) by
saturating reuptake transporters and reducing
autoreceptor inhibition (8).

DA cells in the VTA are capable of both
pacemaker-like and burst firing (9), the latter
being shown to signal novelty and salient stimuli
in whole animal studies (10). DA cells in slices
predominantly display pacemaker-like firing, burst
firing can be induced by surrogate synaptic
stimulation with bath application of glutamatergic
or cholinergic agonists (11;12) that mobilize Ca\textsuperscript{2+}.
Ca\textsuperscript{2+} entry has been shown to be pivotal in
regulating firing patterns of DA neurons. Intracellular administration of Ca\textsuperscript{2+} evokes burst
firing while intracellular Ca\textsuperscript{2+} chelators block it
(13). Our previous study shows that carbachol, a
general cholinergic agonist, induces burst firing
primarily by promoting Ca\textsuperscript{2+} influx through L-type
channels (12). These channels are responsible for
approximately one third of total Ca\textsuperscript{2+} currents of
DA neurons (14-16) and contribute preferentially
to whole-cell Ca\textsuperscript{2+} currents evoked by small
depolarizations (16;17). In line with this, L-type
channels have been shown to be involved in
spontaneous and burst firing (12;18-20).
Protein kinase M and burst firing

Activation of L-type channels has been shown to modulate synaptic strength in DA cells (21). Additionally, L-type channels gate Ca\(^{2+}\)-release from internal Ca\(^{2+}\) stores, activate plasma membrane Ca\(^{2+}\)-dependent K\(^+\) channels, as well as several Ca\(^{2+}\)-dependent kinases such as protein kinase C (PKC), calmodulin kinase II (CaMKII) and protein kinase A (PKA) (22). These kinases are capable of phosphorylating a variety of ion channels to regulate the excitability of neurons (23). Finding the mechanism that controls the firing mode of DA cells would provide a vital means of modulating the system in both normal and disease conditions. Here, we present results that Ca\(^{2+}\) influx through L-type channels activates a Ca\(^{2+}\)-dependent protease which in turn cleaves PKC to generate a labile fragment that is constitutively active (termed protein kinase M, PKM) to induce burst firing in DA neurons.

**EXPERIMENTAL PROCEDURES**

**Slice preparation** - All procedures involving animal handling and tissue harvesting were in accordance with guidelines set by the Institutional Animal Care Committee at the Memorial University of Newfoundland. Sprague-Dawley rat pups (9-21 days old) of either sex were deeply anaesthetized with halothane and killed by chest compression. The skull was quickly opened to expose the brain, which was cooled in situ with ice-cold, carbogenated artificial cerebrospinal fluid (ACSF, composition: 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH\(_2\)PO\(_4\), 1.2 mM MgCl\(_2\), 2.4 mM CaCl\(_2\), 18 mM NaHCO\(_3\), and 11 mM glucose, pH 7.4 when bubbled with 95% O\(_2\) and 5% CO\(_2\)). The brain was removed and a block containing the midbrain was cut on a Leica vibratome (VT 1000, Heidelberg, Germany). Tissue slices were allowed to recover at room temperature (22°C) in carbogenated ACSF for at least 1 h prior to recording. Slices were further trimmed to fit into a recording chamber and continuously perfused with carbogenated ACSF at a rate of 2-3 ml min\(^{-1}\) at room temperature. For PKC depletion experiments, one of the two VTA slices from the same animal was incubated in ACSF with or without 1-2 µM phorbol 12-myristate 13-acetate (PMA) for 20-24 h at room temperature in a partially sealed beaker continuously bubbled with carbogen.

**Patch clamp recording** - All recordings were made from the VTA identified under a dissecting microscope (Leica MZ6). Patch electrodes were prepared from KG-33 glass micropipettes (OD 1.5 mm, Garner Glass CO., Claremont, CA, USA) on a P-97 Brown-Flaming micropipette puller (Sutter Instruments, Novato, CA, USA). For nystatin-perforated patch clamp recording, glass electrodes were filled to the tip with intracellular solution (120 mM potassium acetate, 40 mM HEPES, 5 mM MgCl\(_2\), and 10 mM EGTA with pH adjusted to 7.35 using 0.1 N KOH) and then back-filled with the same solution containing 450 µg ml\(^{-1}\) nystatin and Pluronic F127, yielding a tip resistance of 4-8 MΩ. For conventional whole-cell recording, electrodes were filled with a solution containing 126 mM potassium gluconate, 10 mM KCl, 0.2 mM EGTA, 10 mM HEPES, 4 mM MgATP and 0.3 mM Na\(_3\)GTP with pH adjusted to 7.35 and osmolarity to 295 mOsm, yielding a resistance of 2-4 MΩ. Gigaohm seals were made using a Warner PC-505B (Warner Instruments Inc., Hamden, CT, USA) or a MultiClamp 700B (Axon Instruments, Foster City, CA, USA) amplifier. Signals were sampled at 5 kHz and digitized by DigiData 1320A using pCLAMP (versions 8 and 9) software (Axon Instruments).

Selection of nystatin-perforated cell recordings in current clamp mode was determined by the size of the action potential, since many VTA cells were spontaneously active. After adequate partitioning of nystatin into the membrane, action potentials overshot 0 mV and measured at least 50 mV. Quality of conventional whole-cell recordings was assessed by a brief voltage step (-20 mV, 10 ms) from the holding potential (-55 mV). Only cells that had an access resistance of <30 MΩ and an input resistance of >200 MΩ were included. Cells whose access resistance increased significantly during the course of recording (>20%) were discarded. Episodic protocols were used to induce hyperpolarization activated current (I\(_h\)) and derive passive characteristics of the cell such as current-voltage relationship and input resistance. Current pulses for I\(_h\) induction were of 1 s duration and the intervals between pulses were 8 s to allow complete recovery of I\(_h\) channels. Cells that displayed a prominent I\(_h\) and an apparent DA-
induced hyperpolarization were identified as putative DA cells (11;12;24).

Components of extracellular and intracellular solutions were purchased from bulk distributors Fisher Scientific (Nepean, Ontario, Canada) and VWR International (Mississauga, Ontario, Canada). All other chemicals were obtained from Sigma (St Louis, MO, USA) and Tocris (Ellisville, MO, USA). Chemicals were dissolved in deionized water or DMSO as required. Aliquots of stock solutions were kept at –30°C. Prior to application, an aliquot was diluted to working concentration and applied to the ACSF bath. PKM (Sigma) was kept at –80 °C and was diluted to 1 unit ml⁻¹ immediately before use in the internal solution for conventional whole-cell recording. DA solution was made fresh daily with an equimolar concentration of the antioxidant disodium metabisulfite.

Western blotting - Phosphorylated PKC and PKM were detected by a phospho-PKC (pan) antibody (Cell Signaling, CA, USA) that recognizes PKCa, βI, βII, δ, ε, η and θ isoforms only when phosphorylated at a carboxyl-terminal residue homologous to Ser660 of PKCβII. Ser660 is in the catalytic domain of PKC and allows simultaneous visualization of phosphorylated PKC and PKM. VTA slices from the same animal were hemisected to give equivalent halves for control and experimental groups. Slices were trimmed to contain the VTA and part of the substantia nigra and were homogenized in RIPA [1x PBS, 1% Triton X-100, 50 mM Tris-HCl (pH 7.4), 0.5% deoxycholate, 50 mM β-glycerophosphate, 50 mM sodium fluoride, 5 mM EDTA, 0.1% sodium orthovanadate, 0.1% SDS, 75 ng/ml phenylmethylsulfonyl fluoride (PMSF) and 1x complete protease inhibitor (Roche Diagnostics, QC, Canada)]. Insoluble materials were removed by centrifugation (15,000xg for 10 min), protein concentration for each sample was determined by BCA™ assay using BSA standard (Pierce, Rockford, USA). Equivalent mass of total protein from slice homogenates were separated on an 8.5% acrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane. The blots were blocked with Blotto (5% w/v non-fat milk in TBST; 10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% Tween20) for 1 h at room temperature, then incubated with phosho-PKC (pan) antibody (1: 1,000) diluted in Blotto, rocking overnight at 4°C. Blots were washed 5 times in TBST and followed by goat anti-rabbit HRP-conjugated IgG diluted in Blotto (Cell Signaling, 1: 15,000) for 90 min at room temperature. After five washes in TBST, blots were incubated in enhanced SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, USA) to visualize specific immune complexes using hyperfilm X-ray film (Amersham Biosci., NJ, USA). Blots were then stripped in a solution of 2% SDS, 63 mM Tris-HCl (pH 6.8) and 0.1 M β-mercaptoethanol for 30 min at 50°C. Stripping solution was removed by two washes with TBST at room temperature, followed by blocking with Blotto and incubation with anti-β-actin antibody (Sigma, 1: 4,000) to act as loading controls. Quantification was obtained from densitometric measurements of immunoreactive bands using Gel Logic100 imaging system with Gel Logic200 Software (Kodak, USA).

Data analysis - Electrophysiological data were analyzed offline with Mini Analysis (Synaptosoft Inc., Decatur, GA, USA) and pCLAMP software. Basal firing frequencies were averaged values of at least 5 min stable baseline recording. Ih was measured as the difference in current or voltage between instantaneous and steady-state readings. Analysis of firing behaviour was based on interspike intervals (ISIs) measured with the Mini Analysis program. Averaged as well as instantaneous firing frequencies were derived from those intervals. Coefficient of variance (CV) was calculated as the mean of ISIs over a 1-min period divided by their standard deviation. To compared CV values between cell groups, they were normalized against the mean CV value of the first 5 min. Relative density of ISIs in 2-second bins were plotted to reveal the distribution of a given ISI series, and the resulting histogram was fitted to a Lowess function. Burst firing was defined as two spikes or more in each bursting cycle at a frequency higher than non-bursting periods and separated by a post-burst hyperpolarization. For western blotting analysis, density of phospho-PKM and PKC bands were normalized against the density of β-actin from the same sample.
Protein kinase M and burst firing

Data were expressed as means and standard errors of the mean (SEM). Statistical comparisons of electrophysiological data were performed using two-tailed unpaired Student’s *t* test. Western blotting data were compared using paired *t* test since each control and experimental pair contained hemisected slices from the same animal. Values were considered significant when *P* < 0.05.

**RESULTS**

Except when indicated, experiments were done using the nystatin-perforated whole-cell recording method at room temperature. Dialysis of PKM into recorded cells was done using conventional whole-cell recording. Only DA cells in the VTA identified according to criteria outlined in Materials and Methods section were included. In nystatin-perforated recording, the average hyperpolarization following a brief application of 50 µM DA (within 90 sec) was –8.29±0.57 mV (n=71, excluding cells used for PKC depletion test). Most cells (47 of 71, 66%) were spontaneously active with single spike firing at a low basal firing frequency of 0.41±0.04 Hz; the remainder (24 of 71, 34%) were quiescent during baseline recordings.

**Opening of L-type calcium channels converts firing patterns.** Bath application of 1-4 µM FPL 64176, a benzoylpyrole site L-type calcium channel opener, for 5-10 min converted firing patterns from quiescent state or single spiking to burst firing in 80.3% of treated cells (57 of 71), application of 1 µM FPL 64176 induced burst firing in 63.5% cells (33 of 52). The responses were spike-dependent. Percentage of converted burst firing in spontaneously firing cells (41 of 47, 87.2%) was much higher than that in quiescent cells (16 of 24, 66.7%), a response that appeared to be related to their different resting membrane potentials (–49.35±0.54 mV for spiking cells vs –52.52±0.92 mV for quiescent cells, *P*<0.05). The responses were dose-dependent: 2 µM FPL 64176 induced burst firing in 9 of 17 cells that did not respond to 1 µM and similarly, 4 µM induced burst firing in 4 of 10 cells that did not respond following a dose of 2 µM.

In cells that were spontaneously firing, FPL 64176 first induced a membrane depolarization (2.00±0.38 mV, n=41) accompanied by a 48.8±9.3% increase in firing rate in the first 5 minutes of drug application, burst firing started after a varying period of latency. The lag between the start of drug application and burst firing ranged 5-25 min with an average of 13 min (Fig. 1A, 1F). Within a burst firing cycle, action potentials were fired with increasing frequencies followed by a pronounced post-burst hyperpolarization (Fig. 1B). The average intraburst firing frequency (1.12±0.13 Hz, n=41) was much higher than basal tonic frequency (0.43±0.05 Hz). The development of burst firing in quiescent cells (n=16) was slightly different: they responded to FPL 64176 with a membrane depolarization (1.38±0.38 mV) followed by a sudden appearance of burst firing (Fig. 1D) or irregular single spiking that evolved into burst firing (Fig. 1E). The L-type channel-mediated burst firing was long lasting even after prolonged washout up to 3 hours, however, it was readily inhibited by L-type channel blocker nifedipine at a range of doses (1-10 µM, n=29). Density plots of ISIs in 2-second bins showed an ISI distribution that fits to a single Gaussian distribution under control conditions and to a mixed Gaussian distribution following FPL 64176 application (Fig. 1C). The leftward shift of the main peak indicates higher firing frequencies and the second peak at much lower frequency represents the long pauses of firing between adjacent bursts.

**FPL 64176 induces burst firing independent of an intermediate transmitter.** L-type channels have been shown to enhance a slow NMDA current in DA neurons (21), suggesting that L-type channel opening could induce burst firing by promoting glutamate transmission. Because FPL 64176-induced burst firing was long-lasting, we examined the involvement of synaptic
mechanisms in two ways: whether induced burst firing persisted in the presence of a cocktail containing 100 µM APV, 10 µM CNQX and 100 µM picrotoxin (blocking NMDA, AMPA and GABA<sub>A</sub> receptors, respectively) and whether pretreatment with this cocktail altered the ability of FPL 64176 to induce burst firing. FPL 64176-induced burst firing was robust following the application of the cocktail for 10-30 min (n=7, Fig. 2A). Similarly, cells that were treated with the cocktail for 5-10 minutes prior to FPL 64176 (1 µM) application in the presence of the cocktail still responded with burst firing (n=3). These results suggest that L-type channel opening does not induce burst firing either directly through increased glutamatergic transmission or indirectly by way of GABAergic interneurons in the VTA.

Activation of L-type channels may release DA from the soma and dendrites since this release has been shown to be Ca<sup>2+</sup>-dependent (25;26). DA acting at somatodendritic autoreceptors forms the short-loop negative feedback to regulate the excitability of DA cells, we therefore examined whether D<sub>2</sub> receptors were involved in burst firing. The D<sub>2</sub> receptor antagonist sulpiride (1-10 µM) applied for 10-15 min to 5 burst firing cells induced by FPL 64176 had no effect on firing patterns (Fig. 2B). Two cells to which 10 µM sulpiride was applied for 8 min prior to FPL 64176 application in the presence of sulpiride displayed burst firing similar to that induced by FPL 64176 alone. These data suggest that L-type channel activation does not result in somatodendritic DA release to induce burst firing.

**Internal Ca<sup>2+</sup> stores are not involved.** Ca<sup>2+</sup> entry through L-type channels gates ryanodine receptors on internal Ca<sup>2+</sup> stores to mediate Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (22), we tested whether this was involved in burst firing. Cyclopiazonic acid (CPA) and thapsigargin were used to inhibit the sarcoendoplasmic reticulum Ca<sup>2+</sup>-dependent ATPase (SERCA) that refills internal Ca<sup>2+</sup> stores. After burst firing was induced by FPL 64176, CPA (20-30 µM) applied for 20-60 min (n=4) or thapsigargin (1-2 µM) applied for 50-70 min (n=3) did not alter the induced burst firing (Fig. 2C). In 3 cells that were pretreated with CPA (20 µM) for 30 min, FPL 64176 (1 µM) was still able to induce strong burst firing. These results indicate that internal Ca<sup>2+</sup> stores are not necessary for L-type channel-mediated burst firing.

**Ca<sup>2+</sup>-dependent protein kinase mediates burst firing.** Increased intracellular Ca<sup>2+</sup> activates Ca<sup>2+</sup>-sensitive protein kinases such as PKC, PKA and CaMKII that can phosphorylate ion channels including L-type channels themselves and regulate the excitability of neurons (27-30). After burst firing was induced by FPL 64176, bath application of the PKA inhibitor H-89 (5 or 10 µM) for 40-130 min (n=4), the CaMKII inhibitor KN-93 (1-10 µM) for 25-100 min (n=6), or both for 60-70 min (n=3) did not block burst firing (Fig. 3A). The substrate site PKC inhibitor, chelerythrine (40 µM), applied for 15-44 min (n=6) completely blocked Ca<sup>2+</sup>-induced burst firing and the accompanying membrane potential oscillation. This blockade was reversible since burst firing reappeared after washing out chelerythrine for 18-33 min (Fig. 3B). To establish the role of PKC in burst firing, we further used an inhibitor that binds to the diacylglycerol site of the regulatory domain. Calphostin C (1-2 µM) applied for 40-180 min (n=4) was largely ineffective except in one cell where burst firing slowed marginally but persisted (Fig. 3C). These results suggest that the catalytic subunit of PKC itself or a kinase with a similar substrate site is involved in burst firing.

**Proteolytic cleavage of PKC mediates burst firing.** To solve the apparent inconsistency between the two PKC inhibitors, we considered other modes of PKC activation. Ca<sup>2+</sup> entry has been shown to activate the protease calpain that proteolytically cleaves PKC to form PKM which has been shown to be constitutively active with a very short half-life within the cell (31-34). We therefore tested whether proteolytic cleavage of PKC could explain the inconsistency in response to the two PKC inhibitors. Cells were induced to burst fire by FPL 64176, the calpain inhibitor MDL 28170 (200 µM) was then applied. In all cells tested as such, burst firing reverted to single spike firing in 25-40 min and resumed after washing 20-30 min (n=5, Fig. 4A). Prior application of MDL 28170 (200 µM) for 30-40 min completely prevented FPL 64176-induced (2-4 µM) burst firing (n=3).
To further validate the role of PKM in L-type channel-induced burst firing, we compared the levels of phospho-PKC and PKM by western blotting total protein lysates from slices that were treated with (S)-(−)-Bay K8644 or FPL 64176 alone and with MDL 28170 followed by FPL 64176. As shown in Fig. 4B, phospho-PKC antibody raised against the catalytic domain visualized three bands: two at approximately 82 kDa corresponding to intact PKC isoforms and another at 45 kDa corresponding to the PKC catalytic unit or PKM. Application of L-type channel opener FPL 64176 (2 µM) or (S)-(−)-Bay K8644 (5 µM) for 30 min caused a considerable increase in phospho-PKM expression (Fig. 4C). Densitometry of the bands corresponding to phospho-PKM showed that (S)-(−)-Bay K 8644 (224±61% relative to untreated values, n=8, P<0.05) or FPL 64176 (244±51% relative to untreated values, n=6, P<0.05) significantly increased phospho-PKM levels (Fig. 4D). The increase by FPL 64176 could be completely blocked by prior treatment with the calpain inhibitor MDL 28170 (200 µM for 30 min; 8±1% of FPL 64176 alone, n=5, P<0.0001; Fig. 4C, D). There were no detectable changes in full-length phospho-PKC levels following (S)-(−)-Bay K8644 (102±10% of control values, n=8, P>0.05), FPL 64176 (104±6% of control values, n=6, P>0.05) or MDL 28170 with FPL 64176 (95±2% of FPL 64176 alone, n=5, P>0.05). These data indicate that PKM levels parallel burst firing behavior.

**Burst firing can not be induced after PKC depletion.** Since PKM is generated by cleavage of PKC (33;34), it is an obvious test to determine if burst firing can be induced following PKC depletion. Persistent activation by phorbol esters such as phorbol 12-myristate 13-acetate (PMA), leads to degradation of PKC isoforms that have the diacylglycerol site and is used experimentally to produce a PKC-depleted cell (35;36). Slices were incubated with or without PMA (1-2 µM) for 20-24 hours followed by FPL 64176 (4 µM) to induce burst firing. Regardless of incubation conditions, cells from these slices were no longer spontaneously active although they were capable of firing action potentials (Fig. 5A). There was no difference in resting membrane potential (control: −50.05±1.36 mV, n=6; PMA-treated: −48.25±1.38 mV, n=7, P>0.05) or input resistance (control: 403.24±56.12 MΩ, n=6; PMA-treated: 293.58±42.93 MΩ, n=7, P>0.05) between control and PMA-treated groups. PMA-treated cells had a significantly smaller DA-induced hyperpolarization (control: 12.03±3.43 mV; PMA-treated: 2.99±0.67 mV, P<0.05). Western blots showed a disappearance in PMA-treated slices of a band around 82 kDa and the concomitant decrease of the PKM band at 45 kDa following FPL 64176 (Fig. 5B).

There was a clear difference in FPL 64176-induced firing between the two groups. Of 6 cells from control slices, FPL 64176 (4 µM) induced burst firing in 3 (Fig. 5C1), large membrane potential oscillations in 2 and no change in one cell. Both burst firing and membrane potential oscillation could be blocked by nifedipine (5 µM). Of 7 cells from PMA-treated slices, FPL 64176 (4 µM) only induced an average membrane depolarization of 0.62±0.26 mV with no burst firing or membrane potential oscillation in any of the cells tested (Fig. 5C2).

**Direct loading of PKM induces burst firing.** If PKM links L-type channels and burst firing, it would be expected that direct loading of PKM (1 unit/ml pipette solution) into the cells through the conventional whole-cell recording pipette should induce similar firing mode switching. Out of 7 cells, 3 had single spike firing initially that became irregular and clustered by 3-5 min, with burst firing appearing at 9-12 min. Burst firing was strong and stable for 20-30 min (Fig 6A) and was qualitatively similar to burst firing induced by FPL 64176. When burst firing was prevalent, density plots of ISIs showed a typical two-peak ISI distribution (Fig 6C2). In another 2 cells, single spike firing became burst-like characterized by clustered spikes followed by a pause without a post-burst hyperpolarization. Only in 2 cells was no response seen. The control experiments were done using the same internal pipette solution without PKM and all 6 cells tested maintained regular firing during the 40 min recording period (Fig. 6B). Plotting normalized ISI coefficient of variance values against the average value of the first 5 min (Fig. 6D) revealed that PKM significantly increased variance while it remained unchanged in control cells (unpaired t test on areas-under-the-curve in arbitrary units, control:
Protein kinase M and burst firing

3323.38±291.91, PKM: 5001.95±427.84, \( P<0.05 \), indicating that PKM disrupts regular spiking and promotes burst firing.

**Activation of NMDA and cholinergic receptors increases PKM expression.** To test whether PKM is involved in the physiological regulation of burst firing in DA neurons, glutamate or cholinergic agonists known to induce burst firing in DA cells were used to stimulate VTA slices and the amount of PKM was detected by western blotting. Hemisected slices from the same animal were treated with or without NMDA (20 µM) or carbachol (20 µM) for 20 min to test whether it increased the levels of phosphorylated PKM. As shown in Fig. 7A, application of either glutamate agonist NMDA or cholinergic agonist carbachol caused a considerable increase in phospho-PKM expression. Densitometry of the bands corresponding to phospho-PKM of six independent experiments (Fig. 7B) showed that both NMDA (159±26% relative to untreated values, \( P<0.05 \)) and carbachol (154±41% relative to untreated values, \( P<0.05 \)) significantly increased phospho-PKM levels. Since glutamate and cholinergic inputs are major synaptic modulators of DA cells, these data indicate that PKM is involved in the synaptic regulation of firing behavior of DA cells.

**DISCUSSION**

Burst firing is an important property of DA cells in the VTA to signal novelty and salience that are associated with normal or abnormal expression of motivation and reward. We have previously reported that the cholinergic agonist carbachol induces a calcium-dependent burst firing of DA cells by promoting \( \text{Ca}^{2+} \) influx through L-type channels, this paper studies how L-type channel activation induces burst firing. In this study, we observed that direct activation of L-type channels converted firing patterns of VTA DA cells independent of glutamate, dendritically release DA or \( \text{Ca}^{2+} \) released from internal stores. Nor did \( \text{Ca}^{2+} \) influx through L-type channels activate PKA or CaMKII to induce burst firing. We have shown that \( \text{Ca}^{2+} \) influx through L-type channels activates calpain proteases that in turn cleave PKC, releasing the short lived, active PKM fragment to produce burst firing. Disruption at any point along this chain of events results in an inability of causing burst firing of DA neurons in the VTA. We have also shown that PKM is involved in regulation of DA cells under physiological stimulations where the NMDA type glutamate or cholinergic receptors were activated.

**L-type channel-induced burst firing does not require an intermediate transmitter.** L-type channels are primarily expressed in the soma and dendrites of DA cells in the VTA area (15) although they have shown to modulate synaptic transmission to the VTA (21). Glutamate acting at the NMDA receptors mediates a slow excitatory synaptic transmission to VTA DA cells (37) that can be enhanced by L-type channel activation (21), suggesting that the effects of L-type channel activation could be secondary to increased glutamate transmission. This is particularly relevant since NMDA is one of the pharmacological tools used to induce burst firing in DA neurons (11,20). Our results that combined synaptic blockade at the NMDA, AMPA and GABA \(_A\) site before or after L-type channel activation did not alter burst firing suggest that glutamate transmission is not involved in L-type channel-induced burst firing. Alternatively, activation of L-type channels increases firing (19) and subsequently releases DA from somatodendritic site to terminate firing by \( \text{D}_2 \)-mediated autoinhibition (26). Autoinhibition wanes as DA is taken back by DA transporters (DAT) and a new cycle starts. This mode of action appears logical since all the proposed mechanisms have been shown to work in DA cells. However, L-type channel activation induced strong burst firing that was not blocked by the \( \text{D}_2 \) receptor antagonist sulpiride. Blocking the \( \text{D}_2 \) receptor before application of L-type channel opener did not alter burst firing either, arguing against a role for somatodendritic \( \text{D}_2 \) receptors in L-type channel-induced burst firing.

We have shown previously that burst firing of DA cells depends on \( \text{Ca}^{2+} \) oscillation (12), a phenomenon that could be supported by the so-called \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release from internal stores. The L-type channel has been shown to directly gate \( \text{Ca}^{2+} \) releasing channels on the membrane of sarcoplasmic reticulum (22). The IP\(_3\) channels have a bell-shape sensitivity relationship
with intracellular Ca\(^{2+}\) levels (22) so that their conductance increases initially with higher Ca\(^{2+}\) concentrations, further increases in Ca\(^{2+}\) reduces their conductance capabilities. Also, Ca\(^{2+}\) release from internal stores has been found to mediate a slow inhibition in DA cells taking hundreds of milliseconds to develop (38). This is on a similar time scale to burst firing cycles we observed in slice preparations. These mechanisms can possibly produce a biphasic response that fits the membrane oscillation we observed. In our experiments, burst firing induced by the L-type channel opener FPL 64176 persisted when internal stores were depleted by prolonged inhibition of the refilling enzyme SERCA, suggesting that internal stores do not play an obligatory role in burst firing.

**Ca\(^{2+}\) influx generates PKM to induce burst firing.** The opening of L-type channels did not induce burst firing within the first 5 minutes following FPL 64176 application although Ca\(^{2+}\) influx had already changed the excitability of the cells in the form of membrane depolarization and increased firing frequency. Burst firing emerged 5-25 min following the start of drug application and lasted for hours. The lag seems to indicate that Ca\(^{2+}\) entry activated a signalling cascade that takes time to develop or a second messenger must migrate between the intracellular compartments. Since protein kinase activation requires multiple stages of phosphorylation and translocation (39), the involvement of a Ca\(^{2+}\)-dependent protein kinases presents a reasonable mechanism underlying Ca\(^{2+}\)-dependent burst firing. For example, CaMKII has been shown to become persistent active during Ca\(^{2+}\) oscillation to enhance L-type channels (40). Similarly, PKA has long been associated with adrenergic enhancement of L-type channel activity (41;42). The involvement of these kinases could explain why L-type channels continue operating despite high intracellular Ca\(^{2+}\) levels. Channel phosphorylation has been suggested to reduce the Ca\(^{2+}\)-dependent inactivation of L-type channels (43). That burst firing persisted in the presence PKA and CaMKII blockers supports the concept that they are not necessary for Ca\(^{2+}\)-dependent burst firing.

The striking result that chelerythrine, a PKC catalytic domain inhibitor, reversibly blocked burst firing, whereas calphostin C, a regulatory domain inhibitor did not, raised the possibility that Ca\(^{2+}\) influx through L-type channels activates a PKC-like kinase or PKC operates in an atypical fashion. Elevated intracellular Ca\(^{2+}\) has been shown to activate calpain (44), a protease that cleaves PKC catalytic domain from the regulatory domain (31-33) to generate PKM. Our results support this mode of PKC action and its regulation of burst firing through: 1) L-type channel openers induced burst firing accompanied by increased levels of phosphorylated PKM; 2) inhibition of the Ca\(^{2+}\)-dependent protease calpain reduced the level of phosphorylated PKM and concomitantly blocked burst firing; 3) PKM is generally thought to be a proteolytic product of PKC (31-33) and depletion of PKC isoforms following prolonged incubation with PMA prevented L-type channel-induced burst firing; 4) direct loading of the cell with purified PKM through the recording pipette induced burst firing in a similar manner to Ca\(^{2+}\) influx, with the temporal development of burst firing being consistent with diffusion of PKM into the cell. The evidence collectively indicates that Ca\(^{2+}\) influx through the L-type channel activates proteolytic cleavage of PKC to generate PKM that facilitates the development and maintenance of burst firing of DA cells.

**PKC is functionally important for DA-related conditions.** PKM was found in the rat brain in 1970s (45) but its function remained obscure until recent reports associating it with learning and memory (46;47). Several PKC isoforms have been found to give rise to proteolytic PKM counterparts, it is reasonable to assume that all members of the PKC family are capable of generating their respective PKMs because of their similar structure of the hinge region that joins the regulatory and catalytic domains. Our results did not identify the parent PKC isoform that was cleaved to form PKM, it may involve several PKC isoforms, since many are expressed in DA neurons (48). The PKM used for cellular loading is derived from enzymatic digestion of total PKC, but from the PKC depletion experiments, it appears that the PKM involved in burst firing is generated by the isoforms with the diacylglycerol site. PKC has been implicated in addiction and motivation. Injection of PKC inhibitors into the VTA reduces cocaine-induced DA release in the nucleus accumbens (49) and delays the onset of behavioral
Protein kinase M and burst firing

sensitization (50;51). Repeated administration of cocaine increases overall PKC activity in the VTA which may initiate behavioral sensitization (52). Being persistently active, PKM could fulfill all these behaviors involving PKCs. More importantly, PKM could achieve all this by its role in burst firing reported here as burst firing elevates terminal DA more effectively and all these behaviors involve increased DA transmission. In addition, addiction is a learned behavior and PKM has been shown to facilitate learning and memory in rats (46;47). Taken together, PKC and its cleaved product PKM might play a significant role in central DA transmission and its related pathologies such as drug abuse. It is especially encouraging that activation of the NMDA and cholinergic receptors increased PKM because these receptors not only mediate synaptic regulation of DA cells, but they are also known to induce burst firing in DA cells.

Mechanisms of PKM-induced burst firing. There are many factors contributing to the strength of DA transmission. Besides the efficiency of the release machinery, it is usually agreed that DA reuptake and autoreceptor-mediated inhibition are important regulators of DA transmission. Released DA is rapidly taken back to the terminal by DAT, whereas D2 autoreceptors at both terminal and somatodendritic sites respond to increased DA levels by a negative feedback loop that inhibit DA cell activity. PKC has been shown to modulate both processes. Activation of PKC leads to a decrease in DAT capacity (53;54) due to accelerated internalization (55;56), reduced recycling (57) and degradation (58). Additionally, PKC activation enhances phosphorylation, desensitization and trafficking of D2 receptors (59) resulting in dampened negative feedback. In PMA treated slices, somatodendritic autoreceptors' responses to DA were blunted, it remains to be tested whether these counterparts in the terminal are similarly affected. Together the effects of PKC on DAT and autoreceptor inhibitory feedback, with our results that the proteolytic product of PKC (PKM) induces burst firing, a firing mode that is more effective in increasing DA release, it is a tantalizing possibility that PKC serves as a signaling hub, integrating different aspects of DA transmission to boost its functionality over a long period of time. This postulate further supports the findings that implicate PKC in addiction, a chronic and debilitating condition that has long been thought to be due to enhanced DA transmission.

REFERENCES

1. Kelley, A. E. (2004) Neuron 44, 161-179
2. Koob, G. F. (2000) Ann.N.Y.Acad.Sci. 909, 170-185
3. Seamans, J. K. and Yang, C. R. (2004) Prog.Neurobiol. 74, 1-58
4. Gonon, F. G. (1988) Neuroscience 24, 19-28
5. Suaud-Chagny, M. F., Chergui, K., Chouvet, G., and Gonon, F. (1992) Neuroscience 49, 63-72
6. Garris, P. A., Ciolkowski, E. L., Pastore, P., and Wightman, R. M. (1994) J.Neurosci. 14, 6084-6093
7. Floresco, S. B., West, A. R., Ash, B., Moore, H., and Grace, A. A. (2003) Nat.Neurosci. 6, 968-973
8. Chergui, K., Suaud-Chagny, M. F., and Gonon, F. (1994) Neuroscience 62, 641-645
9. Hyland, B. I., Reynolds, J. N., Hay, J., Perk, C. G., and Miller, R. (2002) Neuroscience 114, 475-492
10. Tobler, P. N., Fiorillo, C. D., and Schultz, W. (2005) Science 307, 1642-1645
11. Johnson, S. W., Seutin, V., and North, R. A. (1992) Science 258, 665-667
12. Zhang, L., Liu, Y., and Chen, X. (2005) J.Physiol 568, 469-481
13. Grace, A. A. and Bunney, B. S. (1984) J.Neurosci. 4, 2877-2890
14. Cardozo, D. L. and Bean, B. P. (1995) J.Neurophysiol. 74, 1137-1148
15. Takada, M., Kang, Y., and Imanishi, M. (2001) Eur.J.Neurosci. 13, 757-762
16. Durante, P., Cardenas, C. G., Whittaker, J. A., Kitai, S. T., and Scroggs, R. S. (2004) J.Neurophysiol. 91, 1450-1454
17. Xu, W. and Lipscombe, D. (2001) J.Neurosci. 21, 5944-5951
Protein kinase M and burst firing

18. Nedergaard, S., Flatman, J. A., and Engberg, I. (1993) *J.Physiol* **466**, 727-747
19. Mercuri, N. B., Bonci, A., Calabresi, P., Stratta, F., Stefani, A., and Bernardi, G. (1994) *Br.J.Pharmacol.* **113**, 831-838
20. Johnson, S. W. and Wu, Y. N. (2004) *Brain Res.* **1019**, 293-296
21. Bonci, A., Grillner, P., Mercuri, N. B., and Bernardi, G. (1998) *J.Neurosci.* **18**, 6693-6703
22. Berridge, M. J., Bootman, M. D., and Roderick, H. L. (2003) *Nat.Rev.Mol.Cell Biol.* **4**, 517-529
23. Levitan, I. B. (2006) *Nat.Rev.Mol.Cell Biol.* **9**, 305-310
24. Lacey, M. G., Mercuri, N. B., and North, R. A. (1989) *J.Neurosci.* **9**, 1233-1241
25. Chen, B. T. and Rice, M. E. (2001) *J.Neurosci.* **21**, 7841-7847
26. Beckstead, M. J., Grandy, D. K., Wickman, K., and Williams, J. T. (1998) *J.Neurosci.* **18**, 6693-6703
27. Dzhura, I., Wu, Y., Colbran, R. J., Balser, J. R., and Anderson, M. E. (2000) *J.Neurosci.* **18**, 6693-6703
28. Young, C. E. and Yang, C. R. (2004) *J.Neurosci.* **24**, 8-23
29. Lee, T. S., Karl, R., Moosmang, S., Lenhardt, P., Klugbauer, N., Hofmann, F., Kleppisch, T., and Welling, A. (2006) *J.Biol.Chem.* **281**, 25560-25567
30. Yang, L., Liu, G., Zakharov, S. I., Morrow, J. P., Rybin, V. O., Steinberg, S. F., and Marx, S. O. (2005) *J.Biol.Chem.* **280**, 207-214
31. Kishimoto, A., Kajikawa, N., Shiota, M., and Nishizuka, Y. (1983) *J.Biol.Chem.* **258**, 1156-1164
32. Al, Z. and Cohen, C. M. (1993) *Biochem.J.* **296 ( Pt 3)**, 675-683
33. Cressman, C. M., Mohan, P. S., Nixon, R. A., and Shea, T. B. (1995) *FEBS Lett.* **367**, 223-227
34. Shea, T. B., Beermann, M. L., Griffin, W. R., and Blumberg, P. M. (1994) *J.Biol.Chem.* **269**, 2118-2124
35. Szallasi, Z., Smith, C. B., Pettit, G. R., and Blumberg, P. M. (1994) *J.Biol.Chem.* **269**, 2118-2124
36. McArdle, C. A. and Conn, P. M. (1989) *Methods Enzymol.* **168**, 287-301
37. Mercuri, N. B., Grillner, P., and Bernardi, G. (1996) *Neuroscience* **74**, 785-792
38. Fiorillo, C. D. and Williams, J. T. (1998) *Nature* **394**, 78-82
39. Liu, W. S. and Heckman, C. A. (1998) *Cell Signal.* **10**, 529-542
40. Hudmon, A., Schulman, H., Kim, J., Maltez, J. M., Tsien, R. W., and Pitt, G. S. (2005) *J.Cell Biol.* **171**, 537-547
41. van der Heyden, M. A., Wijnhoven, T. J., and Opthof, T. (2005) *Cardiovasc.Res.* **65**, 28-39
42. Hoogland, T. M. and Saggau, P. (2004) *J.Neurosci.* **24**, 8416-8427
43. Budde, T., Meuth, S., and Pape, H. C. (2002) *Nat.Rev.Neurosci.* **3**, 873-883
44. Goll, D. E., Thompson, V. F., Li, H., Wei, W., and Cong, J. (2003) *Physiol.Rev.* **83**, 731-801
45. Inoue, M., Kishimoto, A., Takei, Y., and Nishizuka, Y. (1977) *J.Biol.Chem.* **252**, 7610-7616
46. Sacktor, T. C., Osten, P., Valsamis, H., Jiang, X., Naik, M. U., and Sublette, E. (1993) *Proc.Nat.Acad.Sci.U.S.A* **90**, 8342-8346
47. Osten, P., Valsamis, L., Harris, A., and Sacktor, T. C. (1996) *J.Neurosci.* **16**, 2444-2451
48. Yoshihara, C., Saito, N., Taniyama, K., and Tanaka, C. (1991) *J.Neurosci.* **11**, 690-700
49. Steketee, J. D. (1993) *Neuropharmacology* **32**, 1289-1297
50. Steketee, J. D. (1994) *NeuroReport* **6**, 69-72
51. Steketee, J. D. (1997) *Brain Res.Bull.* **43**, 565-571
52. Steketee, J. D., Rowe, L. A., and Chandler, L. J. (1998) *Neuropharmacology* **37**, 339-347
53. Melikian, H. E. and Buckley, K. M. (1999) *J.Neurosci.* **19**, 7699-7710
54. Daniels, G. M. and Amara, S. G. (1999) *J.Biol.Chem.* **274**, 35794-35801
55. Sorkina, T., Hoover, B. R., Zahmiser, N. R., and Sorkin, A. (2005) *Traffic* **6**, 157-170
56. Holton, K. L., Loder, M. K., and Melikian, H. E. (2005) *Nat.Neurosci.* **8**, 881-888
57. Loder, M. K. and Melikian, H. E. (2003) *J.Biol.Chem.* **278**, 22168-22174
58. Miranda, M., Wu, C. C., Sorkina, T., Korstjens, D. R., and Sorkin, A. (2005) *J.Biol.Chem.* **280**, 35617-35624
59. Namkung, Y. and Sibley, D. R. (2004) *J.Biol.Chem.* **279**, 49533-49541

FOOTNOTES
Protein kinase M and burst firing

*The work was supported by grants from NSERC (202999) and CIHR (62277). The authors wish to thank Dr. Y. Peng for statistical advice.

1The abbreviations used are: PKC, protein kinase C; PKM, protein kinase M; DA, dopamine; VTA, ventral tegmental area; CaMKII, calmodulin kinase II; PKA, protein kinase A; ACSF, artificial cerebrospinal fluid; PMA, phorbol 12-myristate 13-acetate; Ih, hyperpolarization activated current; PMSF, phenylmethylsulfonyl fluoride; ISIs, interspike intervals; CV, coefficient of variance; CPA, cyclopiazonic acid; SERCA, sarco-endoreticulum Ca\textsuperscript{2+}-dependent ATPase; DAT, dopamine transporter; SEM, standard error of the mean.

FIGURE LEGENDS

Figure 1. FPL 64176 induces burst firing of VTA DA cells.
A. Continuous current clamp recording from a representative cell showing that FPL 64176 converted regular firing to burst firing which could be blocked by nifedipine. B. Traces on expanded time scales showing regular firing before FPL 64176 application (1), regular firing at higher rates (2) and burst firing after drug application (3) that was brought back to regular firing by nifedipine (4). Note the low frequency of regular firing with a pronounced afterhyperpolarization following each action potential and the clustering of action potentials at increased frequencies followed by a steep post-burst hyperpolarization in burst firing mode. C. Density plot of interspike intervals (ISI) in 2-second bins in control conditions (C1, 76 events) and following FPL 64176 application (C2, 246 events). FPL 64176 dramatically shifted the primary peak to the left and gave rise to a secondary peak corresponding to the frequency of burst firing cycles. D. Continuous current clamp recording from a representative cell showing that FPL 64176 induced sudden burst firing in a quiescent cell. E. Continuous current clamp recording from a representative cell showing that FPL 64176 induced irregular firing that evolved into burst firing in a cell that had no baseline firing. F. Histogram of number of cells (n=57) that started burst firing following the onset of FPL 64176 application. G. Continuous current clamp recording from a representative cell showing that (S)-(-)-Bay K8644 converted regular firing to burst firing.

Figure 2. FPL 64176 induces burst firing independent of glutamate and GABA ionotropic receptors, D\textsubscript{2} DA receptors and internal Ca\textsuperscript{2+} stores.
Continuous current clamp recording from a representative cell showing FPL 64176-induced burst firing that persisted in the presence of a cocktail containing blockers at the GABA\textsubscript{A}, AMPA and NMDA site (A), the D\textsubscript{2} antagonist sulpiride (B) and the SERCA inhibitor thapsigargin (C).

Figure 3. PKC mediates FPL 64176-induced burst firing.
Continuous current clamp recording from a representative cell showing FPL 64176-induced burst firing that persisted in the presence of PKA or CAMKII inhibitors or both (A), was reversibly blocked by the substrate site PKC inhibitor chelerythrine (B), but not by the PKC blocker that binds to the DAG site in the regulatory subunit (C).

Figure 4. Proteolytic cleavage of PKC mediates FPL 64176-induced burst firing.
Continuous current clamp recording from a representative cell showing that FPL 64176-induced burst firing was reversibly blocked by the calpain inhibitor MDL 28170 (A). Western blots showing phosphorylated PKC isoforms at approximate 82 kDa and a smaller band of PKM at approx 45 kDa (B). PKM was increased by FPL 64176 (2 µM, 30 min) or (S)-(-)-Bay K8644 incubation (5 µM, 30 min) and abolished by prior treatment with MDL 28170 (200 µM, 30 min) followed by FPL 64176 (2 µM, 30 min) with MDL 28170 (C). Levels of phosphorylated PKM in response to FPL 64176 (n=6,) or (S)-(-)-Bay K8644 (n=8) relative to control slices and PKM levels with prior MDL 28170 treatment relative to FPL 64176.
Protein kinase M and burst firing

64176 alone (n=5) (D). Levels of PKM were normalized against β-actin values obtained from the same blot.

**Figure 5. FPL 64176 fails to induce burst firing following PKC depletion.**
A. Comparable current-voltage relationships between cells that were incubated in normal ACSF or in PMA 2 µM for 20 h. B. Western blots showing a diminished band of phosphorylated PKC isoforms (B1) and a much smaller phosphorylated PKM increase (B2) following FPL 64176 (4 µM, 30 min) in PMA-treated slices (2 µM, 20 h). C. Continuous current clamp recording from representative cells showing FPL 64176 induced burst firing that could be blocked by nifedipine in ACSF-treated slices (C1) but not in PMA-treated slices (C2).

**Figure 6. Direct loading of purified PKM induces burst firing.**
A. Continuous current clamp recording from a representative cell showing that PKM loading gradually transformed regular firing into burst firing. Traces on expanded time scales showing regular firing at the beginning (1) and burst firing 30 min after going whole cell (2). B. Control whole cell recording showed regular firing throughout the recording period. Traces on expanded time scales showing regular firing at the beginning (1) and 30 min after going whole cell (2). C. Density plot of interspike intervals (ISI) in 2-second bins for the first 3 min (C1, 136 events) and 22-35 min (C2, 370 events) following PKM loading. Note the single ISI distribution in the first 3 min (C1) and PKM loading generated a second distribution at lower frequencies (C2). D. Normalized coefficient of variance (CV, over 1 min periods) against the average value of the first 5 min in control (n=6) or PKM loading groups (n=7) showing PKM increased CV values.

**Figure 7. NMDA or carbachol induces PKM expression.**
A. Western blots showing phosphorylated PKM was increased by NMDA (20 µM, 20 min) or carbachol (20 µM, 20 min) incubation. B. Levels of phosphorylated PKM in response to NMDA (n=6) or carbachol (n=6) relative to control slices.

**Figure 8. Proposed model of PKM-induced bursting.**
Schematic representation of the proposed model of PKM-induced bursting, inferred from our results. Calcium entry through L-type channels activates the Ca²⁺-dependent protease calpain which cleaves PKC into cofactor-independent PKM to induce burst firing in dopamine-responsive neurons in ventral tegmental area.
Figure 1
Figure 2

A

30 mV
2 min
Picrotoxin 100 μM + CNOX 10 μM + APV 100 μM

B

30 mV
1 min
Sulpiride 10 μM

C

30 mV
1 min
Thapsigargin 1 μM
Figure 3
Figure 5
Figure 6
Figure 7

A

p-PKM

β-actin

Carbachol  -  +  -  -
NMDA       -  -  -  +

B

\[ P < 0.05 \]
Figure 8
Calcium influx through L-type channels generates protein kinase M to induce burst firing of dopamine cells in the rat ventral tegmental area
Yudan Liu, Jules Dore and Xihua Chen

J. Biol. Chem. published online January 19, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M610230200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts