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Psychostimulants, L-type Calcium Channels, Kinases, and Phosphatases

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Abstract

There is growing evidence for the role of voltage-gated L-type Ca^{2+} channels in mediating aspects of the addictive properties of psychostimulants. L-type Ca^{2+} channels activate Ca^{2+} second-messenger pathways that regulate protein phosphorylation and thereby activation of target gene expression. Here the authors will review recent progress in our understanding of L-type Ca^{2+} channel-activated signal transduction pathways that contribute to molecular neuroadaptations evident following acute and chronic exposures to psychostimulants.

Keywords

Drug addiction; $\text{Ca}_v1.2$; $\text{Ca}_v1.3$; Ventral tegmental area; Nucleus accumbens; Striatum; Phosphorylation

The psychostimulants amphetamine and cocaine are the most reinforcing drugs known, both in humans and in animal models of drug addiction. These drugs exert their addictive effects by increasing synaptic levels of the neurotransmitters dopamine and glutamate in the reward pathway of the brain (Berke and Hyman 2000; Hyman and Malenka 2001). It is now well established that the life-long behavioral effects resulting from psychostimulant exposure are a result of molecular adaptations that alter normal brain function (Berke and Hyman 2000; Nestler 2001). Many such adaptations have been shown to result from altered regulation of protein phosphorylation and gene expression mediated by dopamine and glutamate, which activate intracellular second-messenger signaling pathways that contribute to both the short- and long-term effects of these drugs. In recent years, the importance of Ca^{2+} neurotransmission via the glutamate receptor subtypes, NMDA and AMPA, in the reinforcing properties of psychostimulants has emerged (Wolf 1998; Carlezon and Nestler 2002; Wolf 2002). In addition to these channels, another route of Ca^{2+} entry in neurons is the voltage-gated L-type Ca^{2+} channel (LTCC), an important mediator of neuronal plasticity (Deisseroth and others 2003; Groth and others 2003). There is growing evidence that LTCCs play an important role in contributing to the behavioral and molecular changes induced by psychostimulants (Licata and Pierce 2003; Rajadhyaksha and others 2004). This review will outline our current understanding of the molecular pathways activated by LTCC following acute and recurrent psychostimulant administration, with the goal of highlighting the importance of LTCCs as a

prime candidate for subserving some of the short-term changes and long-term molecular adaptations induced by amphetamine and cocaine. We will focus our review on the mesoaccumbens and meso-striatal pathways that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and dorsal striatum, respectively, primary pathways implicated in the rewarding and habit-forming aspects of addiction (Everitt and Wolf 2002; Gerdeman and others 2003).

L-type Ca^{2+} Channels, Its Subtypes, and Their Structural Elements

LTCCs belong to the family of voltage-gated Ca^{2+} channels (Ertel and others 2000) that form multisubunit complexes on the neuronal membrane (Fig. 1A) and are activated by membrane depolarization (reviewed in Catterall 1988). They are composed of a Ca^{2+} pore-forming Ca_v or α_1 subunit that exhibits voltage sensitivity. In addition, the complex consists of auxiliary β , α_2 - δ , and γ subunits that regulate the functional properties of the Ca_v subunit (reviewed in Walker and De Waard 1998). The Ca_v subunit is structurally composed of four homologous domains (I–IV) that are linked via cytoplasmic loops (Fig. 1B). Each domain consists of six transmembrane helices with the fourth helix serving as the voltage sensor for the channel. The Ca_v subunit also contains the binding sites for the pharmacological agents (dihydropyridines, phenylalkylamines, and benzothiazepines) commonly used to block and activate the channel. There are two subtypes of LTCCs formed in the brain: they either contain the $\text{Ca}_v1.2$ (α_{1C}) or $\text{Ca}_v1.3$ (α_{1D}) subunits. These two subtypes are activated and blocked by the same pharmacological agents and share a high degree of sequence similarity (Ertel and others 2000) except over a small region that corresponds to the variable region between the second and third transmembrane domains (Fig. 1B). LTCCs have traditionally been classified as high-voltage activated Ca^{2+} channels; however, the availability of subtype-specific antibodies, complementary DNA clones, and generation of knockout and transgenic mice has revealed that $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ have different physiological characteristics (Platzter and others 2000; Koschak and others 2001; Xu and Lipscombe 2001; Lipscombe 2002), neuronal and tissue distributions (Hell and others 1993; Rajadhyaksha and others 2004), and proteins they associate with at neuronal synapses (Olson and others 2005; Zhang and others 2005).

L-type Ca^{2+} Channel-Activated Ca^{2+} Second-Messenger Pathways

Activation of LTCCs results in the influx of intracellular Ca^{2+} that binds the Ca^{2+} -sensitive regulatory protein, calmodulin (CaM; Fig. 2A), which integrates Ca^{2+} signals entering at the membrane with downstream activation of Ca^{2+} /CaM-activated kinase and phosphatase pathways (Xia and Storm 2005). The two major kinase pathways activated by LTCCs are the Ca^{2+} /CaM kinase and the mitogen-activated protein (MAP) kinase (ERK1/2) pathways (Deisseroth and others 2003). Both these pathways activate the transcription factor CREB and CREB-induced gene expression, critical mediators of psychostimulant-induced neuronal and behavioral plasticity (Berke and Hyman 2000; Nestler 2001). In addition, LTCCs also activate the Ca^{2+} /CaM-dependent cal-cineurin (PP2B) pathway (Fig. 2A), a phosphatase pathway also important for neuronal and experience-dependent plasticity (Groth and others 2003).

Both $\text{Ca}_v1.2$ - and $\text{Ca}_v1.3$ -containing channels activate Ca^{2+} signaling pathway, but the functional specificity of the intracellular signal transduction pathways they activate is conferred by their existence as multiprotein signaling complexes (Fig. 2B and C). In general, these complexes have been found to consist of an anchoring or scaffolding protein that secures a kinase and a phosphatase close to the channel, allowing regulation of channel activity (Wong and Scott 2004). In addition, channels associate with downstream transduction molecules that link the channel to specific signaling pathways (Craven and Bredt 1998; Sheng and Pak 2000). $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ have been found to associate with different intracellular targeting molecules, suggesting differential contribution to neuronal signaling. $\text{Ca}_v1.2$ associates with

the microtubule-associated protein MAP2B, a member of the A-kinase anchoring protein (AKAP) family of proteins (Davare and others 1999). $\text{Ca}_v1.2$ also associates with the catalytic subunit of the cyclic-AMP-dependent protein kinase A (PKAc) and the phosphatase, protein phosphatase 2A (PP2A) (Davare and others 1999; Davare and others 2000), allowing regulation of $\text{Ca}_v1.2$ function via activity-dependent phosphorylation and dephosphorylation of a PKA site on the C-terminus of this subunit. In addition, $\text{Ca}_v1.2$ contains the PDZ interaction sequence, Val-Ser-Asn-Leu (VSNL), at its C-terminus that allows interaction of $\text{Ca}_v1.2$ with PDZ domain-containing proteins. Interactions via PDZ sites link channels to second-messenger pathways (Craven and Brecht 1998; Sheng and Pak 2000). To date, two PDZ domain proteins, channel interacting PDZ domain protein and neuronal interleukin-16, that interact with $\text{Ca}_v1.2$ have been identified in the brain (Kurschner and others 1998; Kurschner and Yuzaki 1999). This PDZ interaction site has been found to be critical for $\text{Ca}_v1.2$ activation of CREB protein phosphorylation and CREB-mediated gene expression (Weick and others 2003). $\text{Ca}_v1.3$ contains a PKA-dependent phosphorylation site, but the AKAP and associated kinase and phosphatases are not known (Fig. 2C). $\text{Ca}_v1.3$ also contains a PDZ interaction sequence, Ile-Thr-Thr-Leu (ITTL), albeit different from that of $\text{Ca}_v1.2$, which enables interactions with the synaptic scaffolding protein Shank (SH3 domain and ankyrin repeat containing protein). Shank in turn binds to the adaptor protein, Homer, which is involved in regulation of intracellular Ca^{2+} stores and found to regulate cocaine-induced behaviors (Szumlinski and others 2004).

In the sections below, we will focus on the role of LTCCs in molecular pathways activated by amphetamine and cocaine in the VTA, NAc, and dorsal striatum.

L-type Ca^{2+} Channels, Psychostimulants, and the Ventral Tegmental Area

The VTA serves as the primary neuroanatomical site at which the molecular mechanisms that underlie long-lasting psychostimulant-induced addictive behaviors are initiated (Bonci and others 2003; Vezina 2004). The primary cell type in the VTA is the dopamine neuron, with its cell body and dendrites present in the midbrain and its axons projecting primarily to the NAc, dorsal striatum, and medial prefrontal cortex in the forebrain. Amphetamine and cocaine increase somatodendritic dopamine release in the VTA. Glutamate is released via activation of presynaptic D_1 receptors and activates Ca^{2+} intracellular signaling pathways in VTA cell bodies that play a pivotal role in psychostimulant-induced molecular changes (Licata and Pierce 2003). In this section, we will cover the contribution of LTCCs in psychostimulant-activated Ca^{2+} second-messenger pathways in the VTA and discuss it in the context of its role in psychostimulant-induced behavior.

Acute Psychostimulant Exposure and L-type Ca^{2+} Channel-Activated Molecular Pathways

As shown schematically in Figure 3A, acute administration of amphetamine activates the Ca^{2+} second-messenger pathway in the VTA and has been found to increase CaM mRNA and protein (Michelhaugh and Gnegy 2000). Work from our laboratory has found that amphetamine activates the Ca^{2+} -mediated MAP kinase (ERK1/2) pathway in VTA dopamine neurons (Rajadhyaksha and others 2004). We have further found that LTCCs are not involved in this acute response (Rajadhyaksha and others 2004). This observation is consistent with the presence of very low levels of $\text{Ca}_v1.2$ mRNA and protein, an activator of Ca^{2+} -mediated ERK1/2 phosphorylation, in VTA dopamine neurons (Rajadhyaksha and others 2004). Two alternative routes of ERK1/2 phosphorylation in the VTA in response to acute amphetamine are via NMDA receptors or the neurotrophic factor neurotrophin-3, both important mediators of psychostimulant-induced behaviors (Pierce and others 1999; Sweatt 2001; Thomas and Haganir 2004).

Chronic Psychostimulant Exposure and L-type Ca^{2+} Channel-Activated Molecular Pathways

As shown schematically in Figure 3B, LTCCs have been found to be necessary to induce some of the persistent neuroadaptations evident in the VTA following chronic psychostimulant administration (Licata and Pierce 2003; Rajadhyaksha and others 2004). At a molecular level, work from our laboratory has found that following chronic amphetamine treatment, LTCCs down-regulate ERK1/2 phosphorylation in VTA dopamine neurons, a change that occurs in parallel with an increase in $\text{Ca}_v1.2$ mRNA and protein (Rajadhyaksha and others 2004). The increase in $\text{Ca}_v1.2$ gene expression may be regulated by glutamate signaling, as the $\text{Ca}_v1.2$ gene contains the upstream regulatory sites cyclic AMP and calcium response element (CRE) and Activator Protein 1 (AP1), which bind the transcription factor CREB and the Fos family of proteins, respectively (Liu and others 2000). These regulatory sites and their transcription factors are activated by psychostimulants and glutamate signaling and contribute to some of the long-lasting changes induced by these drugs (Berke and Hyman 2000; Nestler 2001).

We have also found that the decrease in ERK1/2 phosphorylation is accompanied by an increase in calcineurin (PP2B) and MAP kinase phosphatase-1 (MKP-1), regulators of ERK1/2 phosphorylation. As LTCCs have been found to activate PP2B (Graef and others 1999; Chang and Berg 2001; Snyder and others 2003) and play a role in phosphatase-mediated neuronal plasticity (Groth and others 2003), we hypothesize that following chronic psychostimulant exposure, $\text{Ca}_v1.2$ -containing LTCCs in VTA dopamine neurons are involved in activating a phosphatase pathway. This adaptation in the VTA may either represent a homeostatic response to chronic psychostimulant exposure, as has been ascribed to the role of MKP-1 (Bhalla and others 2002), or represent psychostimulant-induced neuronal plasticity via activation of phosphatases as observed for other experiencedependent neuronal events (Groth and others 2003). Figure 3 represents a schematic of our hypotheses. We speculate that one of the pivotal adaptations that occur in VTA dopamine neurons during the transition from acute to chronic psychostimulant exposure involves activation of the $\text{Ca}_v1.2$ gene and its Ca^{2+} -mediated phosphatase pathway.

LTCCs and Psychostimulant-Induced Behavioral Plasticity

Ca^{2+} influx via LTCCs has been found to play an important role in psychostimulant-induced behaviors (Licata and Pierce 2003). In the VTA, the immediate component of acute psychostimulant-activated Ca^{2+} signaling is predominantly mediated via NMDA receptors, whereas the subsequent contribution of recurrent psychostimulant-activated Ca^{2+} signaling is additionally mediated by LTCCs. Consistent with this formulation is the fact that NMDA blockers inhibit both acute and chronic psychostimulant-induced behaviors (Karler and others 1989; Vezina and Queen 2000), whereas LTCC blockers inhibit only chronic, but not acute, psychostimulant-induced behavioral changes (Karler and others 1991; Pierce and others 1998). This finding is consistent with our molecular data identifying a role for LTCCs in chronic, but not acute, amphetamine-mediated ERK1/2 phosphorylation in the VTA (Rajadhyaksha and others 2004). Such findings are also consistent with the observation that MAP kinase (ERK1/2) pathway inhibitors do not block acute cocaine-induced behaviors but inhibit chronic cocaine-induced behaviors (Pierce and others 1999). This suggests that the LTCC-activated MAP kinase pathway in VTA neurons may be selectively activated following recurrent but not acute psychostimulant exposure. In addition, the increase in $\text{Ca}_v1.2$ mRNA and protein observed in the VTA following chronic amphetamine is consistent with the fact that psychostimulant-induced neuroplasticity requires new protein synthesis in the VTA (Sorg and Ulibarri 1995). Based on the above findings, we propose that $\text{Ca}_v1.2$ and ERK1/2 pathways in the VTA contribute to psychostimulant-induced neuronal plasticity that underlies aspects of the long-term alterations in behaviors evident following recurrent drug exposure.

L-type Ca^{2+} Channels, Psychostimulants, the Nucleus Accumbens, and Dorsal Striatum

The nucleus accumbens (ventral striatum) and caudate/putamen (dorsal striatum) represent the primary neuroanatomical sites in the brain at which psychostimulant-induced molecular changes are consolidated. It is these persistent and life-long molecular adaptations that underlie aspects of addictive behaviors, including withdrawal and craving leading to relapse, which may be seen even after long periods of drug abstinence (Hyman and Malenka 2001). Like in the VTA, the primary neurotransmitter pathways that are activated by amphetamine and cocaine in the NAc and dorsal striatum are the dopamine and glutamate pathways. Also like in the VTA, LTCCs mediate components of signaling through both these pathways and contribute to psychostimulant-induced behavior. However, much less is known about the precise contribution of the pathways activated by LTCCs in the NAc and dorsal striatum that result in psychostimulant-induced molecular adaptations.

Psychostimulants and Regulation of L-type Ca^{2+} Channel Phosphorylation

Amphetamine and cocaine release dopamine that activates dopamine D_1 -like and D_2 -like receptor subtype second-messenger pathways (Berke and Hyman 2000). Dopamine D_1 receptors activate the cyclic AMP (cAMP)/PKA pathway that phosphorylates LTCCs at a PKA-specific site, thereby increasing channel activity (Fig. 4; Surmeier and others 1995). Dopamine D_2 receptors have been found to activate PP2B via the phospholipase C pathway, decreasing LTCC activity via PP2B-mediated dephosphorylation (Fig. 4; Hernandez-Lopez and others 2000). In the dorsal striatum, the phosphorylation state of LTCC is also regulated by the CaM binding protein, regulator of calmodulin signaling (RCS) also known as cAMP-regulated phosphoprotein (ARPP-21) (Rakhilin and others 2004). RCS serves as an integrator of Ca^{2+} and cAMP signals. When RCS is phosphorylated by PKA, it becomes an inhibitor of PP2B, one of its target molecules. Acute cocaine and the amphetamine derivative, methamphetamine, have been found to increase phosphorylation of RCS (Caporaso and others 2000). Based on this finding and the regulation of LTCC phosphorylation by RCS, Figure 4 represents our hypothesis of the acute effects of psychostimulants on LTCC phosphorylation state. We speculate that amphetamine and cocaine will increase phosphorylation of RCS, which in turn will inhibit PP2B activity, resulting in an increase in LTCC phosphorylation. This would then activate the LTCC-mediated Ca^{2+} second-messenger pathway and contribute to psychostimulant-induced activation of molecular pathways.

Acute Psychostimulant Exposure and L-type Ca^{2+} Channel-Activated Molecular Pathways

The most extensively studied pathway in the dorsal striatum, and to some extent in the NAc, is the dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein (DARPP-32) pathway (Greengard 2001). DARPP-32 is a phosphoprotein that integrates the cAMP second-messenger pathway activated by dopamine D_1 receptors and the Ca^{2+} pathway activated by NMDA and LTCCs. DARPP-32 modulates multiple downstream targets involved in psychostimulant-induced gene expression, particularly via the transcription factor CREB. LTCCs have been found to influence both dopamine and NMDA neurotransmission (Liu and Graybiel 1996; Rajadhyaksha and others 1999) and, by acutely increasing the levels of intracellular Ca^{2+} as well as by activation of kinases, play a direct role in contributing to activation of some of the downstream targets of these signaling pathways (see Fig. 5A). Although the precise role of the $\text{Ca}_v1.2$ versus $\text{Ca}_v1.3$ subtypes in contributing to psychostimulant-mediated molecular pathways has remained elusive, both of these subtypes have been shown to phosphorylate CREB (Dolmetsch and others 2001; Zhang and others 2005). Regarding the regional specificity of the $\text{Ca}_v1.2$ versus $\text{Ca}_v1.3$ subtypes in contributing to psychostimulant-mediated molecular pathways, a recent study using transgenic mice that

allows specific activation of the $\text{Ca}_v1.3$ subtype has revealed that in the dorsal striatum, $\text{Ca}_v1.2$ activity is required for activation of CREB-induced Fos expression, whereas in the NAc, $\text{Ca}_v1.3$ activity mediates CREB-induced Fos expression (Sinnegger-Brauns and others 2004). This suggests that the $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ pathways may selectively contribute to the actions of psychostimulants in the dorsal striatum and NAc, respectively.

Chronic Psychostimulant Exposure and L-type Ca^{2+} Channel-Activated Molecular Pathways

A clue into the possible role of LTCCs in contributing to the molecular changes that are sustained following recurrent drug exposure, even after long drug-free periods, has come from physiological studies using the behavioral sensitization model of drug addiction (Wolf 2002). Such animal models of addiction share many features of synaptic plasticity evident in animal models of learning and memory, including the induction of longterm depression (LTD), a phenomenon shown in the hippocampus to be mediated by persistent alterations in glutamatergic signaling, which has additionally been associated with decreased levels of intracellular Ca^{2+} and with increased phosphatase activity (Winder and Sweatt 2001). Likewise, it has been found that NAc neurons previously exposed to recurrent cocaine exhibit LTD (Thomas and others 2001) and exhibit persistent alterations in glutamatergic neurotransmission, as well as decreased basal intracellular Ca^{2+} and increased phosphatase signaling (Zhang and others 2002). As LTCCs (Snyder and others 2003) and amphetamine (Wang and Uhl 1998) have been found to acutely increase PP2B activity, respectively, we speculate that the contribution of LTCCs to psychostimulant-induced long-term synaptic plasticity in the NAc may be mediated by persistent activation of the Ca^{2+} /CaM-dependent phosphatase pathway (Fig. 5B). This hypothesis is further supported by the observation that DARPP-32, a downstream target of PP2B, has been found to be dephosphorylated at the PP2B-specific site (Thr 34) in samples taken from the dorsal striatum and NAc 10 days following recurrent cocaine administration (Scheggi and others 2004).

In summary, the findings presented above establish an important role for L-type Ca^{2+} channels and its Ca^{2+} activated pathways in mediating some of the addictive properties of psychostimulants. However, there are many unanswered questions regarding the contribution of $\text{Ca}_v1.2$ versus $\text{Ca}_v1.3$ to the acute and chronic effects of psychostimulants. Future research on molecular correlates of addiction requires consideration of L-type Ca^{2+} channel signal transduction pathways in the VTA in initiating and in the dorsal striatum and NAc in sustaining some of the critical neuroadaptations underlying the transition from the drug-naive to drug-addicted state.

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Fig. 1.

A, Voltage-gated calcium channel subunit composition. Ca²⁺ channels are present as heteromeric complexes with the Ca_v or α₁ subunit forming the voltage-sensitive Ca²⁺ pore that associates with β, α₂-δ and γ auxiliary subunits. *B*, Schematic of the Ca_v peptide. L-type Ca²⁺ channels are composed of either the Ca_v1.2 or Ca_v1.3 subtypes. The Ca_v subunit is a transmembrane protein composed of four homologous domains (I–IV), each containing six transmembrane helices. The fourth helix in each domain acts as the voltage sensor for the channel. The cytoplasmic region between the second and third transmembrane domains contains variable sequences that differentiate Ca_v1.2 and Ca_v1.3 subtypes. COOH = carboxyl.

Fig. 2.

A, Calcium pathways activated by L-type Ca^{2+} channels (LTCCs). Upon activation, L-type Ca^{2+} channels mediate an increase in intracellular Ca^{2+} that binds calmodulin (CaM). $\text{Ca}^{2+}/\text{CaM}$ can initiate either the $\text{Ca}^{2+}/\text{CaM}$ kinase pathway, an activator of protein phosphorylation (shown in green), or the calcineurin (PP2B) phosphatase pathway, an inhibitor of protein phosphorylation (shown in red). $\text{Ca}^{2+}/\text{CaM}$ kinase directly phosphorylates the transcription factor CREB or activates the mitogen-activated protein (MAP) kinase pathway that also activates CREB. Activation of the phosphatase pathway, via protein phosphatase 1 (PP1), dephosphorylates CREB. Hence, depending on the neuronal stimulus, these two pathways regulate the phosphorylation state of CREB that in turn regulates CREB-mediated gene expression, an important mediator of neuronal plasticity. **B** and **C**, Proteins associated with the L-type Ca^{2+} channel subunits, $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$. $\text{Ca}_v1.2$ has been found to associate with the A-kinase anchoring protein (AKAP), MAP2B, the catalytic subunit of protein kinase A (PKAc), and the phosphatase, protein phosphatase 2A (PP2A). Also shown is the PKA phosphorylation site and the PDZ interaction sequence, Val-Ser-Asn-Leu (VSNL), which allow interaction of $\text{Ca}_v1.2$ with PDZ domain-containing proteins. The AKAP, kinase, and phosphatase that interact with $\text{Ca}_v1.3$ have not yet been identified. $\text{Ca}_v1.3$ interacts with the synaptic scaffolding protein Shank (SH3 domain and ankyrin repeat-containing protein) via the PDZ interaction sequence, Ile-Thr-Thr-Leu (ITTL). Shank in turn binds to the adaptor protein, Homer, which is involved in regulation of intracellular Ca^{2+} stores.

Fig. 3.

Schematic representation of molecular changes from acute to chronic amphetamine exposures in ventral tegmental area (VTA) dopaminergic (DA) neurons. *A*, Acute exposure to amphetamine increases somatodendritic dopamine release in the VTA. Glutamate is released via activation of presynaptic D₁ receptors. Glutamate activates the AMPA receptor and depolarizes the VTA neuron, opening NMDA receptors. The NMDA receptor activates the Ca²⁺ second-messenger pathway via calmodulin (CaM) that leads to activation of the CaM kinase (CaMK) and mitogen-activated protein (MAP) kinase pathways. The CaMK pathway phosphorylates CREB (PCREB), which activates gene expression in the nucleus. The CaMK pathway also activates the MAPK pathway that leads to ERK1/2 phosphorylation via activation of MAP kinase kinase kinase (MAPKKK, also known as Raf) and MAP kinase kinase (MEK). Phosphorylated extracellular signal-regulated kinase 1/2 (P-ERK1/2) phosphorylates P-CREB via activation of ribosomal S6 kinase 2 (Rsk-2). Another route of ERK1/2 phosphorylation in the VTA is via activation of the neurotrophic factor (NT) signaling pathway. Activation of these pathways by amphetamine leads to activation of gene expression including the Ca_v1.2 gene. The upstream transcriptional region of Ca_v1.2 gene contains cyclic AMP and calcium response element (CRE) and Activator Protein 1 (AP1) binding sites that bind CREB and the Fos family of transcription factors, activated by amphetamine. *B*, Following chronic exposure to amphetamine, the newly expressed Ca_v1.2 containing L-type Ca²⁺ channels (LTCCs) activates a phosphatase pathway in VTA dopamine neurons. Chronic amphetamine increases

calcineurin (PP2B) expression that increases levels of MAP kinase phosphatase-1 (MKP-1). PERK1/2 also induces expression of MKP-1 that directly dephosphorylates P-ERK1/2. The phosphatase pathway in turn alters the pattern of downstream gene expression that contributes to long-lasting psychostimulant-induced molecular and behavioral changes. GLU = glutamate.

Fig. 4.

Regulation of L-type Ca²⁺ channel (LTCC) phosphorylation by psychostimulants. LTCC phosphorylation and activity are regulated by dopamine D₁ and D₂ receptors. Stimulation of D₁ receptors activates protein kinase A (PKA) that phosphorylates LTCCs, increasing its activity. Stimulation of D₂ receptors activates phospholipase C (PLC)/Ca²⁺ pathway that in turn activates the phosphatase, calcineurin (PP2B). PP2B dephosphorylates LTCCs, decreasing its activity. Acute cocaine has been found to phosphorylate the striatal-enriched phosphoprotein, RCS (regulator of calmodulin signaling), also known as ARPP-21. Phospho-RCS acts as an inhibitor of PP2B, is regulated by D₂ signaling, and has been found to regulate LTCC activity. Hence we speculate that via phospho-RCS, psychostimulants increase phosphorylation of LTCCs, activating LTCC-mediated Ca²⁺ signaling and gene expression.

Fig. 5.

Schematic representation of molecular changes from acute psychostimulant exposure to persistent molecular changes in the nucleus accumbens (NAc) and dorsal striatum. **A**, Acute exposure to psychostimulant increases synaptic dopamine and glutamate in the nucleus accumbens and dorsal striatum. Dopamine binds postsynaptic D₁ receptors and activates the cyclic AMP (cAMP) second-messenger pathway. cAMP activates protein kinase A (PKA) that phosphorylates several target molecules including NMDA and AMPA receptors and L-type Ca²⁺ channels (LTCCs), resulting in an increase in intracellular Ca²⁺ (High [Ca²⁺]_i) and activation of Ca²⁺/CaM (calmodulin) kinases. PKA also phosphorylates downstream signaling molecules including dopamine and adenosine 3', 5'- monophosphate regulated phosphoprotein 32 (DARPP-32) and CREB. Phosphorylation of DARPP-32 at Thr 34 (P-DARPP-32) converts it to a potent inhibitor of protein phosphatase 1 (PP1), a phosphatase that dephosphorylates CREB. Inhibition of PP1 and activation of PKA result in an increase in CREB phosphorylation that activates downstream acute gene expression. **B**, Following chronic psychostimulant exposure, under conditions of low intracellular Ca²⁺ (Low [Ca²⁺]_i), L-type Ca²⁺ channels would activate Ca²⁺/CaM-dependent calcineurin (PP2B), resulting in dephosphorylation of its targets that include AMPA receptors and DARPP-32. DARPP-32 in its dephosphorylated state no longer would inhibit PP1, resulting in dephosphorylation of CREB and alteration of CREB-mediated gene expression. DA = dopamine; GLU = glutamate; CRE = cyclic AMP and calcium response element; AP1 = Activator Protein 1.