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REVIEW

The roles of calcium/calmodulin-dependent and Ras/mitogen-activated protein kinases in the development of psychostimulant-induced behavioral sensitization

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Abstract

Although the development of behavioral sensitization to psychostimulants such as cocaine and amphetamine is confined mainly to one nucleus in the brain, the ventral tegmental area (VTA), this process is nonetheless complex, involving a complicated interplay between neurotransmitters, neuropeptides and trophic factors. In the present review we present the hypothesis that calcium-stimulated second messengers, including the calcium/calmodulin-dependent protein kinases and the Ras/mitogen-activated protein kinases, represent the major biochemical pathways whereby converging extracellular signals are integrated and amplified, resulting in the biochemical and molecular changes in dopaminergic neurons in the VTA that represent the critical neuronal correlates of the development of behavioral sensitization to psychostimulants. Moreover, given the important role of calcium-stimulated second messengers in the expression of behavioral sensitization, these signal transduction systems may represent the biochemical substrate through which the transient neurochemical changes associated with the development of behavioral sensitization are translated into the persistent neurochemical, biochemical and molecular alterations in neuronal function that underlie the long-term expression of psychostimulant-induced behavioral sensitization.

Keywords: amphetamine, cocaine, dopamine, glutamate, L-type calcium channels, nucleus accumbens, ventral tegmental area.

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Repeated exposure to psychostimulants, such as cocaine or amphetamine, leads to augmentation of behavioral activity in a wide range of species. In rodents, repeated intermittent injections of cocaine result in a progressive and enduring augmentation of locomotor and stereotyped behaviors, a phenomenon known as behavioral sensitization. It has been suggested that the neuronal plasticity underlying behavioral sensitization results in the enhancement of the incentive motivational effects of psychostimulants, which contributes to drug craving (Robinson and Berridge 2000). Consistent with this hypothesis, among rats with a previous history of cocaine self-administration there is a correlation between the ability of amphetamine to produce behavioral sensitization and to reinstate drug-seeking behavior, an animal model of drug craving (De Vries *et al*. 1998). Thus, studies examining the mechanisms underlying behavioral sensitization could provide new insight into plasticity in the central nervous system that may help elucidate the mechanisms underlying the shift to compulsive drug use among human psychostimulant addicts.

One of the main effects of cocaine and amphetamine is an increase in dopamine transmission. Dopaminergic projections

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Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate; CaM, calcium/calmodulin complex, CaMK, calcium/calmodulin-dependent protein kinase; CaMKII, calcium/calmodulin-dependent protein kinase II; CREB, cAMP response element binding protein; ERKs, extracellular signal-related kinases; LTP, long-term potentiation; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase kinase; TrkC, tyrosine kinase C; VTA, ventral tegmental area.

from the ventral tegmental area (VTA) to the nucleus accumbens and other forebrain nuclei are critically involved in both the development and long-term expression of behavioral sensitization to psychostimulants (Kalivas and Stewart 1991). The development, or initiation, of behavioral sensitization occurs in the nuclei of the ventral midbrain that contain dopaminergic cell bodies (i.e. the VTA and substantia nigra) (Vanderschuren and Kalivas 2000). The results of hundreds of behavioral, neurochemical, biochemical and molecular experiments indicate that the initiation of behavioral sensitization is a complex process that involves interactions among several neurotransmitters, neuropeptides and neurotrophic factors and their associated receptors and signaling pathways (White and Kalivas 1998; Wolf 1998). To date, dopamine–glutamate interactions in the VTA have received the most attention and several excellent reviews have organized and synthesized the varied and sometimes contradictory information relevant to the development of behavioral sensitization into coherent models that have greatly aided progress in this area (Kalivas 1995; White and Kalivas 1998; Wolf 1998). The present review is an extension of these models. We present evidence indicating that calcium influx into VTA dopamine neurons via α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) and NMDA receptors as well as L-type calcium channels is enhanced during the development of behavioral sensitization leading to persistent activation of calcium/calmodulin-stimulated (CaM) kinases, which collectively play a critical role in psychostimulant-induced neuronal and behavioral plasticity. Moreover, we propose that calcium-stimulated second messengers may be the crucial biochemical link between the development and long-term expression of behavioral sensitization to cocaine and amphetamine.

Changes in VTA dopaminergic and glutamatergic transmission in the VTA during the development (initiation) of psychostimulant-induced behavioral sensitization

Repeated cocaine injections result in a transient increase in basal extracellular dopamine concentrations in the VTA and an enhancement of the ability of cocaine to increase extracellular dopamine in this nucleus (Kalivas and Duffy 1993; Parsons and Justice 1993). These changes in the ability of cocaine to increase extracellular dopamine in the VTA are transient in that they are observed 1 day but not 14 days after the cessation of repeated cocaine injections (Kalivas and Duffy 1993; Parsons and Justice 1993). It is likely that these transient changes in VTA dopamine transmission following repeated cocaine are promoted at least in part by the desensitization of D2 dopamine autoreceptors (White and Wang 1984; Gao *et al*. 1998), which results in membrane depolarization through a decrease in G protein coupling of D2 receptors to potassium channels (Nestler *et al*. 1990; Steketee *et al*. 1990; Striplin and Kalivas 1992). Consistent with these findings, repeated systemic injections of the D2-like receptor agonist quinpirole enhance the behavioral response to a systemic cocaine challenge injection the day after the last injection of quinpirole. In other words, there is cross-sensitization between quinpirole and cocaine (Henry *et al*. 1998). The transience of this phenomenon is highlighted by the fact that quinpirole–cocaine cross-sensitization was not observed 7–30 days after the last quinpirole injection (Pierce *et al*. 1996; Henry *et al*. 1998). D1-like dopamine receptors in the VTA also are involved in the development of behavioral sensitization. Although dopaminergic neurons do not express D1-like dopamine receptors, there is an abundance of D1-like dopamine receptors in the VTA (Mansour *et al*. 1992). D1-like receptor antagonists impair the initiation of behavioral sensitization (Stewart and Vezina 1989; Vezina 1996) and repeated systemic (Henry *et al*. 1998) or intra-VTA (Pierce *et al*. 1996) administrations of the D1 dopamine receptor agonist SKF 38393 enhance the behavioral response to a subsequent systemic cocaine challenge injection (i.e. there is cross-sensitization between SKF 38393 and cocaine). Interestingly, repeated administration of both D1-like and D2-like dopamine receptor agonists produced cross-sensitization to cocaine that persisted longer than pre-treatment with either a D1-like or D2-like dopamine receptor agonist alone (Henry *et al*. 1998).

Based on these findings it was suggested that increased dopamine transmission in the VTA during the development of behavioral sensitization might modulate glutamate release in this nucleus by stimulating pre-synaptic D1 dopamine receptors (Kalivas 1995). Evidence in support of this hypothesis includes a D1 receptor-dependent enhancement of glutamate release in the VTA following repeated cocaine injections (Kalivas and Duffy 1998). With amphetamine, acute and repeated injections both produced delayed and prolonged increases in glutamate efflux in the VTA that were similar in magnitude (Xue *et al*. 1996); these amphetamine-induced increases in VTA glutamate were blocked by a D1 dopamine receptor antagonist (Wolf and Xue 1999). Although it has been suggested that the stimulation of D1-like dopamine receptors located on glutamatergic terminals in the VTA mediates psychostimulant-induced enhancement of glutamate release in this nucleus during the development of behavioral sensitization (Kalivas 1995), it is important to note that there are several discrepancies among the relatively few studies that have addressed the effect of repeated cocaine or amphetamine on glutamate release in the VTA, including the time-course of the psychostimulant-induced enhancement of glutamate release as well as the specific role of D1-like dopamine receptors in this effect (Xue *et al*. 1996; Kalivas and Duffy 1998; Wolf and Xue 1999).

A growing literature indicates that AMPA and NMDA glutamate receptor antagonists impair the development of

behavioral sensitization to cocaine or amphetamine (Karler *et al*. 1989; Stewart and Druhan 1993; Wolf and Jeziorski 1993; Li *et al*. 1997). Consistent with these findings, there is a transient increase in the excitatory effect of glutamate (White *et al*. 1995) or AMPA (Zhang *et al*. 1997) on VTA dopamine neurons in amphetamine and cocaine sensitized rats. In addition, 3 days after the last of five repeated amphetamine injections, intra-VTA AMPA resulted in augmented dopamine and glutamate transmission in the VTA and nucleus accumbens (Giorgetti *et al*. 2001). Finally, a single injection of cocaine induces long-term potentiation (LTP) of AMPA receptor-mediated current in dopaminergic cells in the VTA (Ungless *et al*. 2001). Taken together, these results indicate that enhanced excitatory transmission through ionotropic glutamate receptors in the VTA contributes to the development of behavioral sensitization to psychostimulants.

In summary, the development of behavioral sensitization involves a complex interplay between at least two neurotransmitters, dopamine and glutamate, in the VTA. As depicted in Fig. 1, repeated cocaine or amphetamine injections result in transient increases in extracellular dopamine in the VTA, which may increase glutamate transmission by stimulating pre-synaptic D1 dopamine receptors.

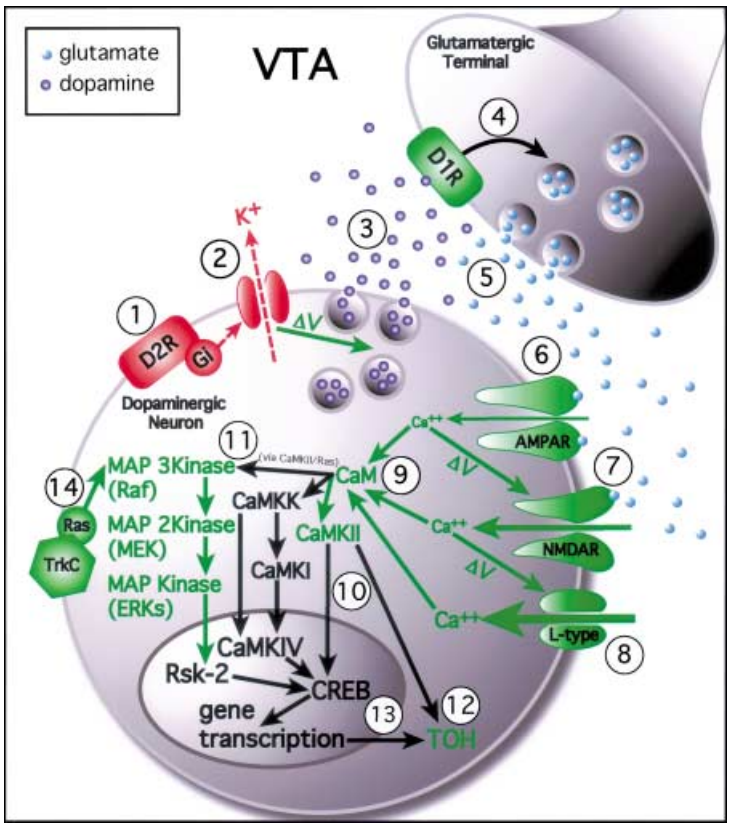

Fig. 1 Interactions between the dopamine and glutamate systems in the VTA that produce some of the biochemical and molecular alterations associated with the development of psychostimulant-induced behavioral sensitization. Repeated psychostimulant injections desensitize D2 dopamine autoreceptors (1) and decrease G-protein mediated K^+ efflux, which depolarizes the dopaminergic cell (2) and promotes somatodendritic dopamine release (3). Extracellular dopamine appears to stimulate D1 receptors located on pre-synaptic glutamate terminals (4) enhancing glutamate release (5). Glutamate activates AMPA (6) and NMDA (7) receptors located on dopaminergic cell bodies and/or dendrites resulting in sodium- and calcium-mediated membrane depolarization, which stimulates voltage-activated calcium channels including L-type calcium channels (8). Calcium influx through AMPA and NMDA receptors as well as L-type calcium channels activates calcium-mediated second messengers such as CaM (9), which stimulates CaMKII. CaMKII, either directly (10) or through the MAP kinase signaling pathway (11), influences a number of intracellular targets, including tyrosine hydroxylase (12), and alters protein expression via transcription factors such as CREB (13). The MAP kinase system also is stimulated by neurotrophin-activated receptors such as TrkC (14). Green lines and symbols represent increased activity during the development of behavioral sensitization, whereas dotted lines and red symbols indicate decreased activity. Black lines and symbols indicate proposed increases in activity. Abbreviations: AMPAR, AMPA glutamate receptor; CaMKK, calcium/calmodulin-dependent kinase kinase; D1R, D1 dopamine receptor; D2R, D2 dopamine autoreceptor; NMDAR, NMDA glutamate receptor; TOH, tyrosine hydroxylase; ΔV, membrane depolarization. See text for additional details and other abbreviations.

Glutamate, calcium and behavioral sensitization to cocaine

A growing body of research indicates that ionotropic glutamate receptors and L-type calcium channels, all of which are calcium permeable, contribute to the development of behavioral sensitization to psychostimulants. As reviewed above, systemic injections of AMPA or NMDA receptor antagonists block the initiation of behavioral sensitization to cocaine or amphetamine (Karler *et al*. 1989; Stewart and Druhan 1993; Wolf and Jeziorski 1993; Li *et al*. 1997). Peripheral injections of L-type calcium channel antagonists also impair the initiation of behavioral sensitization (Karler *et al*. 1991; Reimer and Martin-Iverson 1994). Moreover, microinjection of an AMPA receptor antagonist (Licata and Pierce 2002), an NMDA receptor antagonist (Kalivas and Alesdatter 1993; Vezina and Queen 2000) or an L-type calcium channel antagonist (Licata and Pierce 2002) directly into the VTA attenuates the development of psychostimulant-induced behavioral sensitization. In addition, repeated intra-VTA administration of an L-type calcium channel agonist cross-sensitizes with a subsequent challenge injection of cocaine (Licata *et al*. 2000) and repeated amphetamine injections increase the expression of the α_{1C} L-type calcium channel subunit in the VTA (Rajadhyaksha *et al*. 2002). Taken together, these results suggest that ionotropic glutamate receptors and L-type calcium channels located on dopamine cells in the VTA play critical roles in the initiation of behavioral sensitization to psychostimulants. Indeed, it seems likely that increased activation of L-type calcium channels in the VTA associated with behavioral sensitization results at least partly from enhanced glutamate transmission and depolarization of dopaminergic neurons (Xue *et al*. 1996; Kalivas and Duffy 1998; Wolf and Xue 1999).

We hypothesize that enhanced AMPA and NMDA glutamate receptor stimulation in the VTA during the initiation of behavioral sensitization results in increased activation of L-type calcium channels and calcium-mediated second messengers. As depicted in Fig. 1, AMPA receptors, NMDA receptors and L-type calcium channels all are conduits through which calcium transmission and signaling can be amplified. Stimulation of all AMPA receptors results in membrane depolarization via sodium transmission, whereas only AMPA receptors lacking the GluR2 subunit are calcium permeable (Tanaka *et al*. 2000). Although GluR2 subunits are expressed in dopaminergic neurons in the VTA (Chen *et al*. 2001), it is unclear what proportion of VTA AMPA receptors lack the GluR2 subunit. Membrane depolarization is necessary to remove the tonic block of NMDA receptor channels by magnesium. When activated, NMDA receptors further depolarize the membrane through calcium and sodium influx. The combined membrane depolarization induced by AMPA and NMDA receptor stimulation results in the activation of voltage-dependent channels, including L-type calcium channels. This progression from ionotropic glutamate receptors to L-type calcium channels amplifies the calcium signal. The calcium influx through AMPA receptors is limited because only a subpopulation of these receptors is calcium permeable and they rapidly inactivate (Jones 1998). Although all NMDA receptors are calcium permeable, these receptors also inactivate relatively rapidly (Jones 1998). In contrast, L-type calcium channels inactivate slowly and produce a more sustained influx of calcium than other voltage-dependent calcium channels or ionotropic glutamate receptors (Jones 1998). The sustained increase in intracellular calcium produced by L-type channels leads to the prolonged activation of calcium-mediated second messengers. Intracellular calcium binds calmodulin to become an active complex that can regulate many enzymes including CaM kinase (CaMK) I, II, and IV (Lisman 1994), all of which are abundantly expressed throughout the CNS (Nakamura *et al*. 2000).

Calcium-stimulated second messengers and neuronal plasticity

It is notable that there is considerable overlap between the mechanisms underlying LTP and behavioral sensitization, including a role for ionotropic glutamate receptors (Baudry and Lynch 2001; Everitt and Wolf 2002; Lisman *et al*. 2002). Indeed, recent evidence indicates that exposure to cocaine promotes LTP of AMPA receptor-mediated currents in dopaminergic neurons in the VTA (Ungless *et al*. 2001). There also is considerable evidence indicating that calcium-stimulated second messengers contribute to the induction of LTP (Gnegy 2000; Baudry and Lynch 2001; Lisman *et al*. 2002). Of the CaM kinases, CaMKII in particular has been proposed as a candidate molecule for the long-term storage of information due to its ability to remain phosphorylated in the absence of CaM (Lisman 1994; Lisman *et al*. 2002). This calcium-independent kinase activity is sustained by the multiple catalytic subunits of the CaMKII holoenzyme, which re-phosphorylate adjacent subunits that are inactivated by phosphatases and phosphorylate new subunits that are added during protein turnover (Miller and Kennedy 1986; Lisman 1994). The critical role of this enzyme in neuronal plasticity is supported by experiments using CaMKII-deficient mutant mice, which were shown to have a compromised ability to produce hippocampal LTP (Silva *et al*. 1992b) as well as spatial learning deficiencies (Silva *et al*. 1992a). A growing literature indicates that the CaM kinases also play a critical role in the initiation of behavioral sensitization.

Calcium-stimulated second messengers and behavioral sensitization

As illustrated in Fig. 1, whereas CaMKI and CaMKIV are activated most effectively by CaM-dependent kinase kinase,

CaMKII can be stimulated directly by CaM (Sugita *et al*. 1994). Once activated, CaMKII can phosphorylate a number of intracellular targets including, but not limited to, AMPA receptors (Poncer *et al*. 2002), NMDA receptors (Bayer *et al*. 2001), L-type calcium channels (Dzhura *et al*. 2000) and tyrosine hydroxylase (Griffith and Schulman 1988), the rate limiting enzyme in dopamine synthesis. Based on the role of AMPA, NMDA, and L-type calcium channels in the development of behavioral sensitization, it is likely that calcium-mediated second messengers contribute to this process. Consistent with this hypothesis, administration of the CaMKII inhibitor KN-93 into the VTA prior to daily injections of cocaine impairs the development of behavioral sensitization (Licata and Pierce 2002). Moreover, behavioral sensitization to cocaine is attenuated in CaMKII knockout mice (Licata and Pierce 2002). The effect of amphetamine on calmodulin in the ventral midbrain is complex. Although an acute injection of amphetamine increased calmodulin mRNA and protein in the ventral midbrain (Michelhaugh and Gnegy 2000), these increases were not observed 3 h after a regimen of repeated, daily amphetamine injections (Michelhaugh *et al*. 1998; Ostrander *et al*. 1998; Michelhaugh and Gnegy 2000). Seven to 10 days after the amphetamine injection regimen there was a decrease in calmodulin levels in the ventral midbrain (Michelhaugh *et al*. 1998; Michelhaugh and Gnegy 2000). In addition, repeated amphetamine injections produce changes in the various calmodulin gene mRNAs, which do not always correspond to the amphetamine-induced alterations in calmodulin protein in the ventral midbrain (Michelhaugh *et al*. 1998; Michelhaugh and Gnegy 2000). The effects of acute or repeated cocaine or amphetamine injections on CaMKII protein or mRNA levels in the VTA have not yet been evaluated.

MAP kinase as a calcium/calmodulin-stimulated second messenger and its contribution to the development of behavioral sensitization

As shown in Fig. 1, CaMKII, via activation of Ras, also stimulates the mitogen-activated protein (MAP) kinase second messenger system (Xing *et al*. 1996). Ras activates a MAP 3kinase (Raf) that phosphorylates and activates a MAP kinase kinase (MEK), which in turn phosphorylates the MAP kinases (also known as extracellular signal-related kinases or ERKs) (Seger and Krebs 1995). Recent work from our laboratory indicates that microinjection of a MEK inhibitor into the VTA blocks the initiation of behavioral sensitization to cocaine (Pierce *et al*. 1999), which is consistent with findings showing that repeated cocaine injections increase ERK catalytic activity specifically in the VTA (Berhow *et al*. 1996). The Ras/MAP kinase signal transduction cascade also is activated by stimulation of tyrosine kinase C (TrkC) receptors (see Fig. 1) and repeated intra-VTA administration of the TrkC agonist neurotrophin-3 cross-sensitizes with a subsequent challenge injection of cocaine (Pierce *et al*. 1999). These data, coupled with the results outlined above, indicate that the CaM and MAP kinases, acting either independently or cooperatively, play important roles in promoting the initiation of behavioral sensitization to cocaine.

Targets of CaM and MAP kinases that may play a role in psychostimulant-induced behavioral sensitization

A common target of the CaM and MAP kinases is cAMP response element binding protein (CREB) (Curtis and Finkbeiner 1999), a transcription factor that has been linked to several forms of synaptic plasticity including psychostimulant-induced changes in the mesotelencephalic dopamine systems (Turgeon *et al*. 1997; Pliakas *et al*. 2001). Although all three CaM kinases phosphorylate CREB at serine 133, CaMKI and CaMKIV are more effective activators of CREB-dependent transcription (Sun *et al*. 1996). This is due to the fact that CaMKII also inhibits CREB by phosphorylation at serine 142 (Sun *et al*. 1996). However, CaMKII can stimulate CREB-mediated transcription indirectly via activation of the Ras/MAP kinase second messenger system, which phosphorylates CREB through ribosomal S6 kinase 2 (Xing *et al*. 1996). The influence of psychostimulants on CREB has thus far focused primarily on the striatal complex. However, one study did assess the influence of amphetamine on CREB in the VTA. The results of these experiments indicate that a single injection of amphetamine has no influence on total or phosphorylated CREB in the VTA (Dalley *et al*. 1999). The effect of repeated psychostimulant administrations on CREB levels in the VTA remains unaddressed.

Tyrosine hydroxylase is altered in the VTA following repeated injections of cocaine. Tyrosine hydroxylase immunoreactivity, tyrosine hydroxylase enzymatic activity, and tyrosine hydroxylase mRNA are all increased in rats repeatedly treated with cocaine (Beitner-Johnson *et al*. 1991; Sorg *et al*. 1993; Vrana *et al*. 1993), which indicates that dopamine synthesis is increased in the VTA during the development of behavioral sensitization. This increase in dopamine synthesis likely contributes to the enhancement in basal extracellular dopamine levels in the VTA following repeated cocaine injections (Kalivas and Duffy 1993). Interestingly, calcium influx through L-type calcium channels increases catecholamine synthesis in PC12 cells (McCullough and Westfall 1996), possibly through a CaMKII-mediated phosphorylation of tyrosine hydroxylase (Griffith and Schulman 1988). In primary striatal cultures, calcium influx through L-type calcium channels also increases CREB phosphorylation (Rajadhyaksha *et al*. 1999) and CREB has been shown to regulate tyrosine hydroxylase transcription (Lim *et al*. 2000). Taken together, these results suggest that the calcium influx through ionotropic glutamate receptors and L-type calcium

channels during the development of behavioral sensitization may increase tyrosine hydroxylase activity via CaMKII, either directly or through CREB-mediated transcription. Additional relevant targets for CREB include fos-like proteins (i.e. c-fos, ΔfosB) and neurotensin, which are expressed by dopaminergic cells in the VTA (Kalivas and Miller 1984; Stephenson *et al*. 1999) and have been implicated in the development of neuroadaptations associated with repeated psychostimulant injections (Daunais and McGinty 1994; Kelz *et al*. 1999; Rompre and Perron 2000).

L-type calcium channels and other cocaine regulated behaviors

The information summarized above indicates that calcium influx through L-type calcium channels plays an important role in psychostimulant-induced behavioral and neuronal plasticity. Several studies have shown that L-type calcium channels modulate cocaine-regulated behaviors other than behavioral sensitization. For example, systemic administration of L-type calcium channel antagonists impairs cocaine self-administration (Kuzmin *et al*. 1992; Martellotta *et al*. 1994; Schindler *et al*. 1995), cocaine-induced conditioned place preference (Pani *et al*. 1991; Calcagnetti *et al*. 1995) and the reinstatement of cocaine-seeking behavior (Licata *et al*. 2001). These results highlight the important role of calcium influx through L-type calcium channels in a broad range of behaviors regulated by cocaine. The extent to which the various CaM kinases contribute to these cocaine-mediated behaviors remains to be determined.

Summary and conclusions

During the development of psychostimulant-induced behavioral sensitization there is a transient increase in the excitability of dopaminergic neurons in the VTA. This is the result of a number of factors, including (i) dopamine autoreceptor subsensitivity, (ii) D1 dopamine receptor-mediated enhancement of glutamate release, (iii) increased glutamate transmission through AMPA and NMDA receptors, and (iv) augmented activation of L-type calcium channels. Since AMPA and NMDA receptors as well as L-type calcium channels are calcium permeable, these findings suggest that calcium-stimulated second messengers may play a role in the development of behavioral sensitization. Consistent with this hypothesis, repeated amphetamine injections alter calmodulin protein and mRNA and increase L-type calcium channel subunit mRNA levels in the VTA. Moreover, inhibition of components of the CaM and MAP kinase signaling cascades blocks the development of behavioral sensitization to cocaine and this process is attenuated in CaMKII knockout mice. The numerous targets of the CaM and MAP kinases include CREB, a transcription factor that contributes to psychostimulant-induced neuronal and behavioral plasticity, and tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis. Thus, a persistent augmentation in signaling through the CaM and MAP kinase cascades is likely to have a profound influence on the function of VTA dopaminergic neurons.

One of the critical unresolved issues in the behavioral sensitization literature is the biochemical mechanism underlying the shift from the development of behavioral sensitization, which occurs mainly in the VTA, to expression, which arises in the nucleus accumbens and other dopaminergic terminal regions. In this context, it is interesting to note that a growing literature indicates that L-type calcium channels and calcium-mediated second messengers play important roles in the long term expression of psychostimulant sensitization (Pierce and Kalivas 1997b; Gnegy 2000), including the modulation of dopamine release in the nucleus accumbens (Pierce and Kalivas 1997a). Given the important role of calcium-stimulated kinases in both the development and expression of behavioral sensitization, it is possible that these signaling molecules may provide the biochemical conduit through which the transient neurochemical, biochemical and molecular alterations associated with the development of behavioral sensitization are transferred to the nucleus accumbens, where the main neurophysiological adaptations that drive the long-term expression of behavioral expression occur.

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