REVIEW

mTOR complex 1: a key player in neuroadaptations induced by drugs of abuse

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Abstract

The mammalian (or mechanistic) target of rapamycin (mTOR) complex 1 (mTORC1) is a serine and threonine kinase that regulates cell growth, survival, and proliferation. mTORC1 is a master controller of the translation of a subset of mRNAs. In the central nervous system mTORC1 plays a crucial role in mechanisms underlying learning and memory by controlling synaptic protein synthesis. Here, we review recent evidence suggesting that the mTORC1 signaling pathway promotes neuroadaptations following exposure to a diverse group of drugs of abuse including stimulants, cannabinoids, opiates, and alcohol. We further describe potential molecular mechanisms by which drug-induced mTORC1 activation may alter brain functions. Finally, we propose that mTORC1 is a focal point shared by drugs of abuse to mediate drug-related behaviors such as reward seeking and excessive drug intake, and offer future directions to decipher the contribution of the kinase to mechanisms underlying addiction.

Keywords: addiction, alcohol, drugs of abuse, limbic system, mTOR, translation.

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Accumulating evidence in the past decade suggest that the kinase mTORC1 (mammalian/mechanistic Target of Rapamycin in Complex 1) is a critical mediator of protein synthesis (Ma and Blenis 2009) including dendritic synthesis of synaptic proteins (Costa-Mattioli *et al.* 2009; Liu-Yesucevitz *et al.* 2011). Accordingly, mTORC1 plays a major role in molecular mechanisms underlying normal brain functions such as learning and memory (Costa-Mattioli *et al.* 2009), but also appears to be dysregulated in a number of neurological disorders including epilepsy, Parkinson's disease and Alzheimer's disease (Swiech *et al.* 2008; Hoeffer and Klann 2010; Dazert and Hall 2011; Costa-Mattioli and Monteggia 2013; Maiese *et al.* 2013).

Addiction is psychiatric disorder that manifests itself as compulsive drug seeking and taking despite detrimental negative consequences, resulting in major health and socio-economic impacts worldwide (Spanagel 2009; Koob and Volkow 2010). Although drugs of abuse have diverse chemical structures and pharmacological site of actions, they share behavioral phenotypes. Acute drug use is reinforcing, however, prolonged repeated intake may lead to detrimental behaviors such as uncontrolled drug intake, craving, anxiety, and relapse (Nestler 2005; Koob and Volkow 2010). The limbic system, which includes the hippocampus, amygdala, nucleus accumbens (NAc), and the prefrontal cortex (PFC) plays a key role in circuits that underlie behavioral phenotypes induced by both acute and chronic exposure to drugs of abuse (Spanagel 2009; Koob and Volkow 2010). Recently, a series of studies raised the intriguing possibility that mTORC1 in the limbic system is a central molecular

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determinant of drug-induced neuroadaptations underlying common adverse behavioral phenotypes.

Here, we first briefly review the key features of mTORC1, its main upstream activators and downstream targets and functions. Next, we describe recent findings converging toward the possibility that neuronal mTORC1 is a focal point where signaling pathways altered by drugs of abuse merge and propose potential mechanisms that may underlie mTORC1's function in the onset and maintenance of maladaptive neuroadaptations. Finally, we offer new directions of investigation to further decipher the contribution of neuronal mTORC1 kinase in drug-induced synaptic plasticity.

mTOR in Complex 1

Structural and functional features of the mTOR complexes

mTOR is a large ubiquitous and evolutionary conserved protein kinase, which belongs to the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family (Zoncu *et al.* 2011). mTOR is an atypical kinase as it signals through two distinct multiprotein complexes mTORC1 and mTOR in complex 2 (mTORC2) (Ma and Blenis 2009; Zoncu *et al.* 2011). Both mTORC1 and mTORC2 protein complexes contain the DEP domain-containing mTOR-interacting protein, and the mammalian lethal with SEC13 protein 8 (mLST8 also known as G protein beta-like, GβL) (Zoncu *et al.* 2011). However, the kinase also associates with distinct adaptor proteins that define the complex. Specifically, the regulatory-associated protein of mTOR (RAPTOR) and the 40 kDa Pro-rich Akt substrate (PRAS40) are specific to mTORC1, whereas the rapamycin-insensitive companion of mTOR (RICTOR), the mammalian stress-activated MAP kinase-interacting protein 1 (mSIN1), and the protein observed with RICTOR (PROTOR) are exclusively found in mTORC2 (Ma and Blenis 2009; Zoncu *et al.* 2011). Although mTOR bears the same kinase activity in both complexes, the unique multiprotein network encompassing the kinase results in distinct mTORC1 and mTORC2 substrates and cellular functions (Dowling *et al.* 2010; Sparks and Guertin 2010).

While the role of mTORC2 in addiction is likely to be of interest (Mazei-Robison *et al.* 2011), this review focuses solely on the role of mTORC1 in neuroadaptations induced by drugs of abuse.

Signaling pathways leading to mTORC1 activation

A wide variety of stimuli including nutrients, stress, energy or growth factors converge at the level of mTORC1 to sense and integrate the complex cellular environment to promote an appropriate cellular response. The main upstream activator of mTORC1 is the ras homolog enriched in brain protein (Rheb) (Hay and Sonenberg 2004; Zoncu *et al.* 2011). Rheb is a GTP binding protein and, as such, the enzyme cycles between a GTP-active and a GDP-inactive state. The direct interaction between GTP-bound Rheb and mTORC1 leads to the activation of the kinase (Laplante and Sabatini 2012). The cycling of Rheb between active GTP and inactive GDP-bound states is controlled by a heterodimer composed of the tuberous sclerosis complex 1 (TSC1, also called hamartin) and the tuberous sclerosis complex 2 (TSC2, also called tuberin), which displays a GTPase-activating protein (GAP) activity (Hay and Sonenberg 2004; Kwiatkowski and Manning 2005). Therefore, inhibition of TSC1/TSC2 heterodimer increases the activity of Rheb which subsequently leads to activation of mTORC1 (Laplante and Sabatini 2012).

The activity of TSC2, and consequently the activity of mTORC1, is regulated by phosphorylation. For example, phosphorylation of TSC2 by AMP-activated protein kinase and glycogen synthase kinase 3β results in GAP activation (and thus in mTORC1 inhibition) (Zoncu *et al.* 2011). In contrast, extracellular signal-regulated kinases 1/2 (ERK1/2) or its downstream kinase the 90 kDa ribosomal S6 kinase, as well as AKT (also known as PKB) phosphorylate TSC2 on other distinct sites resulting in the inhibition of TSC2 GAP activity leading to mTORC1 activation (Ma and Blenis 2009; Mendoza *et al.* 2011; Zoncu *et al.* 2011).

Downstream effectors and functions of mTORC1

The best characterized substrates of mTORC1 are the S6 ribosomal kinase 1 (S6K1) and the eukaryotic translation initiation factor-4E binding protein (4E-BP) (Costa-Mattioli *et al.* 2009; Ma and Blenis 2009). Phosphorylation of S6K1 and 4E-BP controls the initiation and elongation of translation of a subset of mRNAs displaying a 5′ terminal oligopyrimidine (TOP) motif (Zoncu *et al.* 2011; Thoreen *et al.* 2012). For instance, mTORC1-induced phosphorylation of 4E-BP allows the interaction of the eukaryotic translation initiation factor 4E (eIF4E) to eIF4G at the 5′ cap structure of mRNAs, a process that is critical for translation initiation (Ma and Blenis 2009). Besides, mTORC1-induced phosphorylation of S6K1 affects translation initiation and elongation by binding or affecting the function of several proteins implicated in these processes such as eukaryotic elongation factor 2 kinase, eIF4B and the S6K1 Aly/REF-like target protein (SKAR) which interacts with the exon junction complex of spliced mRNA (Ma *et al.* 2008; Zoncu *et al.* 2011). mTORC1 activation also increases translational rate by stimulating the production of ribosomal proteins and translation factors (Liao *et al.* 2007), as well as by inducing the transcription of ribosomal RNAs (rRNAs) (Zoncu *et al.* 2011). Furthermore, mTORC1 activity has also been shown to control the transcription of a subset of metabolic genes by regulating the activity of several transcription factors such as the sterol regulatory element-binding proteins (Lamming and Sabatini 2013; Laplante and Sabatini 2013).

In addition to its role in promoting protein synthesis and transcription, mTORC1 is a major regulator of cell

autophagy, a cellular process aimed to recycle intracellular organelles following their lysosomal degradation (Wong and Cuervo 2010; Harris and Rubinsztein 2012). mTORC1 activity negatively regulates autophagy by phosphorylating substrates such as unc-51-like kinase 1 (ULK1), autophagy-related 13 (ATG13) or death-associated protein 1 (Hosokawa *et al.* 2009; Koren *et al.* 2010). Together, these processes allow the cell to control its metabolism, growth capacity, as well as to sustain cell division and accordingly dysregulated mTORC1 signaling has been highlighted in a number of disorders such as cancer, metabolic disorders, neurological diseases, and immunological diseases (Dazert and Hall 2011; Laplante and Sabatini 2012).

mTORC1 in the central nervous system

In agreement with its role in the regulation of cell growth, neuronal mTORC1 impacts the early steps of neuronal development (Jaworski and Sheng 2006; Swiech *et al.* 2008), and mTORC1 is thought to be of potential therapeutic interest in the regeneration of neurons after spinal cord injury (Liu *et al.* 2010; Park *et al.* 2010; Lu *et al.* 2012). In the adult brain, mTORC1 is localized in the cell body but also at the dendrites of neurons where it has been shown to contribute to the induction of the late phase of long-term potentiation (Tang *et al.* 2002; Cammalleri *et al.* 2003; Stoica *et al.* 2011). Therefore, mTORC1 appears to be a critical mediator of synaptic plasticity mainly because of its capacity to promote local dendritic protein synthesis (Gobert *et al.* 2008; Swiech *et al.* 2008; Hoeffer and Klann 2010; Stoica *et al.* 2011) and thus the kinase participates in neural processes that require synaptic protein production such as learning, and memory. Malfunction of this pathway plays a role in the development of neurodegenerative diseases (Swiech *et al.* 2008; Hoeffer and Klann 2010) as well as psychiatric disorders including addiction (Jernigan *et al.* 2011; Dayas *et al.* 2012; Costa-Mattioli and Monteggia 2013).

Rapamycin: a pharmacologic tool to study mTORC1 function in the brain

Rapamycin was first isolated from a bacteria strain of the *Streptomyces genus*, which was collected in the Easter Island soil and initially described as an antifungal agent (Vezina *et al.* 1975). The name "rapamycin" originates "rapa-" which stands for "Rapa Nui", the native name of the Easter Island, and "–mycin" for its antibiotic properties (Vezina *et al.* 1975). The immunosuppressant properties of rapamycin were discovered later on and in 1999 the drug was approved by the Food and Drug Administration (FDA) as treatment to prevent renal allograft rejection (Hartford and Ratain 2007).

Rapamycin acts as an allosteric specific inhibitor of mTORC1, which, in complex with FKBP12 protein (FK 506-binding protein of 12 kDa), binds to the FRB (FKBP12-rapamycin binding) domain of the kinase (Dowling *et al.*

2010; Yip *et al.* 2010). This interaction is thought to modify mTOR conformation, which, in turn not only weakens the integrity of the kinase complex but also prevents the association of its catalytic site with its substrates (Yip *et al.* 2010). Recently, an X-ray structure of the N-terminus-truncated mTOR bound to mLST8 revealed an intrinsically active but poorly accessible kinase catalytic cleft whose access is further restricted by the binding of rapamycin-FKBP12 complex to the FRB domain of mTOR (Yang *et al.* 2013). Rapamycin is a highly valuable pharmacological tool to address the consequence of mTORC1 activation in the brain. Rapamycin crosses the blood–brain barrier, and acutely, the inhibitor does not affect the activity of mTORC2 or other kinases (Davies *et al.* 2000; Dowling *et al.* 2010). In addition, systemic administration of rapamycin does not induce rodent behaviors such as anxiety or general locomotion (Blundell *et al.* 2008; Neasta *et al.* 2010; Lin *et al.* 2014). It should be noted that prolonged treatment with rapamycin was also shown to inhibit mTORC2 activity in non-neuronal tissue (Sarbassov *et al.* 2006; Lamming *et al.* 2012), an effect that was connected with insulin resistance (Lamming *et al.* 2012). However, it is not clear whether prolonged treatment with rapamycin inhibits mTORC2 in all brain structures. For instance, Mazei-Robison *et al.* (2011) found no modification of mTORC2 signaling in the ventral tegmental area (VTA) of mice that were administered with rapamycin daily during 6 days (Mazei-Robison *et al.* 2011).

mTORC1 regulates autophagy in neurons

Autophagy is required for proper cell homeostasis by mediating the turnover of organelles or by clearing misfolded proteins and malfunctioning of this catabolic process has been associated with several neurodegenerative disorders such as Huntington's, Alzheimer's, and Parkinson's diseases (Wong and Cuervo 2010; Harris and Rubinsztein 2012). Similar to its function in non-neuronal cells, mTORC1 also regulates autophagy in neurons (Harris and Rubinsztein 2012). How regulation of autophagy by mTORC1 impacts normal brain functions is still poorly defined but has been recently related to neurotransmission (Hernandez *et al.* 2012; Torres and Sulzer 2012), and perhaps to synaptic plasticity (Shehata *et al.* 2012). From a therapeutic point of view, mTORC1 inhibitors have been reported to improve the symptoms of several neurodegenerative disorders, in part, by increasing the clearance of misfolded toxic proteins (Dehay *et al.* 2010; Spilman *et al.* 2010). Therefore, activation of autophagy in neurons using mTORC1 blockers is considered a promising strategy to fill unmet medical needs for patients affected by a number of neurodegenerative diseases (Wong and Cuervo 2010; Harris and Rubinsztein 2012).

mTORC1 and synaptic transmission

While the role of mTORC1 in the mechanisms that underlie neuronal development and synaptic plasticity has been well

studied, less is known regarding its potential function in basic synaptic transmission. Recent studies addressed this question by the use of transgenic mice in which mTORC1 signaling was altered, together with rapamycin treatment to inhibit the kinase activity. In FKBP12-deficient mice (i.e., conditional knockout), in which mTOR interaction with RAPTOR is enhanced leading to an increased activity of mTORC1, Hoeffer *et al.* (2008) found no change in basal synaptic transmission in hippocampal slices (Hoeffer *et al.* 2008). In contrast, two recent studies reported that mTORC1 activity regulates several features of synaptic transmission at both pre- and post-synaptic levels (Hernandez *et al.* 2012; Weston *et al.* 2012). Although the molecular mechanism underlying this process remains largely unidentified, it may rely on autophagy (Hernandez *et al.* 2012) and/or synapse formation (Jaworski and Sheng 2006; Weston *et al.* 2012). Importantly, mTORC1 appears to regulate synaptic function in glutamatergic, GABAergic, and dopaminergic neurons suggesting that mTORC1 is a universal controller of neurotransmission (Hernandez *et al.* 2012; Weston *et al.* 2012).

mTORC1 in learning and memory

Long-lasting forms of synaptic plasticity and memory require d*e novo* protein synthesis (Costa-Mattioli *et al.* 2009), including specific synthesis at dendrites (Sutton and Schuman 2006). Since mTORC1 controls mRNA translation at the synapse (Liu-Yesucevitz *et al.* 2011), the kinase has been suggested to play an important role in various learning and memory processes. For example, local inhibition of mTORC1 in the rat medial PFC (mPFC) by rapamycin caused a deficit in long-term retention of trace fear memory examined days after conditioning training, whereas short-term trace fear memory and object recognition memory were not affected (Sui *et al.* 2008). Likewise, rapamycin leads to impairment of spatial memory retrieval but not acquisition in mice when given systemically (Deli *et al.* 2012) and was shown to impair novel object recognition when infused into the basolateral amygdala or dorsal hippocampus of rats (Myskiw *et al.* 2008; Jobim *et al.* 2012b). Moreover, mTORC1 activation was also implicated in the consolidation and reconsolidation of fear- and drug-related memories (Blundell *et al.* 2008; Slipczuk *et al.* 2009; Glover *et al.* 2010; Gafford *et al.* 2011; Stoica *et al.* 2011; Zhu *et al.* 2011; Mac Callum *et al.* 2013; Lin *et al.* 2014). For example, mTORC1 inhibition in the hippocampus or the amygdala of rats was shown to impair consolidation and reconsolidation of long-term memory in an inhibitory avoidance-learning task (Bekinschtein *et al.* 2007; Slipczuk *et al.* 2009; Jobim *et al.* 2012a). In addition, mTORC1 inhibition during memory consolidation attenuated long-term taste memory in a conditioned taste aversion paradigm, and novel-taste learning induced a correlative activation of mTORC1 in the gustatory cortex

(Belelovsky *et al.* 2009). Taken together, these studies suggest that mTORC1 is critical for memory consolidation, reconsolidation, storage, and/or retrieval processes, whereas its role in initial learning (acquisition) is yet to be fully characterized.

Interestingly, mice with an increased mTORC1 activity (FKBP12-deficient mice) displayed enhanced long-term contextual fear memory retention, as well as compulsive-like perseverative behaviors in several tasks including the marble burying assay, object recognition task, Morris water maze, and Y maze reversal task (Hoeffer *et al.* 2008). However, no difference between FKBP12-deficient and wild-type mice was found in short-term contextual memory and in short- and long-term cued memory (Hoeffer *et al.* 2008). Together, these findings suggest that over activation of the mTORC1 pathway may result in persistent memory and perseverative behaviors.

While the above-mentioned studies were conducted using acute rapamycin treatments, it was recently reported that chronic rapamycin, given *via* food supplementation, enhanced spatial learning and memory as measured in Morris water maze and passive avoidance (Spilman *et al.* 2010; Halloran *et al.* 2012). In line with this finding, lifelong rapamycin administration was shown to ameliorate age-dependent spatial learning and memory deficits by reducing interleukin-1 beta and enhancing NMDA signaling (Majumder *et al.* 2012). Thus, these studies raise the possibility that long-term chronic inhibition of mTORC1 leads to cognitive enhancement and/or repair, in contrast to the memory impairing effect of acute inhibition of the kinase.

Drugs of abuse and mTORC1

Rodent models of drug-induced modifications of behavior

Repeated exposure to drugs of abuse promotes modifications of animal behavior that model certain aspects of addiction (Steketee and Kalivas 2011). For instance, recurring exposure of rodents to drugs of abuse results in sensitization, a progressive enhancement of responsiveness to the drug (Vanderschuren and Pierce 2010). Sensitization is thought to be related in part to adaptive changes in gene expression and synaptic organization induced by repeated activation of the reward/reinforcement system by drugs of abuse (Sanchis-Segura and Spanagel 2006; Vanderschuren and Pierce 2010). Conditioned placed preference (CPP) measures the preference of an animal to a context that was previously associated with a reward and is used to evaluate the reinforcing properties of a drug and reward-seeking behavior (Sanchis-Segura and Spanagel 2006; Tzschentke 2007). Similar to sensitization, induction and expression of CPP induced by repeated administration of a drug are thought to depend on drug-induced neuroadaptations in relevant brain areas such as the reward circuit (Hyman *et al.* 2006).

Activation of mTORC1-mediated signaling pathway by drugs of abuse

As described above, mTORC1 is known for its role in promoting neuroadaptations that underlie learning and memory processes by stimulating mRNA translation into proteins (Costa-Mattioli *et al.* 2009; Hoeffer and Klann 2010). Since neural circuitry and molecular mechanisms of learning and memory share similarities with those underlying drug addiction (Hyman *et al.* 2006), an attractive corollary would be that mTORC1 also orchestrates key signaling events leading to drug-induced maladaptive neuroadaptations. Recently, several studies have evaluated this possibility by examining mTORC1 activity in the brain limbic system following acute exposure to drugs of abuse. For instance, Wu *et al.* (2011) reported an enhancement in the phosphorylation level of the ribosomal protein S6, a substrate of S6K1, within the NAc, cortex and VTA of rats 1 h after a single systemic administration of cocaine (Wu *et al.* 2011). Importantly, this increase was completely abolished by a systemic pre-treatment with the selective mTORC1 inhibitor, rapamycin, indicating that mTORC1 is activated in several brain nuclei that compose the limbic system. The activation of mTORC1 appears to be transient, at least in the NAc, as S6K1 and S6 were not phosphorylated 24 h following acute administration of cocaine (Bailey *et al.* 2012). In line with this observation, Puighermanal *et al.* (2009) found that a single systemic administration of Tetrahydrocannabinol (THC) also led to a rapid increase (within 30 min) of mTORC1 activity in the hippocampus before returning to the basal level 4–6 h after drug treatment (Puighermanal *et al.* 2009). Interestingly, THC-mediated stimulation of mTORC1 is not restricted to hippocampus as an increased activity of the kinase was also detected in the striatum, the frontal cortex and the amygdala (Puighermanal *et al.* 2013). We showed that a single systemic administration of a non-hypnotic dose of alcohol induced a rapid activation of mTORC1 signaling in the NAc as reflected by an enhancement of the phosphorylation level of its two substrates S6K1 and 4E-BP (Neasta *et al.* 2010). In contrast to these previous findings and ours, Goncalves *et al.* (2012) observed a decrease in mTORC1 activity in the hippocampus of mice 24 h after an acute dose of methamphetamine (Goncalves *et al.* 2012), however, the effect of this drug would need to be further evaluated in other brain structures. Nevertheless, at this point, it is striking to note that three different classes of drugs of abuse, namely, cocaine, THC, and alcohol with distinct primary cellular targets in the brain (Hyman *et al.* 2006) appear to have similar actions on the mTORC1-mediated signaling pathway in the limbic system. This observation therefore raises the possibility that some detrimental behaviors arising following repeated exposure to drugs of abuse may be mediated, at least in part, through mTORC1 activation in key addiction-related brain regions. In agreement with this hypothesis, we observed that excessive alcohol drinking, a hallmark of alcohol abuse (Spanagel 2009), triggers mTORC1 activation in the NAc which lasts at least 24 h after alcohol withdrawal (Neasta *et al.* 2010). Likewise, phosphorylation of S6K1 was detected in the NAc 3 days after the last of five administrations of methamphetamine (Narita *et al.* 2005). Similarly, Puighermanal *et al.* (2013) reported a small but not significant increase of mTORC1 activity in the hippocampus of mice that were chronically administered THC when assessed 3 days after the last drug treatment (Puighermanal *et al.* 2013). Finally, a 5-day morphine treatment (via subcutaneous pellet) was recently reported to activate mTORC1 signaling in rat VTA (but not in the NAc) as assessed 24 h after the last morphine pellet implantation (Mazei-Robison *et al.* 2011). In contrast, Bailey *et al.* (2012) did not observe mTORC1 activation in the NAc or the PFC of mice when assessed 24 h after the last cocaine administration (Bailey *et al.* 2012). Nevertheless, together the studies above suggest that a variety of drugs produce acute but also long-lasting activation of the mTORC1 signaling pathway (Fig. 1).

Possible molecular mechanisms underlying mTORC1 activation by drugs of abuse

The potential role of PI3K and AKT kinases in THC, morphine, and alcohol-mediated mTORC1 activation

mTORC1 is a downstream target of both ERK1/2 and AKT signaling (Mendoza *et al.* 2011). Acute exposure to psychostimulants, THC and opiates resulted in ERK1/2 activation in the NAc (Valjent *et al.* 2001, 2004). In contrast, acute systemic administration and excessive intake of alcohol activate the PI3K/AKT signaling cascade within the NAc (Cozzoli *et al.* 2009; Neasta *et al.* 2011; Ron and Messing 2013). THC administration induced both ERK1/2 (Derkinderen *et al.* 2003) and PI3K/AKT (Ozaita *et al.* 2007) activation along with the phosphorylation of S6K1 in the hippocampus (Puighermanal *et al.* 2009). However, THC-induced phosphorylation of S6K1 on threonine 389 (a marker of mTORC1 activity) was not blocked by SL327, a selective inhibitor of ERK1/2 (Puighermanal *et al.* 2009). Although Puighermanal *et al.* (2009) did not test the consequence of PI3K and/or AKT inhibition on S6K1 phosphorylation, this finding implies that THC-induced mTORC1 activation is likely to result from the activation of the PI3K/AKT cascade. It is plausible, however, that ERK1/2 cooperates with PI3K/AKT/mTORC1 to activate S6K1 and to induce mRNA translation (Puighermanal *et al.* 2009). The contribution of the PI3K/AKT pathway to mTORC1 signaling has also been suggested for morphine. Specifically, Cui *et al.* (2010) showed that local infusion of the PI3K inhibitor, LY294002, within the hippocampus blocked mTORC1 activation observed following morphine place preference (Cui *et al.* 2010), although it is noteworthy to point out that the lack of mTORC1 activation could not be directly because of PI3K inhibition but rather to the fact that
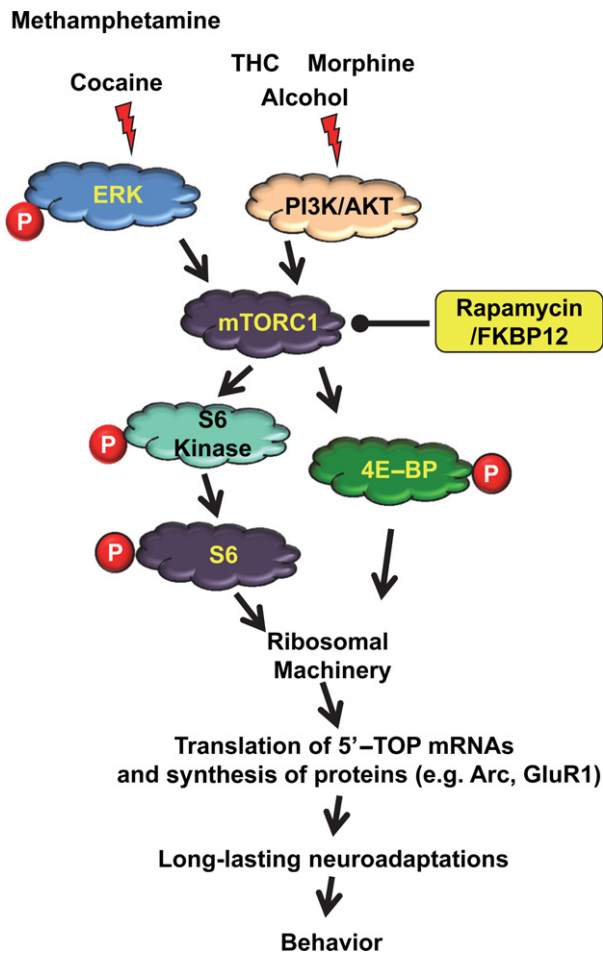
Fig. 1 A graphical model depicting the signaling cascades that activate mammalian/mechanistic Target of Rapamycin in Complex 1 (mTORC1) in response to exposure to drugs of abuse and a possible mechanism by which mTORC1 serves as a central player in drugs of abuse-dependent neuroadaptations that underlie phenotypes of drug addiction such as drug seeking and relapse.

animals did not respond to the drug-paired context as they did not express morphine place preference. Finally, we generated indirect evidence suggesting that mTORC1 activation in response to alcohol exposure is the result of the activation of the PI3K/AKT pathway. Specifically, we showed that acute systemic administration and recurring cycles of voluntary consumption of alcohol led to the activation of the small G protein H-Ras, the upstream activator of the PI3K/AKT signaling, in the NAc (Neasta *et al.* 2011; Ben Hamida *et al.* 2012). The same alcohol exposure paradigms resulted in the activation of PI3K/AKT but not ERK1/2 in the NAc (Neasta *et al.* 2011). Further studies are needed, however, to confirm that H-Ras/PI3K/ AKT cascade is indeed upstream of mTORC1 in the NAc in response to alcohol. Nevertheless, together these studies strongly suggest that the PI3K/AKT pathway is likely to contribute to mTORC1 activation in several limbic structures following exposure to THC, morphine, and alcohol (Fig. 1).

The potential role of ERK1/2 kinases in cocaine-mediated mTORC1 activation

Several studies suggest that ERK1/2 activation may be necessary for cocaine-mediated induction of mTORC1 signaling. First, acute administration of cocaine induces a rapid activation of both ERK1/2 (Valjent *et al.* 2000, 2004; Girault *et al.* 2007) and mTORC1 (Wu *et al.* 2011) in brain structures that control behavioral responses to drugs of abuse. Secondly, activation of both ERK1/2 (Lu *et al.* 2006; Girault *et al.* 2007) and mTORC1 (Wang *et al.* 2010b; Wu *et al.* 2011; Bailey *et al.* 2012) in the limbic system promote neuroadaptations that underlie certain behavioral alterations induced by repeated cocaine exposure. Finally, the activation of ERK1/2 (Miller and Marshall 2005; Lu *et al.* 2006) and mTORC1 (Wang *et al.* 2010b) is specifically localized to the NAc core of animals exposed to a cocaine-paired cue. This tight connection raises the possibility that ERK1/2 signaling activation is necessary for mTORC1 by cocaine (Fig. 1).

The potential role of NMDA receptor in drug-mediated mTORC1 signaling

Several studies have shown that mTORC1 signaling can be triggered in response to activation of the NMDA receptor (Cammalleri *et al.* 2003; Gong *et al.* 2006) whose activity is an important mediator of cocaine and alcohol-dependent behaviors (Wang *et al.* 2007, 2010a; Stuber *et al.* 2010). Accordingly, Wang *et al.* (2010b) proposed that cocaine-paired cue activates mTORC1 through NMDA receptor stimulation (Wang *et al.* 2010b). Furthermore, inhibition of the NMDA receptor with MK801, a potent non-competitive antagonist, prevented the activation of mTORC1 in the hippocampus following THC administration (Puighermanal *et al.* 2009). In contrast, acute alcohol exposure, which has been shown to be a potent inhibitor of NMDA receptor function (Lovinger *et al.* 1989; Ron and Wang 2009) promotes mTORC1 activation (Neasta *et al.* 2010), and interestingly, the rapid antidepressant effect of ketamine, a non-competitive NMDA receptor antagonist, is thought to be mediated through mTORC1 stimulation (Li *et al.* 2010; Autry *et al.* 2011). Therefore, it is likely that drugs of abuse activate mTORC1 through different but cross-talking mechanisms.

mTORC1 and drug–related behaviors

As mentioned above, sensitization and CPP paradigms were used to uncover the functional role of mTORC1 in alterations of animal behavior following repeated drug exposure (Table 1). Wu *et al.* (2011) showed that when rapamycin is administered to rats along with cocaine during the acquisition phase, animals did not express locomotor sensitization when tested after 2 weeks of withdrawal (Wu

Table 1 Summary of rapamycin effects on drug-related behaviors. i.p. denotes intraperitoneal, ↓ denotes an inhibition

Substance of abuse	Animal model	Rapamycin administration	Effect of rapamycin on drug-related behaviors	References
Alcohol	Mice	i.p.	↓ Alcohol consumption	Neasta *et al.* (2010)
			↓ Expression of locomotor sensitization	
			↓ Expression of CPP	
	Rats	i.p.	↓ Alcohol seeking and consumption in operant procedure	
		Intra-NAc	↓ Consumption in two-bottle choice procedure	
		Intra-NAc	↓ Consumption in operant procedure	unpublished data
		i.p. and intra-CeA	↓ Reconsolidation of alcohol-associated memories	Barak *et al.* (2013)
Cocaine	Mice	i.p.	↓ Expression of locomotor sensitization	Bailey *et al.* (2012)
			↓ Expression of CPP	
			No change on acquisition of locomotor sensitization	
			No change on acquisition of CPP	
	Rats	i.p.	↓ Acquisition of locomotor sensitization	Wu *et al.* (2011)
			↓ Expression of locomotor sensitization	
		i.p.	↓ Reconsolidation of cocaine-associated memories	Lin *et al.* (2014)
		Intra-NAc core	↓ Cue-induced reinstatement of cocaine seeking	Wang *et al.* (2010b)
		Intra-NAc shell	No change on cue-induced reinstatement of cocaine seeking	
Morphine	Rats	Intra-hippocampal	↓ Acquisition of CPP	Cui *et al.*, (2010)
		i.p.	↓ Reconsolidation of morphine-associated memories	Lin *et al.* (2014)
Methamphetamine	Rats	Intra-NAc	↓ Acquisition of sensitization	Narita *et al.* (2005)
			No change on acquisition of CPP	

et al. 2011). In contrast, Bailey *et al.* (2012) did not observe that rapamycin affects the acquisition of cocaine sensitization in mice (Bailey *et al.* 2012). Therefore, the functional role of mTORC1 in the mechanisms that promote long-lasting neuroadaptations that are necessary for the acquisition of cocaine sensitization needs to be further clarified. However, systemic administration of rapamycin for 4 days (Wu *et al.* 2011) or only 1 h (Bailey *et al.* 2012) prior to the challenge drug administration, was found to inhibit the expression of cocaine locomotor sensitization in both rats and mice. In line with these findings, we previously found that a single systemic administration of rapamycin attenuates the expression of alcohol-induced locomotor sensitization (Neasta *et al.* 2010), although rapamycin did not affect the locomotor hyperactivity typically observed following acute systemic administration of cocaine or alcohol (Neasta *et al.* 2010; Bailey *et al.* 2012), suggesting that mTORC1 activation is specifically implicated in neural plasticity underlying the expression of cocaine and alcohol locomotor sensitization, but not in the acute locomotor effect of these substances. Finally, systemic mTORC1 inhibition decreased the expression of cocaine and alcohol place preference but not the acquisition of cocaine place preference (Neasta *et al.* 2010; Bailey *et al.* 2012). Altogether, these data indicate that mTORC1 regulates certain but not all neural processes underlying drug-related behaviors and further suggest that local infusion of rapamycin in specific brain structures is

necessary to clarify the contribution of the kinase to these processes.

As a complementary approach to systemic administration of rapamycin, local infusion of this inhibitor in relevant brain regions has been used to explore the functional relevance of the local increase in mTORC1 activity following exposure to drugs of abuse (Table 1). Specifically, intra-hippocampal infusion of rapamycin prior to morphine administration blocked the acquisition of CPP along with mTORC1 activation in rats (Cui *et al.* 2010). In contrast, intra-NAc infusion of rapamycin only affected the acquisition of sensitization elicited by methamphetamine but not the acquisition of methamphetamine CPP (Narita *et al.* 2005). Together, these data suggest that focal activation of mTORC1 rather than a global increase kinase activity is sufficient to elicit drug-related behaviors. Thus, to avoid discrepancy between studies, special attention must be paid to accurately locate the subregions where drugs trigger mTORC1 activation, and in parallel determine whether local administration of rapamycin is sufficient to inhibit both mTORC1 activation and drug-related behaviors.

Furthermore, mTORC1 also contributes to mechanisms that underlie alcohol and cocaine-seeking and intake. Specifically, systemic (Neasta *et al.* 2010) or intra-NAc (unpublished observation) administration of rapamycin reduces alcohol seeking and intake in a rat operant self-administration procedure. Likewise, a single systemic administration of

rapamycin also reduced voluntary excessive alcohol drinking in the home cage of both mice and rats, and similar results were obtained when rapamycin was infused directly into the NAc of rats (Neasta *et al.* 2010). Finally, focal inhibition of mTORC1 within the NAc core was shown to suppress cue-induced reinstatement of cocaine seeking (Wang *et al.* 2010b). Together, these data suggest that mTORC1 plays an important role in a number of drug-related behaviors.

Finally, it is important to note that the inhibition of mTORC1 by rapamycin did not produce non-specific modifications of rodent behavior. Specifically, we showed that rapamycin is neither rewarding nor aversive *per se* as reflected in lack of place preference or aversion to this drug in mice and rats (Neasta *et al.* 2010; Barak *et al.* 2013). Moreover, systemic mTORC1 inhibition did not affect the motivation of rats to obtain a natural reward, and did not affect taste palatability, locomotor activity, and coordination in mice (Neasta *et al.* 2010; Barak *et al.* 2013). Together, these studies suggest that mTORC1 activation plays a unique role in drug intake and addiction-related behaviors, rather than in general reward or non-specific behaviors.

mTORC1 and drug-associated memory

The essential role of mTORC1 in mechanisms that underlie drug-associated memories is strengthened by evidence showing that exposure to a drug context or drug-related cues also activate this pathway. Specifically, Cui *et al.* (2010) reported that mTORC1 is activated in the hippocampus of animals exposed to a morphine-paired compartment following acquisition of morphine place preference (Cui *et al.* 2010). Although this study did not rule out the possibility that acquisition of morphine CPP induced a long-lasting activation of mTORC1 in the hippocampus, the data indicate that a mere reexposure of animals to a context associated with the drug leads to mTORC1 activation. Consistent with this hypothesis, Wang *et al.* (2010b) found that exposure of animals to a cocaine-related cue activated mTORC1 signaling pathway focally within the NAc core (Wang *et al.* 2010b). Recently, we found that mTORC1 is activated following retrieval of alcohol-associated memories in rats with a history of excessive alcohol intake, specifically in the central nucleus of the amygdala (CeA) and in the prelimbic (PrL) and orbitofrontal (OFC) regions of the prefrontal cortex (Barak *et al.* 2013). The alcohol-associated memories were retrieved after a period of abstinence by reexposure of the rats to the alcohol-associated context (operant chambers), as well as presentation of a small, non-pharmacologically active quantity of alcohol, serving as an odor-taste cue (Barak *et al.* 2013). Interestingly, when the memory was retrieved solely by the odor-taste cue (i.e., in the home cage), mTORC1 was activated exclusively in the CeA, but not in the cortical regions (Barak *et al.* 2013), suggesting that activation of the kinase in the PrL and OFC plays a role in retrieval of alcohol-associated contextual and instrumental cues that are specific for the operant chamber. Importantly, countering this memory retrieval-induced mTORC1 activation, by systemic or CeA inhibition of mTORC1 immediately after memory retrieval, disrupted the reconsolidation of alcohol-associated memories, leading to a long-lasting suppression of relapse (Barak *et al.* 2013). Together, these data indicate that mTORC1 plays a crucial role in the retrieval and reconsolidation of alcohol and drug-related memories. Because relapse to drug seeking and consumption is frequently caused by the retrieval of drug-associated memories, these findings strongly suggest that the mTORC1 pathway contributes to the neurobiological processes underlying relapse after a period of abstinence.

mTORC1-dependent mRNA translation – a potential mechanism underlying the actions of drugs of abuse

As reviewed above, the best characterized substrates of mTORC1 are S6K1 and 4E-BP, proteins that are part of the translation initiation machinery, the rate-limiting step of protein synthesis (Costa-Mattioli *et al.* 2009; Ma and Blenis 2009). In neurons, S6K1 and 4E-BP are expressed in the cell body and at the synapse (Tang *et al.* 2002; Schratt *et al.* 2004), where *de novo* protein synthesis is a key process that contributes to the molecular mechanisms underlying long-lasting neuroadaptations (Costa-Mattioli *et al.* 2009; Liu-Yesucevitz *et al.* 2011). Therefore, it is plausible that mTORC1 contributes to the mechanisms underlying drug effects, at least in part, by stimulating translation initiation of synaptic 5′ TOP mRNAs (Gobert *et al.* 2008; Thoreen *et al.* 2012). Accordingly, Puighermanal *et al.* (2009) data suggest that THC-mediated activation of the translation initiation machinery in the hippocampus relies, at least in part, on mTORC1 activation (Puighermanal *et al.* 2009). However, identification of proteins whose synthesis is controlled by mTORC1 following exposure to drugs of abuse is only now starting to unravel. We recently found that the protein levels of scaffolding protein Homer (Szumlinski *et al.* 2006), whose mRNA translation was shown to be mTORC1-dependent (Schratt *et al.* 2004), were up-regulated in the NAc of rodents that consumed large amounts of alcohol (Neasta *et al.* 2010). Interestingly, the increase in the level of Homer was detected even 24 h after withdrawal, and importantly, alcohol-mediated increase of Homer was not observed in animals that were pre-treated with rapamycin (Neasta *et al.* 2010). Other potential downstream effectors of mTORC1 are the subunits of the α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor. In fact, AMPA receptor subunit 1 (GluR1) and GluR2 synthesis were reported to depend on mTORC1 (Mameli *et al.* 2007; Slipczuk *et al.* 2009). Regarding GluR1, we observed that voluntary consumption of alcohol led to a robust increase in the immunoreactivty of GluR1, which was also maintained 24 h after withdrawal (Neasta *et al.* 2010). Furthermore, more recently we found that retrieval of alcohol-associated

memories in rats with a history of excessive alcohol intake resulted in an mTORC1-dependent increase in the protein levels of the activity-regulated cytoskeleton-associated protein (Arc) (Takei *et al.* 2004), in the amygdala, OFC and mPFC (Barak *et al.* 2013). Interestingly, retrieval of alcohol-associated memories produced increased levels of the protein GluR1 as well as post-synaptic protein 95 (PSD-95), whose synthesis was also shown to be mTORC1 dependent (Lee *et al.* 2005), in the amygdala and OFC (Barak *et al.* 2013). Together these studies imply that mTORC1 mediates drug-related maladaptive neuroadaptations, at least in part, by increasing the translation rate of specific mRNAs and thus the expression level of a subset of proteins that play a crucial role in synaptic plasticity (Fig. 1). Identifying such downstream mTORC1 effectors is of particular interest not only to obtain new insights about the molecular basis of maladaptive neuroadaptations but also more generally to understand the means by which mTORC1 regulates neuronal function and plasticity.

Summary and future directions

An intriguing paradox in the neurobiological basis of addiction is the fact that recurring exposure to chemically diverse substances such as drugs of abuse can induce similar debilitating behaviors. As reviewed here, recent findings suggest that mTORC1 kinase in the limbic system could be a focal point of the distinct signaling cascades altered by drugs of abuse following binding to their respective brain targets (Fig. 1). This possibility stems from evidence showing that whereas substances of abuse as diverse as cocaine, THC and alcohol that acutely trigger disparate or intersecting signaling events, all appear to activate mTORC1 in several brain regions. Secondly, inhibiting drug-mediated mTORC1 stimulation with rapamycin blocked the development and expression of several drug-related behaviors in both mice and rats. Thus, and as proposed in other tissues (Zoncu *et al.* 2011), neuronal mTORC1 seems to be capable of being activated via diverse stimuli and it is tempting to speculate that mTORC1-mediated downstream responses, that need to be defined, are then common mechanisms of actions that are shared by several drugs of abuse. We therefore propose that mTORC1 is potentially a common focal point of neuronal signaling that governs drug-induced neuroplasticity (Fig. 1). Importantly, once set up, these neuroadaptations appear to be labile or at least may be inhibited. This is suggested by the fact that, even after being acquired, drug-related behaviors are still sensitive to mTORC1 inhibition by rapamycin (Fig. 1). Therefore, the molecular mechanisms that underlie the stabilization and/or the expression of these adverse behaviors probably rely, at least in part, on mTORC1 signaling (Fig. 1). This latter hypothesis obviously makes mTORC1 and its downstream effectors very valuable targets for the treatment of symptoms that are shared among drug and alcohol addiction.

However, many remaining questions need to be addressed. For example, it is plausible that activation of mTORC1 by drugs of abuse also affects neuronal function via translational-independent mechanisms, e.g., phosphorylation of relevant substrates and transcription. Global phosphoproteomics approaches were recently implemented to identify rapamycin-sensitive phosphorylation of proteins although these were carried out using non-neuronal cells (Demirkan *et al.* 2011; Hsu *et al.* 2011; Yu *et al.* 2011). Therefore, a global analysis aimed to identify mTORC1 substrates in neuronal cells, especially at the synaptic level, would be of great interest for a better understanding of mTORC1 function in the nervous system in general and in addiction in particular. Furthermore, as mentioned above, mTORC1 activity has been linked to rRNA transcription (Zoncu *et al.* 2011), and the expression of numerous metabolic genes is controlled by mTORC1 (Lamming and Sabatini 2013; Laplante and Sabatini 2013). These processes are important for non-neuronal cell growth and cycle, but could also be implicated in neuronal structural changes that are observed in several brain structures after repeated exposure to drugs (Russo *et al.* 2010). Thus far, only few studies have explored the role of mTORC1 in regulating the transcription of genes in neurons (Jaworski and Sheng 2006; Domanskyi *et al.* 2011). Therefore, the possible transcriptional contribution of mTORC1 in neuroplasticity induced by drugs of abuse needs further investigation.

Another critical question is whether mTORC1 activation is a direct consequence of the drug binding to its receptor, and an example to this possibility is the opioid peptide, DAMGO, activating mTORC1 in cells that heterologously express the mu opioid receptor (Polakiewicz *et al.* 1998). However, mTORC1 can also be activated in response to animal exposure to a drug-paired context (i.e., in absence of the drug) (Cui *et al.* 2010; Wang *et al.* 2010b; Barak *et al.* 2013) suggesting that, in certain cases, the binding of the drug to its target is not directly responsible of mTORC1 activation. Furthermore, and as reviewed above, Puighermanal *et al.* (2009) proposed that binding of THC to the cannabinoid receptor inhibits GABAergic interneurons which consequently leads to an increased release of glutamate in the hippocampus, and that the subsequent activation of the NMDA receptor results in the activation of mTORC1 signaling (Puighermanal *et al.* 2009). In addition, dopamine neurotransmission has been linked to mTORC1 signaling in the striatum (Santini *et al.* 2009). As all drugs of abuse acutely increase extracellular dopamine levels in the NAc (Nestler 2005; Hyman *et al.* 2006), it would be of great interest to test whether drug-induced mTORC1 activation in the NAc depends on the dopaminergic system. Finally, the neurotrophin brain-derived neurotrophic factor, which plays a crucial role in drug-induced synaptic plasticity (Autry and Monteggia 2012) regulates the mRNA translation of several synaptic proteins through an mTORC1-dependent

mechanism (Schratt *et al.* 2004; Takei *et al.* 2004). Therefore, it would be also of interest to test whether activation of mTORC1 in certain limbic structures in response to drugs of abuse is a consequence of brain-derived neurotrophic factor signaling.

In conclusion, the recent evidence indicating that mTORC1 kinase is an important contributor to mechanisms underlying drug-related behaviors open new areas of investigation in the neurobiology of addiction and its treatment. However, the fact mTORC1 controls a wide number of vital processes in all organs and that rapamycin and its derivatives are immunosuppressant and promote insulin resistance are obviously an issue for their long-term use to treat addiction. Therefore, identifying the downstream effectors and the detailed mechanisms by which mTORC1 specifically mediate drug-behavioral plasticity will offer potential new candidates and pharmaceutical strategies to help individual to cope with drug addiction.

Acknowledgements and conflicts of interest disclosure

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