CaM Kinases: From Memories to Addiction

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Drug addiction is a major psychiatric disorder with a neurobiological basis that is still insufficiently understood. Initially, non-addicted, controlled drug consumption and drug instrumentalization are established. They comprise highly systematic behaviours acquired by learning and the establishment of drug memories. Ca²⁺/calmodulin-dependent protein kinases (CaMKs) are important Ca²⁺ sensors translating glutamatergic activation into synaptic plasticity during learning and memory formation. Here we review the role of CaMKs in the establishment of drug-related behaviours in animal models and in humans. Converging evidence now shows that CaMKs are a crucial mechanism of how addictive drugs induce synaptic plasticity and establish various types of drug memories. Thereby, CaMKs are not only molecular relays for glutamatergic activity but they also directly control dopaminergic and serotonergic activity in the mesolimbic reward system. They can now be considered as major

molecular pathways translating normal memory formation into establishment of drug memories and possibly transition to drug addiction.

Trends

Ca²⁺/calmodulin-dependent protein kinases (CaMKs) translate glutamatergic activation into synaptic plasticity during learning and memory formation.

Addictive drugs increase CaMK activity, foremost that of α CaMKII.

The CaMKII increase is a necessary prerequisite for the learning and consolidation of drug memories.

During withdrawal, CaMKII activity declines, but rises again during reinstatement of drug seeking and consumption.

CaMKs control dopaminergic and serotonergic activity in the mesolimbic system.

Drug Use and Addiction

Drug addiction is a major psychiatric disorder for which only limited therapies are currently available [1,2]. A striking criterion for drug abuse and addiction is that it severely threatens one's own and others well-being and health. As such, there is a persistent need to treat drug addiction effectively, and ideally reverse the behavioural repertoire of an affected individual back to normal.

An important feature of drug addiction is that it develops from a behavioural repertoire, which is considered to be normal in many societies of the world: the controlled consumption and instrumentalization of psychoactive drugs [3,4]. Establishing and maintaining controlled drug consumption is based on systematic learning and memory retrieval of distinct behaviours, related to drug seeking, preparation, and consumption in a specific context [5,6]. Thereby, information is encoded within different behavioural systems, which can be summarised as 'drug memories' [5,7–9] (Box 1). It is believed that an intensification of these memories together with a loss of impulse control (compulsivity) is responsible for the transition from controlled drug use to addiction [10–12]. Understanding how these drug memories are established and how they may take control over a normal behavioural repertoire should,

therefore, allow to improve the prevention of addiction and to develop new and more effective treatments [13].

From Memories to Drug Memories

It was suggested that anatomical pathways, micromorphological adaptations, as well as molecular mechanisms in the brain, overlap between normal learning and memory and drug memories [14–16]. A crucial player for memory formation in the brain is the glutamatergic system and its plasticity based on experience [17]. Addiction research considered glutamate as an important mediator during the establishment of drug use, but also during escalation of consumption and addictive states [18]. The most abundant excitatory neurotransmitter in the brain, glutamate, activates α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors to generate intracellular Ca^{2+} transients. This cascade is pivotal for long-term potentiation (LTP) and subsequent alterations in gene expression that are the basis for morphological adaptations at the synapse during learning [17]. A major pathway for this is the Ca^{2+} activation of calmodulin (CaM) and subsequent activation of Ca^{2+} /CaM-dependent kinases (CaMKs). Here, we review how this memory pathway controls the establishment of drug addiction-related behaviours. To determine whether this role is drug-specific or works as a common principle, CaMK function for different drug classes is compared.

Molecular Neurobiology of CaMKs

Calcium signalling through NMDA receptors is a fundamental step for inducing long-lasting synaptic plasticity, which is thought to be a key mechanism underlying learning and memory [19]. As a result of high levels of CaM at the synapse, Ca^{2+} influx through NMDA receptors leads to formation of Ca^{2+} /CaM complexes [20], which activate CaMKs. Among the CaMKs, CaMKII has received most attention because this protein is very abundant in the brain [21] and because this multifunctional kinase has remarkable biochemical properties [22]. CaMKII is a holoenzyme that consists of 12 subunits [23]. α CaMKII and β CaMKII are the most abundant CaMKII subunits in the brain [21]. While α CaMKII is expressed only in glutamatergic neurons [24], β CaMKII occurs in inhibitory [25] and in excitatory neurons [26]. β CaMKII, but not α CaMKII, binds to F-actin. This localises the CaMKII holoenzyme. The binding to F-actin is relieved by binding of Ca^{2+} /CaM [22]. The dissociation of CaMKII from F-actin is thought to regulate actin polymerisation that shapes synapse morphology [27].

α CaMKII, but not β CaMKII, activity is crucial for CaMKII function [26]. α CaMKII activity is primarily regulated by autophosphorylation at threonine 286 (T286), which results from an intersubunit kinase reaction within the holoenzyme. T286 autophosphorylation switches the kinase from Ca^{2+} /CaM dependence to independence [28]. Currently, it is thought that T286-autophosphorylated α CaMKII prolongs CaMKII activity at the synapse after the Ca^{2+} stimulus [29]. This leads to glutamate receptor trafficking to the postsynaptic density, resulting in enhanced synaptic transmission [20]. Moreover, CaMKII activity is also regulated by ‘inhibitory’ autophosphorylation at T305/306 [30], endogenous CaMKII inhibitor proteins [31], and phosphatase activity [32]. Given its somewhat remarkable role in learning and memory, most addiction-related research has involved CaMKII, which is also the focus of this review.

In addition to regulation of CaMKII, Ca^{2+} /CaM also activates other members of the CaMK cascade. CaMKI, CaMKIV, and Ca^{2+} /CaM kinase kinase (CaMKK) belong to this kinase cascade. CaMKK phosphorylates CaMKI and CaMKIV to activate these kinases [33]. This kinase cascade may have proofreading character in that only ‘real’ strong Ca^{2+} signals can induce it. Once activated, CaMKI and CaMKIV regulate gene transcription in the nucleus and local protein synthesis, which are needed for long-lasting synaptic plasticity [22].

CaMKs and Their Role in Memories

Mouse molecular genetic manipulations have now established that the CaMKs have a fundamental function in hippocampus-dependent learning and memory [22,34]. Loss of α CaMKII and β CaMKII expression impairs contextual and spatial memory formation [26,34,35]. A point mutation that prevents the T286 autophosphorylation of α CaMKII severely impairs spatial and contextual memory formation [36,37]. In addition to having a role in memory formation, the T286 autophosphorylation has also been suggested to mediate memory storage and maintenance [20]. However, in the absence of T286 autophosphorylation, hippocampus-dependent long-term memory can still be formed after massed training [38]. Thus, CaMKII activity appears to be primarily important for the acceleration of memory formation but not for memory storage *per se* [39].

Members of the CaMK cascade are important for consolidation of hippocampus-dependent long-term memory. However, each member of this kinase cascade appears to have a specific role in consolidation rather than being important for all types of hippocampal memory consolidations. Accordingly, CaMKIV is required for consolidation of contextual, but not spatial, memory [40,41]. Further, the β -isoform of CaMKK is likely to contribute to

consolidation of spatial memory and memory of the delay version of the social transmission of food preferences task [42]. Interestingly, it is only required for long-term memory formation in males but not in females [43], thus suggesting that memory consolidation mechanisms differ between the sexes [44]. Similarly, α CaMKK is required for contextual, but not spatial, long-term memory formation in males but not in females [45,46]. Genetic studies now support that CaMKs also play an important role in human learning and memory (Box 2).

CaMKs and Their Role in Drug Memories

Distinct types of drug memories have been identified and grouped according to the system for normal memories (i.e., non-drug-related memories) in humans (Box 1). Animal models of drug use and addiction, however, depict complex behaviours, which normally involve several types of drug memories (**behavioural paradigms to test drug use and addiction behaviours**; see Glossary) [47]. Neurobiological mechanisms may, therefore, overlap between single types of drug memories [5–8].

Psychostimulants

Psychostimulants have common behavioural and subjective effects. They increase behavioural activity and arousal, and can also induce euphoria in humans. Drugs that share these effects include cocaine and amphetamine (AMPH) together with its derivatives [1]. An acute injection of cocaine [48] or AMPH [49] induces an increase in T286-phosphorylated α CaMKII levels in the striatum of rats, but has no effect on total α CaMKII levels (Figure 1). Chronic cocaine or AMPH administration and **self-administration** increases levels of α CaMKII in the nucleus accumbens (NAc) shell but not the NAc core of rats. This effect is paralleled by phosphorylation of the GluA1 AMPA receptor subunit and persists several weeks after drug administration (Table 1) [50–52]. An increase in α CaMKII activity leads to an upregulation of functional AMPA receptors [53] and receptor conductance [54]. After 7 days of withdrawal from repeated cocaine administration, but not after fewer days, levels of CaMKII and phosphorylated CaMKII (pCaMKII) are increased in the NAc, prefrontal cortex (PFC), or dorsal striatum (DS). Both return to control levels several weeks after withdrawal [55–57]. The **reinstatement of self-administration** such as cocaine seeking by a priming injection of cocaine induces an increase in pCaMKII in the NAc shell but not in the NAc core of rats [58].

Locomotor Sensitisation

Psychostimulant-enhanced Ca^{2+} influx through AMPA, NMDA, and L-type Ca^{2+} channels leads to activation of CaM in dopaminergic neurons of the ventral tegmental area (VTA). Subsequent activation of CaMKII enhances phosphorylation of various targets and promotes sensitisation of hyperlocomotor responses in a context-dependent way [59] (Table 1). CaMKII activation in the VTA, NAc, and hippocampus appears to be of crucial importance for **locomotor sensitisation** to cocaine and AMPH [60–64]. The sensitisation of the AMPH-induced locomotor activation is blunted in αCaMKII -deficient mice [65]. Transient inhibition of αCaMKII in the NAc shell reverses the increase in pGluA1 levels and attenuates AMPH-induced locomotor sensitisation [66]. αCaMKII autophosphorylation-deficient mice have preserved sensitisation of the hyperlocomotor effects of cocaine [67]. Mice that lack CaMKIV in dopamine D1 receptor-expressing neurons do not sensitise to the hyperlocomotor effects of cocaine [68].

Conditioned Place Preference

CaMKII activation in the VTA, NAc, and hippocampus appears to be of crucial importance for **conditioned place preference** (CPP) establishment after cocaine and AMPH treatment [61–64]. αCaMKII -deficient mice are not impaired in AMPH- or cocaine-induced CPP when only endpoints of learning are considered [65]. However, αCaMKII autophosphorylation-deficient mice are significantly delayed in their ability to establish cocaine-induced CPP [67]. Mice with a striatum-specific autonomously active CaMKII show potentiated cocaine CPP after low, but not after high, doses of cocaine [64]. Several studies identified the VTA–NAc axis as a crucial pathway of CaMKII action in the establishment of cocaine-induced synaptic plasticity and CPP [69,70]. Local injection of the CaMKII inhibitory peptide TatCN21 into the VTA blocks establishment of cocaine CPP and cocaine-evoked depression of excitatory synaptic transmission in the NAc shell [70].

Mice lacking CaMKIV in D1 receptor-expressing neurons show enhanced establishment and reinstatement of cocaine CPP [68]. However, reduced CaMKIV activity is also associated with enhanced social anxiety [71] and reduced fear learning [41]. These findings suggest that CaMKIV may work as a resilience factor against cocaine addiction possibly by stabilising emotional tone.

Self-Administration

The activation of CaMKII in the VTA, NAc, and hippocampus appears to be of crucial importance for self-administration of cocaine and AMPH [61–64]. Transient overexpression of α CaMKII in the NAc shell increases not only pGluA1 levels but also AMPH self-administration in rats [72]. Transient inhibition of α CaMKII in the NAc shell reduces AMPH self-administration in sensitised rats [51,66]. Cocaine effects on α CaMKII, but not β CaMKII, expression are suggested to be essential for motivation to self-administer cocaine. α CaMKII expression correlates with break points during cocaine self-administration. In turn, virus-mediated downregulation of α CaMKII expression in the NAc shell reduced break points [73]. In the NAc shell, α CaMKII binds directly to D3 receptors. This binding is Ca^{2+} -sensitive and can be enhanced by autophosphorylation of α CaMKII. Recruitment of α CaMKII to D3 receptors transiently inhibits receptor efficacy [74] (Table 1). Downstream α CaMKII action, reduced D3 receptor function at the level of the NAc was associated with enhanced impulsivity and cocaine reinforcement [10], which may facilitate the transition to compulsive psychostimulant self-administration [11]. While there is no direct evidence that CaMK-dependent drug memories override normal CaMK-dependent memories, as would be expected in the time course of addiction establishment, indirect evidence suggests at least a competition at the level of CaMK-induced morphological changes. It was shown that AMPH self-administration, which leads to dendritic arborisation and an increase in spine density in the neocortex and NAc, would prevent morphological changes induced by normal learning [75,76]. Whether CaMK-dependent drug memories are stronger than normal memories and, therefore, induce a shift in behavioural repertoire from normal to addiction-related behaviours, is currently unclear. Other factors, such as loss of impulse control over established drug-related behaviours, are under serotonergic control [12], which is, in turn, CaMK-dependent [129,132].

Reinstatement of Self-Administration

The reinstatement of cocaine seeking by D1 receptor activation in the NAc shell is mediated by L-type Ca^{2+} channels and subsequent phosphorylation of α CaMKII at T286 and of AMPA GluA1 subunits (Table 1). Blocking CaMKII in the NAc shell, but not in the basolateral amygdala (BLA), attenuates cocaine-induced reinstatement of cocaine seeking [58,77]. This suggests a strong link between dopaminergic and glutamatergic signalling in the reinstatement of cocaine seeking mediated by CaMKII [58].

Dopamine Activity

The mesocorticolimbic dopamine system plays a crucial role in the establishment of psychostimulant abuse-related behaviours [1]. Basal dopamine levels in the DS of α CaMKII-deficient mice are significantly enhanced compared with wild-type (WT) mice [65]. This is not attributable to an altered dopamine synthesis rate or enhanced dopamine transporter (DAT)-mediated dopamine uptake [65,78]. However, enhanced vesicular dopamine release is found in α CaMKII-deficient mice, which may explain the enhanced extracellular dopamine levels [65].

The AMPH-induced increase in extracellular dopamine levels is markedly reduced in striatal synaptosomes, in brain slices, and *in vivo* in α CaMKII-deficient mice. This is in line with findings showing that α CaMKII and its T286 autophosphorylation are essential requirements for AMPH-triggered substrate efflux at the DAT [65,78]. Withdrawal from repeated cocaine or AMPH treatment sensitised striatal dopamine responses to an AMPH challenge in rats. This sensitisation could be blocked with the rather unspecific CaMKII inhibitor KN-93 [79,80], suggesting that CaMKII plays a role in neurochemical sensitisation during chronic psychostimulant exposure.

The lack of α CaMKII autophosphorylation enhances basal dopamine levels in the NAc and PFC of mice. The cocaine-induced dopamine increase in both brain regions, however, is attenuated. Reduced neurotransmitter activation is associated with lower c-Fos activation, as an indicator of reduced cellular activation, after cocaine in the NAc and hippocampus [67,81]. CaMKII phosphorylates tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, and the DAT [82,83]. Since TH, DAT, CaM, and CaMKII are coexpressed in mesolimbic projections [78,84], it has been suggested that dopamine synthesis is regulated by CaMKII-controlled TH activity [85]. An increase in basal dopamine levels may, thus, limit the capacity for the drug-induced dopamine increase. The α CaMKII-mediated DAT phosphorylation is essential for an AMPH-induced dopamine increase, but not for regulation of basal dopamine efflux [78,84]. The DAT is a binding partner of α CaMKII in dopaminergic neurons. CaMKII has a strong influence on dopaminergic activity. However, there is also some feedback, in that D2, D3, and D4 receptors regulate CaMKII activity [74,86–89]. It should be noted that CaMKs also interact with other neurotransmitter systems that are pivotal for drug-related behaviours (Table 1).

Serotonin Activity

In the absence of a dopamine response, rewarding effects of psychoactive drugs can be mediated by the serotonergic system [12,83]. The lack of α CaMKII autophosphorylation enhanced basal serotonin (5-HT) levels in the NAc and PFC of mice. By contrast, the cocaine-induced 5-HT increase in both brain regions was abolished in α CaMKII^{T286A} mice [67]. Tryptophan hydroxylase 2 (TPH2) is the rate-limiting enzyme in the biosynthesis of 5-HT in the brain. Its activity is under the control of CaMKII [126]. A lack of α CaMKII autophosphorylation may limit the capacity of transmitter available for release into the extracellular space upon excitation. This can lead to reduced 5-HT activation, which may account for the delay in the establishment of the rewarding effects of cocaine [12,67]. α CaMKII binds to serotonin transporters (SERTs) in neurons, but with lower affinity than to the DAT. Pharmacological inhibition of CaMKII impairs the AMPH-induced SERT efflux, but not substrate uptake [127]. While CaMKII has a strong influence on serotonergic activity, there is also a feedback regulation of CaMKII activity by 5-HT_{1A} receptors [128,129].

Opioids

Activation of opioid receptors induces a Ca²⁺ transient that also activates CaMKs [90] (Figure 1). μ -Opioid receptors, which are colocalised with CaMKII in neurons [91], are also targets for CaMKII [92] (Table 1). The CaMKII-mediated phosphorylation of μ -opioid receptors contributes to functional desensitisation after activation [93]. An acute morphine application enhances autophosphorylation and activity of α CaMKII in the striatum, hippocampus, and PFC of rats [94–97], without affecting α CaMKII gene expression [98]. Chronic morphine administration during CPP learning increases CaMKII and pCaMKII in the ventral striatum and dorsal striatum, as well as in the hippocampus of rats [97,99]. This is paralleled by an increase in α CaMKII mRNA expression [98]. Chronic morphine treatment increases expression of CaMKIV in the CA3 region of the hippocampus of mice, while it decreases it in the DS and other brain areas [100]. Withdrawal from repeated morphine and heroin administration or naloxone precipitated withdrawal increases CaMKII activity and mRNA levels in the hippocampus and medial PFC (mPFC) of rats [94,98,101,102]. Heroin withdrawal has no effects on β CaMKII or CaMKIV levels in the mPFC [102], while CaMKII activity returned to control levels within a week of withdrawal [16]. The reinstatement of morphine self-administration by a priming injection of morphine induces an increase in T286 p- α CaMKII, but not p- β CaMKII, levels in the NAc shell of rats compared with levels during extinction [103].

Conditioned Place Preference

The establishment of morphine-induced CPP depends on CaMKII in the brain [99]. Pharmacological inhibition of CaMKII in the hippocampus and amygdala blocks the establishment and reinstatement of morphine-induced CPP [104]. Pharmacological inhibition of CaMKII in the mPFC, but not in the BLA, blocks the establishment of morphine- and heroin-induced CPP in naive rats. By contrast, in chronically treated and withdrawn rats, mPFC blockade of CaMKII has no effect on CPP establishment. Intra-BLA pharmacological blockade of CaMKII, however, blocks morphine and heroin CPP establishment [102,105]. This effect is most evident in the late phase of CPP consolidation 12 h postconditioning [106]. CaMKIV is required for the establishment of morphine-induced CPP in mice, since CaMKIV knockout (KO) mice show a significantly reduced CPP for morphine [107].

Self-Administration

Inhibition of CaMKII activity in the NAc shell reduces the reinstatement of morphine seeking in rats [70,103]. This effect is potentially mediated by a decrease in T286 α CaMKII phosphorylation in the NAc [70].

Withdrawal and Abstinence

Withdrawal from chronic morphine administration induces withdrawal symptoms. The development of withdrawal symptoms is blocked by intrahippocampal injection of the unspecific CaMKII inhibitor KN-62 [92]. After 7 days of morphine treatment, naloxone precipitates withdrawal symptoms, such as wet dog shakes, paw tremors, or jumping. There is no difference in these withdrawal symptoms between CaMKIV KO and WT mice, which suggests that CaMKIV does not play a role in morphine withdrawal behaviours [107].

Alcohol

Acute alcohol application does not affect CaMKII activity in astrocytes [108]. By contrast, repeated alcohol administration enhances CaMKII activity in neurons and astrocytes (Figure 1) [108,109] and increases the phosphorylation rate [110]. Free choice alcohol consumption increased protein levels of α CaMKII, but not β CaMKII, in the amygdala and NAc of mice [111]. Operant alcohol self-administration increases p- α CaMKII levels in the amygdala without changing total α CaMKII levels [111].

Conditioned Place Preference

CPP measures the learning and expression of an association between the incentive properties of the drug with environmental cues. Altered CPP may, thus, result from changes in learning and memory and/or changes in the rewarding value of a drug [47]. α CaMKII autophosphorylation plays a special role in the speed at which alcohol induces CPP in mice. Preference for an alcohol-paired environment was established after seven conditioning trials in WT mice. Surprisingly, α CaMKII autophosphorylation-deficient mice (α CaMKII^{T286A}) establish a profound alcohol CPP after only a single alcohol conditioning trial [112]. The disparity between the reported establishment of alcohol consumption [113] and CPP [112] may be explained by the acute effects of alcohol in the CPP paradigm. CPP involves several learning processes and is not simply a result of incentive-driven behaviour [47]. α CaMKII^{T286A} mice display enhanced activity and hyperarousal in response to potentially threatening environments, while showing no altered behaviour in well-habituated environments [114]. Arguably, one key component to compulsive drug seeking is the alleviation of a negative affective state resulting in negative reinforcement [115]. Alcohol drinking behaviour was measured in a familiar home cage environment while CPP rewarding effects were measured in a less familiar environment, which might together with the injection process represent an aversive/threatening stimulus. This view is supported by the observed hyperactivity in α CaMKII^{T286A} mice during CPP baseline and novel open field exposure, but not in familiar home cages [114]. In contrast to WT mice, alcohol has an acute and persistent sedating effect in α CaMKII^{T286A} mice. One possible explanation of this effect is that alcohol alleviated the threat-induced behavioural responses in α CaMKII^{T286A} mice in a potentially aversive new test situation, thus driving CPP learning [47].

Self-Administration

CaMKII activity and autophosphorylation in the amygdala is required for operant alcohol self-administration as local pharmacological inhibition of both decreased alcohol-reinforced responding [111]. α CaMKII^{T286A} mice showed initially reduced alcohol consumption in a two-bottle free-choice procedure. This effect is persistent until animals are withdrawn and reinstated twice to alcohol [113]. These findings suggest that α CaMKII autophosphorylation controls the speed at which alcohol consumption is established, in a similar way that it may control the establishment of non-drug-related learning [28].

Dopamine Activity

There is a significantly reduced dopamine response to alcohol in the NAc of α CaMKII^{T286A} mice [113]. An analysis of the cellular activation of the VTA, as an origin of the mesolimbic dopamine projections, revealed an enhanced activation after acute and subchronic alcohol exposure in the rostral, but not caudal, VTA in α CaMKII^{T286A} mice, which appears to be driven predominantly by GABAergic neurons [113]. Also, cellular activation after alcohol in the hippocampus is reduced in α CaMKII^{T286A} mice [81].

Serotonin Activity

The lack of α CaMKII autophosphorylation provoked an alcohol-induced 5-HT increase in the PFC that was not observed in WT animals [113]. High alcohol consumption has been associated with deficiencies in brain 5-HT levels and turnover [130]. An induced 5-HT increase, in turn, can reduce alcohol consumption [131]. The alcohol-induced 5-HT increases in the PFC of α CaMKII^{T286A} mice may, therefore, contribute to the reduced alcohol consumption observed in these mice. There is a strong link between CaMKII and serotonergic function in the brain. CaMKII is required for the phosphorylation and activation of TPH, the rate-limiting enzyme in the biosynthesis of 5-HT [126]. Pharmacological inhibition of CaMKII has been shown to increase the firing rate of 5-HT neurons [132]. This supports the view that a reduced CaMKII function may increase 5-HT neuronal firing and terminal 5-HT release, which then inhibits alcohol consumption. These data may suggest that α CaMKII autophosphorylation controls the speed at which an alcohol preference is established, but not the capacity to consume alcohol, at least in part, by a serotonergic mechanism.

Nicotine

Acute subcutaneous administration of nicotine increases activity of CaMKII in the VTA, NAc, and amygdala in mice. Nicotine acute effects on CaMKII activity mainly depend on β 2 acetylcholine receptor subunits, but less on α 7 subunits [116]. It was also suggested that the nicotine-induced β 2-mediated phosphorylation of cAMP response element-binding protein (CREB), which is required for the rewarding properties of nicotine [117,118], is mediated by CaMKII activation in the VTA and NAc [116]. CaMKII appears to play a role in nicotine withdrawal behaviours. Pharmacological inhibition of CaMKII reduced somatic nicotine withdrawal symptoms, but enhanced anxiety [119].

Concluding Remarks

CaMKs are abundant proteins in the brain. In neurons they are a crucial transducer of Ca^{2+} transients into the activation of a plethora of functional targets involved in acute molecular and long-term plastic changes at synapses that are the basis for learning and memory. Accumulating research has now provided evidence for the view that the same CaMKs also serve acute and long-term molecular plasticity that directly supports the various types of drug memories. Causal sequences are now emerging: many (if not all) addictive drugs increase CaMK activity, foremost that of αCaMKII , by either enhanced expression, enhanced phosphorylation, or both. This increase lasts during periods of drug intake and is a necessary prerequisite for the learning and consolidation of numerous drug-related behaviours. During withdrawal, CaMK activity usually declines with some delay, to rise again during reinstatement of drug seeking and consumption. Also, this behaviour is facilitated by a boost of CaMK activity. Various studies have now identified binding partners and phosphorylation targets of CaMKs. These not only comprise glutamatergic receptors. Important evidence has now demonstrated an extensive control of dopaminergic and serotonergic activity in the mesolimbic system, which is a crucial mediator of drug reward. CaMK activation of these targets may, thus, provide a large number of parallel functional pathways by which a drug-induced Ca^{2+} transient may control drug memory establishment and consolidation and, eventually, pave the way to drug addiction. This would suggest that CaMKs are excellent pharmacological targets to prevent the establishment of drug memories. However, there are obstacles that currently limit this possibility. CaMK-dependent drug memories compete with normal memories and use the same downstream pathways. Currently, there is no CaMK that is exclusively activated by drugs and not during normal memory formation. One way to circumvent this problem could be the tailoring of CaMK-based pharmacotherapies to settings and time points where little normal learning is required and where drug memories can be specifically activated and pharmacologically attenuated (see Outstanding Questions). Another future perspective would be the development of subtype-specific ligands that can target major CaMKs more selectively and spare others that are primarily involved in other behaviours [120].

Outstanding Questions

Most research revealed that α CaMKII plays an important facilitating role in drug memory establishment. CaMKIV rather appears a resilience mechanism. What is the role of other CaMKs, such as CaMKI and CaMKK, in drug memories?

α CaMKII is a crucial mediator of LTP, which has been shown to be an important mechanism in normal learning. Does α CaMKII also drive drug-induced LTP and is this LTP a necessary condition for distinct types of drug memories to be established?

There is a dissociation in CaMKII activation between brain areas during different stages of the drug-taking time line. Most evidence suggests that the VTA and NAc shell act as a crucial anatomical locus for drug memories. However, is there a functional impact of drug-induced CaMK activity changes in other brain areas, such as the hippocampus, amygdala, or PFC?

Currently available CaMKII inhibitors are not specific for CaMKII. They also block other CaMKs and other molecules. However, many of the mechanistic findings rely on these inhibitors. Can findings be confirmed with more specific inhibitors?

Research has focused mostly on cocaine, AMPH, morphine, heroin, and alcohol. Do emerging principles of, for example, α CaMKII action also apply for other widely abused drugs, such as cannabis, γ -hydroxybutyrate, methamphetamine, or hallucinogenic drugs?

The strong overlap in the function of CaMKs between normal learning and memory and drug memories makes it difficult to use them as plain pharmacological targets. However, would there be time windows, for example, in therapeutic settings, when selective inhibition/stimulation of CaMKs could be beneficial for addiction therapy?

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Box 1. Drug Memories and Related Preclinical Tests

Parallel to non-drug-related memory systems [121], two major drug memory categories can be distinguished: a declarative drug memory and a non-declarative drug memory [5–8]. The declarative drug memory should comprise a semantic memory for drug facts and a memory for drug episodes. The semantic memory for drugs contains all impersonal facts, rules, and concepts involving drugs, for example, their names, where they come from, recommended doses, what others report about its effects, and the social rules of their consumption. The episodic drug memory comprises the memories of personally experienced episodes with the drug. It is an autobiographical memory on the ‘what’, ‘where’, and ‘when’ of the personal drug encounters. This may include memories of subjectively experienced acute drug effects, for example, the mental states the drug induced [9].

The non-declarative drug memory contains engrams of the classically conditioned drug memory, instrumentally conditioned drug memory, habit memory, procedural drug memories, and drug priming memories [8]. Classically conditioned drug memories may contain all drug effects that refer to the process of Pavlovian conditioning. These may include, for example, the sensitisation of the acute locomotor effects, drug tolerance, partially conditioned place preference, conditioned locomotor activity, conditioned emotional and physiological responses, and conditioned withdrawal effects. Instrumentally conditioned drug memories comprise engrams established by instrumental conditioning. Major behaviours induced by these engrams are drug-seeking behaviours and drug self-administration. These memories also include drug cues that can serve as secondary reinforcers, as in CPP [47], or that can reinstate drug-seeking and drug self-administration behaviour. Drug habit memories refer to instrumental behaviour that is no longer goal directed, but stimulus controlled and independent from its behavioural consequences. This type of memory plays an important role in the transition from controlled to compulsive drug use and addiction. Procedural drug memories comprise all memories for skills involved in handling a drug. This may range from its production (e.g., cooking up heroin) to the method of self-administration (e.g., snorting cocaine). Drug priming memories refer to those engrams whose activation by a small amount of the drug, which would not induce major subjective and behavioural effects in drug naive individuals, can induce drug-related behaviours (e.g., reinstate drug seeking, CPP, or self-administration) and subjective effects in experienced users.

Box 2. CaMKs Shaping Human Memories and Drug Memories

Genetic association studies provide evidence for the association between CaMKs and memory performance in humans. Easton *et al.* [122] provided the first evidence of α CaMKII involvement in human memory functions by demonstrating an association between CAMK2A gene single nucleotide polymorphisms (SNPs) and spatial as well as non-spatial working memory in two populations of young healthy subjects. In line with the association findings in healthy humans, two other CAMK2A SNPs have recently been associated with the risk of Alzheimer's disease, although in relatively small samples [123].

In a translational effort, postmortem evidence and genetic associations of CaMK gene polymorphisms with various drug use and addiction-related behaviours have been found. Increased NAc levels of CaMKII were reported for cocaine-dependent humans compared with controls in a postmortem study [52]. Recently, an association between the CAMK2A SNP, rs3776823, and the slope time by which severe cocaine consumption was established was found in two independent samples of Brazilian and Swiss cocaine users [67]. These findings suggest that polymorphisms in the CAMK2A gene may contribute to the speed of acquiring a severe level of cocaine intake once consumption has commenced. A genetic study with 670 cocaine-dependent individuals and 726 controls from São Paulo, Brazil revealed a significant association of cocaine abuse with the rs919334 SNP in the CaMKIV gene promoter region [68].

In agreement with animal data, several associations between SNPs in the human CAMK2A gene and alcohol use and dependence were found. Seven SNPs typed from the CAMK2A gene were found to be significantly associated with alcohol dependence [113]. Notably, the rs10463293 SNP has previously been associated with working memory performance [122], while SNP rs3756577 has previously been suggested to influence the risk for Alzheimer's disease [123]. A set of 13 CAMK2A SNPs were found to predict the number of alcohol drinking days per month in a sample of adults living in the USA [124]. The SNP with the lowest *P* value (rs7711562) was also significantly associated with alcohol dependence in a study by Easton *et al.* [113]. While emerging evidence reveals that CaMKs play an important role in drug use behaviour and addiction, CaMKs are also shown to be crucially involved in other psychiatric disorders that are frequently comorbid with drug addiction [125]. These findings suggest a significant overlap between CaMK genetic mechanisms controlling normal learning and memory and those involved in drug memories in humans.

Glossary

Behavioural paradigms to test drug use and addiction behaviours: drug use subsumes numerous distinct behaviours. These behaviours can be investigated separately in animal models. The most important of them have been used to discover the role of CaMKs.

Conditioned place preference: measures the incentive properties of a drug by its conditioning to a spatial context (place in a box). Thereby, an increase in the time spent in a previously drug-paired environment indicates incentive properties of a drug. CPP learning and expression can be tested separately and involves both classical and instrumental conditioning [47].

Locomotor sensitisation: measures the progressive augmentation of hyperlocomotor effects. Sensitisation can be context-dependent involving a classical conditioning or context-independent that is assumed not to result from a learning process but from a sensitisation of neuronal circuits in the brain to the drug. The establishment and expression of sensitisation is part of psychoactive drug addiction [7,8].

Reinstatement of self-administration: measures the ability of a stimulus that was paired with the availability of the drug to reinstate self-administration behaviour after a period when the drug was not available and the behaviour extinguished. Such a stimulus can be sensory (e.g., a cue) or intrinsic (the effects of the drug itself). Reinstatement is believed to model, in particular, relapse behaviour [7,8].

Self-administration: measures if and at which work load (e.g., bar pressing) animals learn to self-administer a drug. The route of self-administration differs between drugs, for example, oral for alcohol and intravenous by an implanted catheter for other drugs. Rapid learning and a high motivation to work for the drug indicate strong reinforcing effects of the drug and, thus, a high potential for addiction. It is believed that instrumental conditioning is the major learning mechanism for operant drug self-administration [7,8].

Figure 1. α CaMKII Activity during Long-Term Repeated Exposure to Major Addictive Drugs and its Functional Consequences. (A). Psychostimulants such as cocaine or amphetamine increase activity of α CaMKII in the nucleus accumbens (Ncl. acc.)/shell of the brain after acute and chronic psychostimulant administration (red arrows indicate single drug episodes). Thereby, an increase in α CaMKII activity can be induced by enhanced phosphorylation and/or expression. The activity increase is paralleled by phosphorylation of various targets, such as α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluA1

subunits, extracellular signal-regulated kinase 1, CREB, and many more [48,49]. Interestingly, GluA1 Ser831 phosphorylation is one of the prerequisites for appetitive incentive learning [133]. Overall, this appears to facilitate the establishment and expression of numerous drug-related behaviours (↑). After cessation of drug administration, αCaMKII activity declines to basal levels. During reinstatement of drug self-administration, however, it is increased again. Importantly, the action of αCaMKII appears brain area-specific, no such response or facilitation of behaviour was found, for example, in the nucleus accumbens core. (B) Opioid drugs such as morphine or heroin increase the activity of αCaMKII in the hippocampus and nucleus accumbens (Ncl. acc.)/shell of the brain after chronic drug administration. This increase appears to facilitate the establishment of the rewarding effects of the drugs. After cessation of drug administration, αCaMKII activity remains elevated before it eventually declines to basal levels. This elevation was shown to contribute to the expression of withdrawal symptoms. During reinstatement of drug self-administration αCaMKII activity is increased again, which is required for this drug-related behaviour. Importantly, the action of αCaMKII appears brain area-specific, no such response or facilitation of behaviour was found, for example, in the nucleus accumbens core. (C) Alcohol increases the activity of αCaMKII in the amygdala and nucleus accumbens (Ncl. acc.) after chronic drug administration. Alcohol consumption also increased Ser831 phosphorylation of AMPA GluA1 subunits, which are coexpressed with αCaMKII in neurons [104]. These effects appear to facilitate the establishment of some drug-related behaviours (↑), but may also limit other behaviours (↓). αCaMKII activity during withdrawal and reinstatement and its contribution to alcohol-related behaviours at these time points is currently unclear and awaits further investigation. Abbreviations: CaMK, Ca²⁺/calmodulin-dependent protein kinase; CREB, cAMP response element-binding protein.

Table 1. Major Binding Partners and Phosphorylation Targets of αCaMKII in the Brain^a

Transmitter System	Target	Target Function	Phosphorylation Site	Effect on Function	Refs
Glutamate	GluR1	AMPA receptor subunit	Ser831	↑	[48,53,54,111,134–139]

	NR2B	NMDA receptor subunit	Ser1303	↑	[88,140,141]
Dopamine	Tyrosine hydroxylase	Dopamine synthesis		↑	[82,84,86,142–144]
	Dopamine transporter	Dopamine clearance	N terminal serines*	↑ (AMPH-induced DA efflux)	[65,78,85]
	D3 receptor	Dopamine receptor	Third intracellular loop*	↓	[74]
Serotonin	Tryptophan hydroxylase 2	Serotonin synthesis	Ser19	↑	[145,146]
	Serotonin transporter	Serotonin clearance	N terminal serines*	↑ (AMPH-induced 5-HT efflux) – (substrate uptake)	[127]
Endogenous opioids	-Opiate receptor	Opiate receptor	Ser261 and Ser266	↑ Desensitisation	[92,93,126,147–149]
GABA	GABA _A receptor	GABA receptor	β2 subunit	↑	[150]
General	L-type Ca ²⁺ channel	Ca ²⁺ channel		↑	[151]
	BK channel	High conductance Ca ²⁺ -activated Voltage-gated K ⁺ channel	Thr107	↑	[152]
				↓	[153]

	SynGAP	Ras-GTPase activating protein		↓	[154]
	FosB	Transcription factor	Ser27*	↑	[52]

^a↑, enhanced function; ↓, reduced function; –, no effect on function; *, targeted by α CaMKII; AMPH, amphetamine; DA, dopamine.