Protein kinase M ζ and the maintenance of long-term memory

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24 **Abstract**

25 Although various molecules have been found to mediate the processes of
26 memory acquisition and consolidation, the molecular mechanism to
27 maintain memory still remains elusive. In recent years, a molecular
28 pathway focusing on protein kinase M ζ (PKM ζ) has become of interest to
29 researchers because of its potential role in long-term memory
30 maintenance. PKM ζ is an isoform of protein kinase C (PKC) and has a
31 related structure that influences its function in maintaining memory.
32 Considerable evidence has been gathered on PKM ζ activity, including
33 loss of function studies using PKM ζ inhibitors, such as PKM ζ inhibitory
34 peptide (ZIP), suggesting PKM ζ plays an important role in long-term
35 memory maintenance. This review provides an overview of the role of
36 PKM ζ in long-term memory and outlines the molecular structure of
37 PKM ζ , the molecular mechanism of PKM ζ in long-term memory
38 maintenance and future directions of PKM ζ research.

39

40 **Keyword**

41 Protein kinase M ζ (PKM ζ); maintenance of long-term memory; zeta
42 inhibitory peptide (ZIP)

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Introduction

How do neurons alter their morphology and function to maintain long-term memory? How do molecular synaptic signals contribute? These questions have always been of interest to neuroscientists. In 1973, Timothy Bliss and Terje Lomo found that synaptic discharges of rabbit hippocampal cells were enhanced by electrical stimulation *in vivo* (Bliss and Lomo 1973). The mechanism behind this enhancement process was called long-term potentiation (LTP).

LTP leads to an increased responsiveness of post-synaptic cells by increasing the membrane potential above the baseline, leading to a lower number of chemical signals to generate an action potential in the post-synaptic cell. During LTP, chemical synaptic activity produces a long-lasting increase in signal transmission between two neurons and allows for an activation loop with increased efficiency in post-synaptic neurons to be associated with memory. The LTP mechanism is widely considered one of the major cellular mechanisms that underlies learning and memory and a crucial entry point for the studying of long-term memory (Frey and Morris 1997, Morris 2003).

There are two types of glutamate receptors that participate in the induction of LTP: N-methyl-D-aspartate (NMDA) receptors and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors. Regarding NMDA receptors, glutamate is released from the presynaptic

membrane, binds to the receptor and Mg^{2+} is removed from the channel of the receptor. Then extracellular Na^+ and Ca^{2+} flow through the postsynaptic membrane via the NMDA receptor channel. The Ca^{2+} increase in the postsynaptic neuron is the critical trigger for LTP (Malinow et al. 1989, Mayford et al. 1995). Regarding AMPA receptors, glutamate acts on the receptors causing Na^+ channels to open and Na^+ to flow into the post-synaptic neuron inducing the depolarization of the postsynaptic membrane (Lisman et al. 2003, Malenka and Nicoll 1999).

In addition, there are a number of kinases, including phosphoinositide-3-kinase (PI3-Kinase), α -calcium-calmodulin-dependent protein kinase (CaMK II), and protein kinase A (PKA), that also play roles in triggering LTP (Lisman et al. 2012, Malinow et al. 1989, Mayford et al. 1995, Thomas et al. 1994).

LTP is sustained for a few seconds to several days *in vivo*. In comparison with long-term memory, which lasts for weeks, months or years, it can be asserted that transcription and protein synthesis are required in the late stages of LTP in order to maintain a long-term memory.

Protein kinase C (PKC) represents an important family of genetic signalling modulators that have been identified as a potential therapeutic target for psychiatric disorders, cognitive dysfunction and cancer. As such, PKC activators have been considered candidates for

cognitive-enhancement and anti-dementia therapeutics (Mochly-Rosen et al. 2012, Sun and Alkon 2010). Increases of PKC activity have been shown to increase postsynaptic transport and PKC inhibition have been shown to inhibit LTP, suggesting that PKC signalling might modulate long-term activity in neurons and synapses (Sacktor 2012).

The PKC family can be divided into three subclasses according to the signal sensitivity of the protein: (1) conventional PKCs, which are sensitive to Ca^{2+} and diacylglycerol (DAG); (2) non-conventional or novel PKCs, which are only activated by DAG; and (3) atypical PKCs, which are insensitive to both Ca^{2+} and DAG.

The structure of full-length PKCs is comprised of three domains: (1) an N-terminal regulatory domain; (2) a C-terminal catalytic domain; and (3) a hinge region tethering the N- and C-terminal domains. The N-terminal regulatory domain has a sequence that acts as a pseudosubstrate and binds to a region on the C-terminal catalytic domain in order to maintain PKC in an inactive state. When second messengers (such as Ca^{2+} and/or DAG) bind the active site on the regulatory N-terminal domain, the molecular structure of PKCs is thought to undergo a conformational change such that the N-terminal regulatory domain separates from the C-terminal catalytic domain in order to activate PKCs by unblocking the kinase catalytic sites (Sacktor 2012).

Protein kinase M ζ (PKM ζ) is an autonomously active atypical PKC ζ

isoform that has become of interest to researchers because of its role in the maintenance of long-term memory (Migues et al. 2010, Oster et al. 2004, Pastalkova et al. 2006, Sacktor 2011, Shema et al. 2007, Yao et al. 2008). Structurally, PKM ζ lacks the N-terminal regulatory domain and contains only part of the hinge region and the C-terminal catalytic domain of most PKCs (Hernandez et al. 2003). As such, PKM ζ has a persistent activity independent of second messenger signalling due to the absence of the N-terminal regulatory domain needed to block the catalytic sites of the kinase. Interestingly, it has been found that the persistent activity of PKM ζ affects synapses further maintaining long-term memory (Sacktor 2012), suggesting that it might be a molecular basis of memory maintenance.

Early studies suggested that PKM ζ activity was dependent on the proteolytic activity of calpain, a Ca²⁺ dependent protease active during LTP, to fragment PKC ζ in the hinge region (Kishimoto et al. 1983). However, future studies reported that there was no distribution of PKC in the mouse hippocampus (Oster et al. 2004), despite there being a large amount of PKM ζ synthesis reported during LTP (Li L. et al. 2014). Moreover, Ca²⁺ flow into the postsynaptic membrane is transient, suggesting that postsynaptic Ca²⁺ dependent activity would be limited for a long time during LTP and would limit the potential of calpain to cleave PKC ζ into PKM ζ .

It has since been reported that PKM ζ is synthesized via the translation of PKM ζ mRNA, where the transcriptional promoter of PKM ζ mRNA is located within the PKC ζ gene (Hernandez et al. 2003). The PKC ζ gene contains 18 exons: exon 1 encodes the 5'-UTR and a part of N-terminal domain of PKC ζ ; exons 2–4 encode the N-terminal regulatory domain of PKC ζ ; exons 6–8 encode the hinge region; exons 9–16 encode the C-terminal catalytic domain of PKC ζ ; and exons 17–18 encode the 3'-UTR. Exon'1, located on a large intron between exon 4 and exon 5 of the PKC ζ gene sequence, contains the PKM ζ promoter and encodes the 5'-UTR of PKM ζ . Transcription initiated from exon'1 to exon 18 generates PKM ζ mRNA (Hernandez et al. 2003).

The PKM ζ mRNA is then transported and specifically localised to the dendritic area, instead of being directly translated within the cell body (Sacktor 2011). In a study of rat hippocampal slices, researchers found that large-scale PKM ζ synthesis occurred 10–40 minutes after receiving electrical stimulation (Ling et al. 2002). This involved a large number of PKM ζ being synthesised in a short window of time, suggesting that the PKM ζ was translated from mRNA in the dendritic area. Two kinds of cis-acting dendrite targeting elements (DTE) are found on the PKM ζ mRNA: M zeta DTE1 and M zeta DTE2. Both play crucial roles in locating PKM ζ mRNA (Muslimov et al. 2004). M zeta DTE1 resides in a 499-nt segment spanning the 5'-UTR and ORF of PKM ζ . It is important

to transport PKM ζ mRNA from the soma to the dendrite area. M zeta DTE2 resides in a 42-nt segment of the 3'-UTR and is required for the distal dendrite localization of PKM ζ mRNA. Dendritic BC1 RNA can partially match M zeta DTE1, which delivers the PKM ζ mRNA to the site of protein synthesis, depending on the sequential participation of microtubules and actin filaments.

PKM ζ is mainly distributed in the prefrontal cortex, hippocampus and cerebellum. Its expression has been found in pyramidal cells in CA1 and CA3 of the hippocampal region, granular cells of the dentate gyrus, pyramidal cells in the neocortex and Purkinje cells in the cerebellar region (Hernandez et al. 2014, Naik et al. 2000, Oster et al. 2004, Yoshihama et al. 2009). PKM ζ is distributed widely in the soma and dendrites, but no expression of PKM ζ has been found in axons of the hippocampus. The expression of PKM ζ is largest in the postsynaptic region and dendrites (Hernandez et al. 2014) and has been observed in dot-like structures/clusters in the dendrites. PKM ζ is highly expressed in the endoplasmic reticulum as well, but is barely visible in the Golgi and mitochondria. In the nuclei, the expression of PKM ζ varies (Hernandez et al. 2014). This distribution of PKM ζ in neurons supports the possibility that PKM ζ plays a role in LTP and long-term memory.

PKM ζ and long-term memory

177 A study in rats has shown a high expression level of PKM ζ in the
178 prefrontal cortex and hippocampus after the animals were trained in a
179 delayed nonmatch-to-sample (DNMS) task (Wang D. C. et al. 2014). A
180 similar result was found in rats trained in a radial arm maze task (Ling et
181 al. 2002). Morrison and his colleagues found that higher proportions of
182 dendritic spines co-expressed GluR2 and PKM ζ in the hippocampal
183 dentate gyrus of monkeys considered to be fast learners in a DNMS task.
184 In contrast, aged monkeys expressed a lower density of synaptic GluR2,
185 as judged by immunogold labelling. This lower density correlated with a
186 lower recognition accuracy in aged monkeys (Hara et al. 2012) indicating
187 a role of PKMzeta in long term memory and/or LTP.

188 PKM ζ inhibitors have often been used to inhibit PKM ζ activity *in*
189 *vitro* in order to biochemically disrupt LTP. PKM ζ has proved to be
190 essential for various forms of memory maintenance because PKM ζ
191 inhibitors have erased long-term memory (Parsons and Davis 2011, von
192 Kraus et al. 2010). One of the PKM ζ inhibitors commonly used is Zeta
193 Inhibitory Peptide (ZIP) (Pastalkova et al. 2006). ZIP contains a 13 amino
194 acid sequence from the regulatory domain of the full-length PKC ζ . ZIP
195 has been found to penetrate into neurons and to specifically interact with
196 the catalytic domain of PKM ζ . A high-dose of scrambled ZIP was found
197 to have a similar effect as ZIP. Scrambled ZIP was also used to reduce the
198 effect of ZIP efficiency by three- to four-fold (Yao et al. 2013).

Chelerythrine, another widely utilised inhibitor of PKM ζ , has also been found to specifically interact with the catalytic domain of PKM ζ , thereby inhibiting PKM ζ activity (Yao et al. 2013). By using PKM ζ inhibitors, researchers have found evidence that PKM ζ plays an important role in maintaining various kinds of long-term memories (von Kraus et al. 2010).

Injecting ZIP into the dorsal hippocampus of rats was found to effectively erase one day and six days of long-term memory of an object's location without altering the object's identity recognition memory (Hardt et al. 2010). This suggests that PKM ζ is closely related to reference memory and is likely a key protein in the molecular mechanisms of storing long-term memory, but has no significant effect on working memory (Unpublished data). The repeated finding that PKM ζ is over-expressed within the hippocampus of animals that are trained in memory tasks suggests that PKM ζ plays a role in various memory processes.

PKM ζ related experiments have also taken aim at the amygdala and the insular cortex. Injection of ZIP into basolateral amygdala (BLA) to suppress the activity of PKM ζ the day after establishing auditory and contextual fear conditioning paradigms in rats was found to impair the maintenance of these two types of fear-conditioned memories (Serrano et al. 2008). Because rats have a habit of avoiding light, giving rats a foot shock in a dark room and keeping them safe in a bright room will lead to

the rats establishing an inhibitory avoidance of the dark and a preference to stay in the bright room. 22 hours after training, rats that received an injection of ZIP into the BLA displayed impaired memory with respect to the long-term inhibitory avoidance of the dark by restoring the habit of avoiding the light (Serrano et al. 2008). This suggests that the long-term emotional memory was lost after PKM ζ was inactivated in the amygdala. Moreover, rats injected with ZIP in the insular cortex at four separate time points – three days, seven days, 25 days and 30 days - after establishing a conditioned taste aversion (CTA) were found to have the CTA eliminated (Shema et al. 2007).

The sensorimotor cortex is involved in procedural learning. Francis and his colleagues trained rats in a “pronation-grasp-supination” task to obtain food pellets on a reaching platform. When rats were able to feed in the device and reached the criterion of the task, ZIP was injected into the sensorimotor cortex. Behavioural tests found that the rats lost the ability to correctly perform the reaching task after being injected with ZIP (von Kraus et al. 2010). This demonstrated that maintaining sensorimotor memory was dependent on the persistent activity of PKM ζ .

Extinction is a special form of learning that can interfere with conditioned responses (Quirk and Mueller 2008). Previous studies have shown that the infralimbic cortex is the key area related to extinction learning. After establishing morphine conditioned place preference (CPP)

and conditioned place aversion (CPA), rats underwent CPP and CPA extinction processes. The rats were injected with ZIP in their infralimbic cortex after they showed extinction in the CPP and CPA. Interestingly, the rats regained the established CPP and CPA (He et al. 2011). This result revealed that, similar to other forms of memory, PKM ζ was required to maintain the extinction memory of CPP and CPA in the infralimbic cortex.

Drug addiction is a serious social problem that has been an interest to memory researchers because of the parallels between learning and memory paradigms and drug dependency and seeking behaviours. There has been a consensus formed that drug addiction is dependent on specific brain regions, including the nucleus accumbens, amygdala and insular cortex. The nucleus accumbens (NAc) is widely considered an important area linked to drug addiction. Injecting ZIP into the nucleus accumbens shell was found to abolish cocaine-induced CPP and ZIP injections into the NAc core resulted in an earlier onset of CPP extinction (Shabashov et al. 2012). Similarly, impaired expression of morphine CPP, cocaine CPP and high-fat food CPP was found after an injection of ZIP into the rat nucleus accumbens core. It was concluded that the persistent effect of PKM ζ activity was mediated through GluR2-containing AMPA receptors (Li Y. Q. et al. 2011). These results showed that PKM ζ might play an important role in drug associated memory.

A single application of ZIP blocked the development of cocaine sensitization and sustained ZIP inhibition produced an almost complete blockade of cocaine sensitization in mice (Howell et al. 2014). This suggested a role of PKM ζ and PKC λ/ι in addiction. Another study showed that methamphetamine (MA) treated mice had poor working memory after 5 weeks of MA abstinence. PKM ζ and GluR2 were both found to be significantly reduced in the hippocampus after MA administration. This suggested the poor working memory performance was related to reduced PKM ζ activity. The study also showed that PKM ζ may have a potential role in modulating dopamine and glutamate receptors after MA treatment (Braren et al. 2014). ZIP microinfusions into the ventral tegmental area (VTA) after cocaine sensitization, but before the withdrawal period, decreased locomotor activity on a subsequent cocaine challenge. In contrast, ZIP microinfusions into the nucleus accumbens core after the withdrawal period disrupted the expression of locomotor sensitization (Velez-Hernandez et al. 2013). In conclusion, PKM ζ may have a time-dependent function during addiction that is modulated by the AMPA receptor.

How PKM ζ maintains long-term memory

Researchers have found using confocal microscopy that PKM ζ overexpression changes the protrusion length and morphology of

dendritic spines in cultured neurons (Ron et al. 2012). The length of the spine stem has been considered closely related to the stability of the input and output of information. Protrusions are roughly divided into five different types according to their shape and size: (1) stubby, (2) mushroom, (3) short, (4) long and (5) filopodia. The morphology criteria determines whether the dendritic spine is mature and the presence of stubby and mushroom type protrusions indicate that spines stably support memory storage. Overexpression of PKM ζ significantly reduced the average length of spines and increased the number of stubby type spines (Holtmaat et al. 2005), which potentially provides evidence of PKM ζ maintaining LTP via morphology changes of the dendritic spine.

Molecular changes in postsynaptic density (PSD) have also been found to contribute to LTP and long-term memory (Shao et al. 2012). PSD-95, the major scaffolding protein in PSD, can anchor postsynaptic signal molecules and receptors in postsynaptic membranes (Ehrlich and Malinow 2004). The size of PSD-95 clusters significantly increased in primary cultures of rat hippocampal neurons overexpressing PKM ζ . This PKM ζ -induced increase of PSD-95 clusters was reversed after injection of ZIP or chelerythrine. However, neither ZIP nor chelerythrine affected the basal size of the PSD-95 cluster and the level of PSD-95 expression (Shao et al. 2012) suggesting that regulation of PSD-95 by PKM ζ might occur during post-translational modifications rather than during the

synthesis of PSD-95. Recently, a hypothesis that PKM ζ promotes the transport of PSD-95 to the post-synaptic membrane and increases the size of PSD-95 clusters that provide more insertion sites for many kinds of postsynaptic signal molecules and receptors, including AMPA receptors, has been developed (Ehrlich and Malinow 2004). This increase in PSD is necessary to maintain LTP and long-term memory.

There is also a considerable amount of evidence indicating that PKM ζ maintained long-term memory by regulating the trafficking of GluR2-dependent AMPA receptors (Braren et al. 2014, Hara et al. 2012, Li Y. Q. et al. 2011, Miguez et al. 2010, Santerre et al. 2014). PKM ζ increased the level of GluR2-dependent AMPA receptors at the postsynaptic membrane in order to maintain LTP and long-term memory. ZIP-mediated inactivation of PKM ζ triggered the loss of GluR2 from GluR2-dependent AMPA receptors (Miguez et al. 2010).

There are two ways to transport AMPA receptors to postsynaptic sites – one way is via the vesicle exocytosis pathway (Luscher et al. 1999) and the other is through the interaction between N-ethylmaleimide-sensitive factor (NSF) and GluR2-dependent AMPA receptors (Song et al. 1998). It has been found that PKM ζ inhibition led to a decline in NSF expression levels and that NSF/GluR2-dependent AMPA receptors trafficking was depressed, while no significant reversal of the vesicle exocytosis pathway was found under PKM ζ inhibition (Yao

et al. 2008). Interactions between AMPA receptors and proteins interacting with PKC1 (PICK1) keep AMPA receptors in the postsynaptic AMPA receptor pool. It is possible that PKM ζ activity is to signal NSF to disrupt the binding between GluR2-dependent AMPA receptors and PICK1.

PKM ζ has autonomous activity because of its molecular structure. Almost all AGC proteins (protein kinase A/PKG/PKC superfamily members) have maximised activity when related sites on their catalytic domains are phosphorylated. PKM ζ also has this characteristic. The enzyme involved in the positive feedback loop to maximize PKM ζ activity is phosphoinositide-dependent protein kinase-1 (PDK1). Increasing the synthesis of PKM ζ and PDK1 is irreplaceable to the PKM ζ dependent maintenance phase of LTP (Sacktor 2012).

CaMK II, PI3-Kinase, PKA and PKM ζ itself have been found to regulate the synthesis of PKM ζ (Kelly et al. 2007). However, these proteins have yet to be found to influence the phosphorylation level of PKM ζ (i.e. the ratio of phospho-PKM ζ to PKM ζ). This means that the synthesis of PKM ζ is the rate-limiting step after LTP is triggered.

Summarizing the above, researchers have obtained a possible molecular pathway for PKM ζ in the maintenance of long-term memory (Sacktor 2011). After synthesis, PKM ζ is phosphorylated by PDK1 to maximize activity of PKM ζ . Then PKM ζ binds to NSF and GluR2 to

signal GluR2-dependent AMPA receptors away from PCK1 and promote the trafficking of GluR2-dependent AMPA receptors to the postsynaptic membrane. Once PKM ζ is synthesised and phosphorylated by PDK1, the positive feedback loop is triggered rapidly – phosphorylation of PKM ζ increases PKM ζ synthesis resulting in a maximal activity state and AMPA receptors are persistently inserted in appropriate sites of the postsynaptic membrane. Because of these processes, it is hypothesized that LTP is sustained in the neuron and long-term memory is maintained at the behavioural level.

Controversies regarding PKM ζ

A large number of studies have confirmed that PKM ζ plays an important role in the maintenance of many types of long-term memories in different brain regions. However, some recent studies have questioned this hypothesis. These studies suggest, at a minimum, that mechanisms of memory storage and maintenance may not simply be under PKM ζ mechanistic control.

It is widely considered that the hippocampus is the key area related to contextual fear association memory. A study has found that injecting ZIP into the hippocampus failed to affect contextual fear association memory (Serrano et al. 2008). One explanation of this finding is that the role of the hippocampus performs memory encoding instead of memory

storage or maintenance. This explains why inhibiting PKM ζ by ZIP in the hippocampus does not affect contextual fear association memory.

Another conflicting explanation is that PKM ζ does not participate in the process of memory maintenance (Kwapis and Helmstetter 2014). This interpretation has been confirmed in some experiments. Injection of ZIP into the hippocampus was not found to erase spatial reference memory in a water maze task (Serrano et al. 2008). Injection of ZIP in the insular cortex was found to inhibit olfactory fear memory when tested two hours, one day, and two days after ZIP infusion. However, this memory was intact when tested 10 days or 15 days after ZIP injection (Parsons and Davis 2011). This suggests that a window of time for ZIP-induced memory impairment may exist. It is possible that the memory might be lost when it was retrieved because PKM ζ was inhibited. Therefore, PKM ζ may be involved in the process of memory reestablishment instead of the process of memory maintenance.

Most studies that utilized PKM ζ inhibitors, such as ZIP and chelerythrine, have provided supportive evidence for a role of PKM ζ in maintaining long-term memory. Investigators have made Prkcz null mice that lost both PKM ζ and PKC ζ activity by knocking out exon 9 of the Prkcz gene (Lee et al. 2013). These Prkcz null mice showed normal learning and memory behaviours in tests for fear conditioning cues, novel object location recognition and cocaine CPP paradigms, which is

consistent with the behaviour of wild-type mice. Interestingly, injection of ZIP into the nucleus accumbens of the *Prkcz* null mice destroyed an established cocaine CPP (Lee et al. 2013). Furthermore, Lee *et al.* found that the *Prkcz* null mice consumed more ethanol than their wild-type littermates in 24-hour and 4-hour intermittent access procedures (Lee et al. 2014). These results indicated that lack of PKM ζ failed to impair learning and memory in *Prkcz* null mice and that ethanol-induced PKM ζ might act in a negative feedback loop to limit binge-like ethanol consumption. ZIP still had the effect of limiting reward memory after deletion of *Prkcz* (Lee et al. 2013, Volk et al. 2013). The researchers assume that there may be another mechanism to maintain long-term memory in the case of PKM ζ absence.

PKC λ/ι is a kind of atypical PKC that has a distribution in brain areas similar to the distribution of PKM ζ . Studies have found that high dose treatments of ZIP inhibited the activity of PKC λ/ι (Oster et al. 2004). Therefore, it is possible that PKC λ/ι supplements the process of maintaining memory when PKM ζ is absent. PKC λ/ι is a member of PKC superfamily that has a full-length peptide backbone with an intact regulatory domain, unlike the truncated PKM ζ , which is its natural pseudosubstrate. This means that PKC λ/ι may not have persistent activity to maintain LTP. During LTP, no significant increase in the expression level of PKC λ/ι has been reported. While this does not absolutely rule out

the possibility that PKC λ/ι plays a role in long-term memory, research is needed to ascertain the role of PKC λ/ι in memory maintenance.

In addition, limited research into the effects of PKM ζ on memory has been done in non-human primates, which are likely to be better models due to its relative homology to human. It is therefore worthwhile to test the role of PKM ζ in long-term memory in monkeys (Wang J. H. 2014).

To date, our group has tested rhesus monkeys in a morphine conditioned place preference (CPP) behavioural paradigm to study drug addiction by analysing their preference for the morphine chamber. The insular cortex is well known to be involved in addiction (Abdollahi et al. 2015, Contreras et al. 2007, Gray and Critchley 2007, Naqvi and Bechara 2010, Naqvi et al. 2007). Injection of ZIP into the insula of these macaques (*Macaca mulatta*), after they had successfully established morphine CPP, was found to reduce the preference for the morphine chamber (unpublished data). The results of a post-mortem western blot analysis showed that PKM ζ in the hippocampus was significantly increased after morphine CPP compared to the control group. In the nucleus accumbens, there was an upward trend in the expression levels of PKC λ/ι and GluR2. In the amygdala, there was an upward trend in the expression of PKM ζ and GluR2. While in the insula, there was no significant change in expression levels of PKM ζ and PKC λ/ι during

morphine CPP and after the injection of ZIP (unpublished data). These results suggest that PKM ζ and PKC λ/ι might not play a key role in maintaining CPP long-term memory in the insular cortex. However, it is possible that PKM ζ in the hippocampus might maintain long-term spatial memory. This implies that different areas of the brain may have different molecular mechanisms for maintaining memory.

The concept of ZIP-specific blocking of PKM ζ has also been challenged. It has been reported that high doses of ZIP inhibited the activity of CaMK II (Kwapis and Helmstetter 2014). In future studies, other inhibitors of PKM ζ need to be used to identify the function of PKM ζ in morphine-induced long-term memory in monkeys.

Until now, the mechanism of maintaining long-term memory surrounding PKM ζ seems rather simple, especially in comparison with the several molecular signalling pathways identified in the acquisition and consolidation of memory. It is foreseeable that the process of maintaining long-term memory will inevitably be found to be more complicated than we currently understand. In the field of long-term memory maintenance, the findings may be just the tip of the iceberg. Despite the progress made in the understanding of the PKM ζ associated mechanism, it is likely to be just one part of the whole molecular signalling pathways involved. Future works will hopefully lead to the discovery of all the parts of this molecular network that governs the

maintainance of long-term memory.

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