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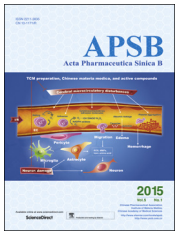

REVIEW

S-palmitoylation regulates AMPA receptors trafficking and function: a novel insight into synaptic regulation and therapeutics

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Abstract Glutamate acting on AMPA-type ionotropic glutamate receptor (AMPAR) mediates the majority of fast excitatory synaptic transmission in the mammalian central nervous system. Dynamic regulation of AMPAR by post-translational modifications is one of the key elements that allow the nervous system to adapt to environment stimulations. *S*-palmitoylation, an important lipid modification by post-translational addition of a long-chain fatty acid to a cysteine residue, regulates AMPA receptor trafficking, which dynamically affects multiple fundamental brain functions, such as learning and memory. *In vivo*, *S*-palmitoylation is controlled by palmitoyl acyl transferases and palmitoyl thioesterases. In this review, we highlight advances in the mechanisms for dynamic AMPA receptors palmitoylation,

Abbreviations: ABE, acyl-biotinyl exchange; ABP, AMPA receptor binding protein; AD, Alzheimer's disease; AKAP79/150, A-kinase anchoring protein 79/150; AMPAR, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; APT1, acyl-protein thioesterase-1; APT2, acyl-protein thioesterase-2; CP-AMPARs, Ca^{2+}-permeable AMPARs; DHHC, aspartate-histidine-histidine-cysteine; FMRP, fragile X mental retardation protein; FXS, Fragile X syndrome; GAP-43, growth associated protein-43; GRIP, glutamate receptor interacting protein; LTD, long-term depression; LTP, long-term potentiation; 17-ODYA, 17-octadecynoic acid; PATs, palmitoyl acyl transferases; PDZ, postsynaptic density-95/discs large/zona occludens-1; PICK1, protein interacting with C-kinase 1; PKA, protein kinase A; PKC, protein kinase C; PPT1, palmitoyl-protein thioesterase-1; PSD-95, postsynaptic density-95; Ras, rat sarcoma; SNAP-23, soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor protein-23

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and discuss how palmitoylation affects AMPA receptors function at synapses in recent years. Pharmacological regulation of *S*-palmitoylation may serve as a novel therapeutic strategy for neurobiological diseases.

1. Introduction

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system. As an important ionotropic glutamate receptor, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) mediates the fast excitatory synaptic transmission in the mammalian brain[1]. The plenty of AMPARs at synapses plays a pivotal role in determining synaptic efficacy. An abundance of convincing evidence has shown that AMPAR is a crucial factor in normal cellular and synaptic activities and in the pathogenesis of multifarious neuropsychiatric and neurodegenerative diseases[1]. Only 30%–50% of AMPARs are expressed on the surface of neurons and a significant proportion of intracellular receptors are located in dendrites. Dynamics of AMPARs at synapse provide a compelling mechanism for understanding the cellular basis of neuropsychiatric and neurodegenerative diseases. *S*-palmitoylation, a principal type of lipid modifications, controls functions of various neuronal proteins by affecting their surface trafficking, including AMPARs. This process is mainly mediated by post-translational addition of a long-chain fatty acid to a cysteine residue of AMPARs or its regulators *via* a thioester linkage. Considering the critical role of AMPARs in the central nerve system function and neurobiological diseases, regulation of their dynamical trafficking by *S*-palmitoylation also serves as a predominant determinant of multiple fundamental brain functions and pathological process. Pharmacological regulation of *S*-palmitoylation may emerge as a potential therapeutic strategy for neurobiological diseases in the future.

2. What is palmitoylation?

Protein lipid modification, one important post-translational modification, commonly includes isoprenylation[2], myristoylation[3], glycophosphatidyl inositol and palmitoylation[4–6]. Among them, palmitoylation is a sort of major lipid modifications of proteins. It is defined as the covalent attachment of saturated 16-carbon palmitic acid to specific cysteine and less frequently to serine and threonine residues of proteins[4,7]. From aspect of chemical biology, palmitoylation increases the hydrophobicity of targeted proteins and facilitates their membrane association (Fig. 1A). Depending on the site of palmitoylation, it can be divided into *N*-palmitoylation and *S*-palmitoylation (Fig. 1B). *N*-palmitoylation, through the formation of a stable N-amide bond, was discovered *via* the analysis of the secreted morphogen Sonic Hedgehog[8]. Furthermore, the *N*-palmitoylation of sonic hedgehog proteins is stable and irreversible[9]. On the contrary, *S*-palmitoylation, through the formation of a labile thioester bond, is a distinctive, reversible lipid modification[4], and potentially regulates the function of proteins *via* cycles of palmitoylation and depalmitoylation catalyzed by protein palmitoyltransferases and protein thioesterases respectively (Fig. 1C). This review focuses on the effect of *S*-palmitoylation on AMPARs function.

2.1. Palmitoylation-related enzymes

As a class of aspartate-histidine-histidine-cysteine (DHHC) proteins (also known as ZDHHC proteins)[10–15], palmitoyl acyl transferases (PATs)[14,15] containing a genetically conserved DHHC cysteine-rich domain (the catalytic center of the enzyme)[16] catalyze palmitoylation of multiple targets *in vivo*. PATs were first discovered in *Saccharomyces cerevisiae*[12,13] and subsequently in various of mammalian cells[11,17–23]. So far, 23 mammalian DHHC proteins and their targets have been discovered[11,24] (Fig. 1D). For example, in the past several decades, several studies have demonstrated that AMPARs subunits-GluA1 and GluA2 can be palmitoylated by DHHC3, which regulates AMPARs surface expression[21]. PSD95, as a scaffolding protein, was also palmitoylated by DHHC 2, 15, 3, and 7[25]. Recent study shows that DHHC8 can palmitoylate protein interacting with C-kinase 1 (PICK1), as a PDZ domain-containing protein, which is required for cerebellar long-term depression (LTD) in mouse[26]. Palmitoylation of both AMPAR subunits and synaptic scaffolding proteins affects synaptic function assembly[25,26].

2.2. Depalmitoylation-related enzymes

Depalmitoylation is catalyzed by palmitoyl protein thioesterases, such as palmitoyl-protein thioesterase-1 (PPT1), acyl-protein thioesterase-1 (APT1) and acyl-protein thioesterase-2 (APT2). Palmitoyl protein thioesterases remove thioester-linked saturated 16-carbon palmitic acid from modified cysteine residues in proteins or peptides during lysosomal degradation. APT1 is a unique thioesterase which is engaged in depalmitoylation of cytoplasmic proteins, such as Ras (rat sarcoma), G_{α} subunits, endothelial nitric oxide synthase and SNAP-23 (soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor protein-23)[27–30]. APT2 catalyzes the depalmitoylation of peripheral membrane-associated GAP-43 (growth associated protein-43)[28]. Besides as a thioesterase, PPT1 is a lysosomal enzyme associated with the degradation of palmitoylated proteins[28,31], and the deficit of PPT1 causes neuronal ceroid lipofuscinosis of infants[30].

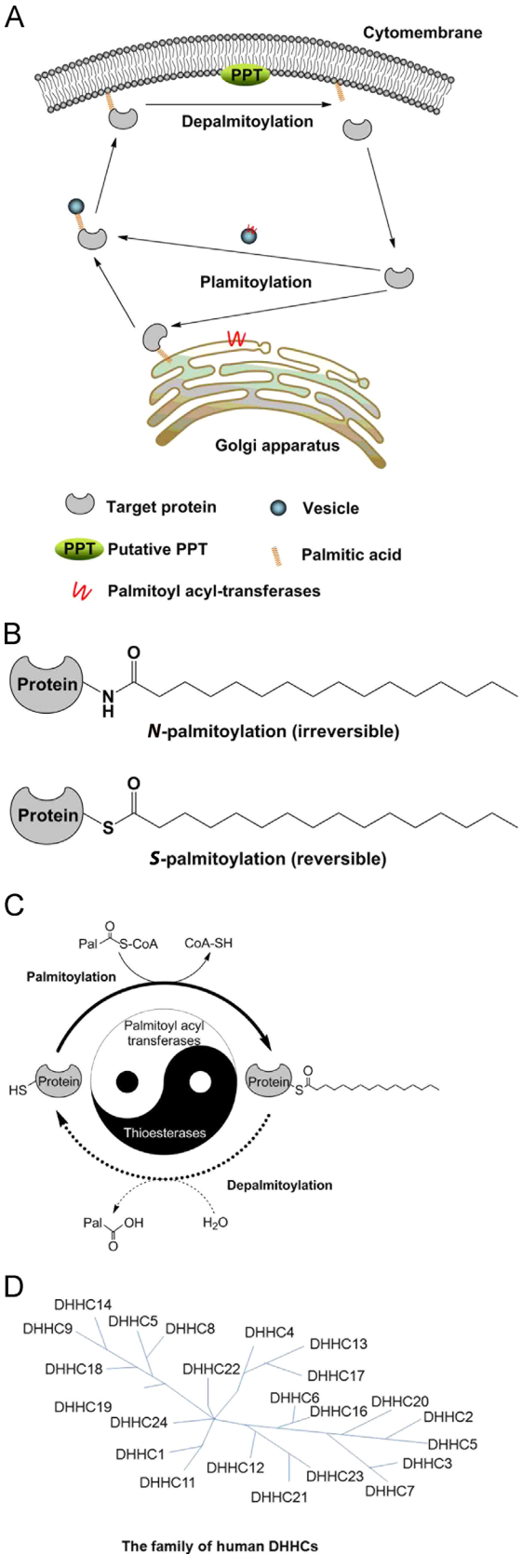
3. Direct regulation of AMPARs trafficking by self-palmitoylation

AMPARs are heterotetrameric and ionotropic glutamate receptors, consisted of 4 subunits: GluA1-4[32]. In mammals, AMPARs are highly conserved. GluA4-containing forms predominantly appear in early postnatal development, while heteromers of GluA1-GluA2 and GluA2-GluA3 mainly exist in the mature mammalian hippocampus[33,34].

AMPARs surface delivery is a crucial procedure in the synaptic transmission and synaptic plasticity. Palmitoylation of AMPARs is a subunit-specific process which affects its trafficking[35]. Thus, palmitoylation of AMPARs plays a crucial role in the regulation of AMPARs function[21,35,36]. The dynamic regulation of palmitoylation and depalmitoylation provides a pivotal mechanism for synaptic plasticity.

Two sites of AMPARs can be directly palmitoylated. One site is the cysteine-610 in the second transmembrane domain of GluA1 and GluA2. The other is different between GluA1 and GluA2. GluA1 (Fig. 2A) and GluA2 (Fig. 2B) are palmitoylated at cysteine-811 and cysteine-836 respectively in the juxta-transmembrane region of the C-terminal cytoplasmic tail[21]. In GluA1, palmitoylation of cysteine-811 indirectly affects AMPARs trafficking to the cell surface through decreasing interaction with the protein band 4.1N[21], which is relative to stabilize surface expression of GluA1[11] (Fig. 2A).

Notably, neuronal activity highly regulates palmitoylation of AMPARs. Depalmitoylation of AMPARs is rapidly induced by the stimulation of glutamate. But the level of total receptors in neuronal cultures are not altered[21,35]. DHHC3, as a PAT, catalyzes palmitoylation of the transmembrane domain site of AMPARs. Thus, it may negatively regulate AMPARs trafficking, and affect expression of AMPARs on the plasma membrane[21]. However, its precise role in synaptic plasticity remains yet largely unknown.



4. Indirect regulation of AMPARs trafficking *via* palmitoylation and AMPAR-interacting proteins

Many AMPAR-interacting proteins that control surface insertion of AMPARs have been identified, such as postsynaptic density-95 (PSD-95), glutamate receptor interacting protein (GRIP)/AMPA receptor binding protein (ABP), PICK1, 4.1N and the A-kinase anchoring protein 79/150 (AKAP79/150). Palmitoylation of these proteins facilitates their membrane association, stabilizes their postsynaptic density and increases their interactions with intracellular receptors[15]. Thus, palmitoylation of AMPAR-associated proteins always produce a contrary effect in contrast to the palmitoylation of AMPARs.

4.1. The palmitoylation of PSD-95 and AMPARs trafficking

PSD-95 is a major scaffolding protein in postsynaptic density, and its palmitoylation is pivotal for AMPARs trafficking[37]. The surface expression of AMPARs is dynamically increased by palmitoylation of PSD-95[25]. The palmitoylated sites of PSD-95 are cysteines-3 and -5 at the N-terminus of the protein. The mutation of the palmitoylated sites on PSD-95 blocks its palmitoylation, and notably decreases surface expression of AMPARs[37].

Figure 1 PATs and cycles of palmitoylation-depalmitoylation. (A) The schematic diagram of palmitoylation-depalmitoylation cycles. (B) The classification of palmitoylation. Palmitoylation divides into *N*-palmitoylation (through the formation of a stable N-amide bond) and *S*-palmitoylation (through the formation of a labile thioester bond). (C) The reaction process between palmitates and proteins in *S*-palmitoylation. *S*-palmitoylation is a reversible lipid modification, and potentially regulates the function of proteins *via* cycles of palmitoylation and depalmitoylation catalyzed by protein palmitoyltransferases and protein thioesterases respectively. (D) The phylogenetic tree of the human DHHC protein family. According to the alignment of the DHHC-CRD core domains, the 23 DHHC proteins are classified into several subfamilies.

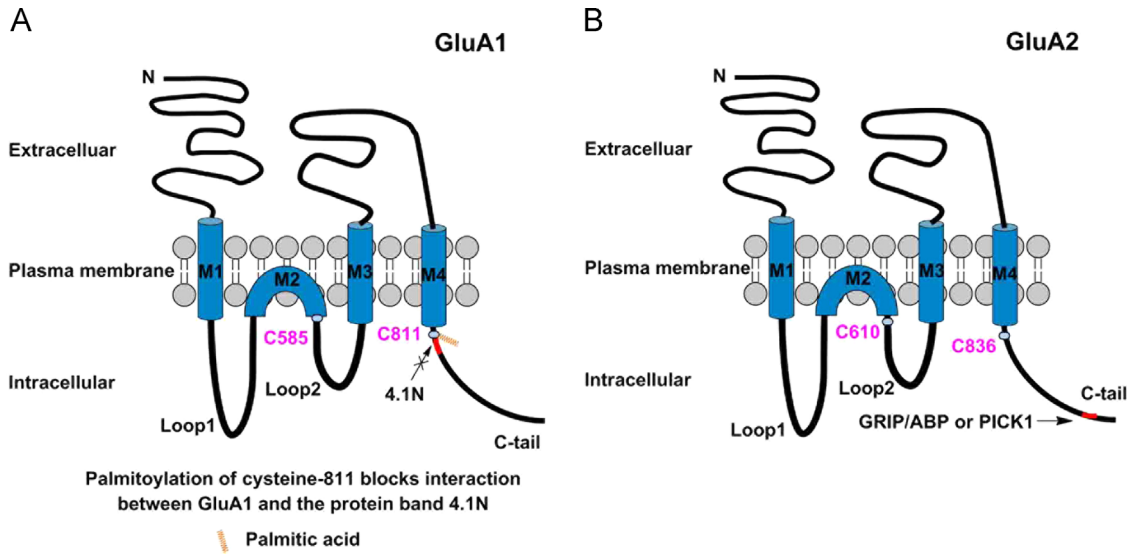


Figure 2 Topology and palmitoylation of AMPAR subunits. (A) Schematic of the GluA1 subunit. The cysteine residues of palmitoylation are indicated in purple. (B) Schematic of the GluA2 subunit. The cysteine residues of palmitoylation are highlighted in purple. Subdomains, mediating the interaction with 4.1N protein (A) or GRIP/ABP or PICK1 (B), are marked in red.

Moreover, Ca^{2+}/calmodulin can promote dissociation of PSD-95 from the postsynaptic membrane *via* binding to the N-terminus of PSD-95, and preventing palmitoylation of PSD-95[38]. It affects the surface expression of AMPARs.

The N-terminal palmitoylation is essential for stabilization of PSD-95 within the postsynaptic density[39]. And DHHC 2, 3, 5, 7, 8, and 15, a series of DHHC-PAT family members, catalyze PSD-95 palmitoylation[15]. Among these DHHCs, DHHC3 and DHHC2 are both essential in the process of postsynaptic accumulation of PSD-95, but only DHHC2 is implicated in the palmitoylation of PSD-95 in response to the decreasing synaptic activity[40].

The decreasing neuronal activity initiates a rapid mobilization of dendritic DHHC2 close to the postsynaptic membranes, therefore mediating robust palmitoylation and improving synaptic accumulation of PSD-95. Finally, it contributes to the increasing surface expression of AMPARs after neuronal stimulation[40].

Regulation of PSD-95 palmitoylation may serve as a novel target for controlling AMPARs surface delivery. Although the lack of selective pharmacological antagonist, we may use the specific peptide to inhibit palmitoylation of PSD-95 by intervening interaction between DHHCs and PSD-95 in the future.

4.2. The palmitoylation of GRIP/ABP and AMPARs trafficking

GRIP also called ABP, with a multi-PDZ domain scaffold, links and stabilizes AMPAR GluA2/3 subunits at synapses. Palmitoylated N-terminal splice variant expression specifically induces multiple changes relative to non-palmitoylated form, contributing to increase of synaptic transmission and AMPARs surface trafficking, as well as development of presynapse and postsynapse[41].

GRIP1 targets to the endosome, and controls the dynamic recycling of internalized AMPARs back to the plasma membrane[42]. GRIP1b mediates NMDA-induced AMPARs internalization, and GRIP1a inhibits this process[43]. Furthermore, GRIP1b, targeting to trafficking endosomes, palmitoylated by DHHC5/8, mediates activity-dependent AMPARs trafficking[44].

4.3. The palmitoylation of PICK1 and AMPARs trafficking

PICK1, a key candidate as a bidirectional regulator of synaptic AMPARs trafficking, mediates the trafficking of GluR2/3 and participate in many physiological and pathological processes. As a postsynaptic density-95/discs large/zona occludens-1 (PDZ) domain protein, PICK1 binds directly with the C termini of the GluA2 and GluA3 subunits of AMPARs[45,46]. PICK1 plays an inverse role in regulating the membrane expression of GluA2-containing and GluA2-lacking Ca^{2+}-permeable AMPARs (CP-AMPARs). The membrane expression of GluA2 was decreased in PICK1 over-expressed neurons, while the surface expression of CP-AMPARs was increased[47]. On the contrary, knockout of PICK1 reduced surface expression of CP-AMPARs in cultured neurons, but the levels of surface GluA2/3 were elevated[48,49]. Palmitoylation on cysteine-414[50] juxta-C terminus of PICK1 by DHHC8[26] contributes to the internalization of postsynaptic GluA2-containing AMPARs[51], which is essential for cerebellar LTD.

4.4. 4.1N and palmitoylation of AMPARs

4.1N, consisting in major neurons of the adult mouse brain, is a neuronal homolog of 4.1R[52]. Besides binding to the actin cytoskeleton, 4.1N selectively interacts with the membrane proximal region of GluA1, but not GluA2[53,54]. 4.1N regulates AMPARs trafficking through providing a pivotal link between AMPARs and the actin cytoskeleton. Consequently, 4.1N is essential to GluA1 insertion. Depalmitoylation of the C811 residue of GluA1 facilitates the interaction between GluA1 and 4.1N. The relationship between 4.1N and palmitoylation is close to AMPARs trafficking and synaptic plasticity.

4.5. The palmitoylation of AKAP79/150 and AMPARs trafficking

AKAP79/150, encoded by the *AKAP5* gene, is a sort of scaffold protein that expressed in human and rodent, respectively. It targets kinases such as protein kinase A (PKA), protein kinase C (PKC),

and calcineurin to the PSD to regulate its phosphorylation, which controls trafficking process of AMPARs[55–61]. The neuronal activity regulates palmitoylation of AKAP79/150, and mediates its targeting to postsynaptic membrane lipid rafts and dendritic endosomes. Crucially, spine enlargement, endosome recycling, and AMPARs trafficking pathways associated with long-term potentiation (LTP) are regulated by the palmitoylation of AKAP79/150[62].

5. AMPARs trafficking in disease

Disorder of the synaptic AMPARs trafficking contributes to cognitive dysfunction in Alzheimer's disease (AD). The expression level of AMPAR subunits, such as GluA1, GluA2 and GluA2/3, was decreased in CA1 of hippocampus, the subiculum and entorhinal cortex of patients with AD[63,64].

Fragile X syndrome (FXS), caused by the loss of fragile X mental retardation protein (FMRP)[65], also associates with dysregulation of AMPARs trafficking. The translation level of GluA1 and GluA2 subunits was significantly increased[65], but the surface expression level of GluA1 is reduced in the amygdala of fragile X mental retardation 1 (*Fmr1*) knock-out mice[66].

However, there are few reports on palmitoylation regulates AMPARs trafficking in neurobiological diseases.

6. Conclusions and perspectives

We have seen considerable steps forward in our understanding of the extent and roles of palmitoylation of AMPARs and their regulators in past several decades. The development of two complemental methods have been used in the global palmitoyl proteomic analysis. The acyl-biotinyl exchange (ABE) method[67–69] can be applied to analyze palmitoylated proteins from any cell-free protein extract. In contrast to the ABE method, metabolic labeling with the 17-octadecynoic acid (17-ODYA, as the palmitic acid analog)[70,71] can identify dynamically palmitoylated proteins *in vivo*.

The large DHHC family plays essential roles in a range of physiological functions, and several DHHC genes are closely associated with diseases, such as cancers[72], schizophrenia[73,74], mental retardation[75,76], and Huntington's disease[77]. However, how the DHHCs dynamically regulate palmitoylation of targeted proteins in several diseases including neuropsychiatric disorder yet remains elusive.

Palmitoylation of postsynaptic proteins, such as PSD-95 and GRIP1, may up-regulate the membrane expression of AMPARs, and enhance the synaptic function[15]. Consequently, palmitoylation-dependent regulation of AMPARs trafficking inevitably plays pivotal roles in physiological activities of neurons and synapses, and in the pathogenesis of multiple neuropsychiatric and neurodegenerative diseases, such as cocaine addiction[78].

Both the pharmacological antagonist of PATs 2-bromopalmitate[44] and shRNA knockdown or rescue approaches[62] are widely used to explore the roles of specific palmitoylation events. Recently, 2-bromopalmitate analogs (1,2-bromohexadec-15-ynoic acid and 2-bromooctadec-17-ynoic acid), serve as novel and chemical tools to probe PATs in cell signaling and diseases[79]. But it is a pity that the 2-bromopalmitate analogs are not selective to pharmacologically antagonize one of PATs. Considering the key role of palmitoylation in the regulation of AMPARs function, these PATs inhibitors may serve as medicinal approaches to rescue neuropsychiatric and neurodegenerative diseases in the future.

Regardless of these progresses in research on palmitoylation of AMPARs, there are many mechanisms on dynamical regulation of between palmitoylation and depalmitoylation remain elusive. Therefore what primary challenges lie ahead?

First and foremost, a selective pharmacological antagonist for PATs is lacking. 2-Bromopalmitate, pervasively used in palmitoylation of studies, can block all of PATs. It causes a serious trouble for our research on a specific type of PAT. Consequently, it is high time that we found specific and selective pharmacological antagonists for PATs which is helpful for the studies on palmitoylation of AMPARs, even for all of researches about palmitoylation.

Besides, until now, most studies focused on the physiological role of palmitoylation, but little reports about the role of palmitoylation in pathogenesis of neuropsychiatric disorders, including major depressive disorder, drug addiction and post-traumatic stress disorder, have been revealed. Surface delivery of AMPARs plays a principal role in pathogenesis of neuropsychiatric disorders; interestingly, palmitoylation regulates AMPARs trafficking. Therefore, we should pay more attention to the role of palmitoylation in the neuropsychiatric diseases. A clarification for the relationship between palmitoylation in the blood and neuropsychiatric disorders will ultimately translate AMPARs modifications from laboratory to bedside. Furthermore, palmitoylating/depalmitoylating enzymes associated with AMPAR trafficking might become potential therapeutic targets of neuropsychiatric disorders in the future.

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