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The diversity of linkage-specific polyubiquitin chains and their role in synaptic plasticity and memory formation

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Abstract

Over the last 20 years, a number of studies have provided strong support for protein degradation mediated by the ubiquitin-proteasome system in synaptic plasticity and memory formation. In this system, target substrates become covalently modified by the small protein ubiquitin through a series of enzymatic reactions involving hundreds of different ligases. While some substrates will acquire only a single ubiquitin, most will be marked by multiple ubiquitin modifications, which link together at specific lysine sites or the N-terminal methionine on the previous ubiquitin to form a polyubiquitin chain. There are at least eight known linkage-specific polyubiquitin chains a target protein can acquire, many of which are independent of the proteasome, and these chains can be homogenous, mixed, or branched in nature, all of which result in different functional outcomes and fates for the target substrate. However, as the focus has remained on protein degradation, much remains unknown about the role of these diverse ubiquitin chains in the brain, particularly during activity- and learning-dependent synaptic plasticity. Here, we review the different types and functions of ubiquitin chains and summarize evidence suggesting a role for these diverse ubiquitin modifications in synaptic plasticity and memory formation. We conclude by discussing how technological limitations have limited our ability to identify and elucidate the role of different ubiquitin chains in the brain and speculate on the future directions and implications of understanding linkage-specific ubiquitin modifications in activity- and learning-dependent synaptic plasticity.

Keywords

Ubiquitin; memory; transcription; protein degradation

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1. Introduction

The neurobiological basis of memory formation has been extensively studied, and we now have a good understanding of the molecular mechanisms involved in the processes of long-term memory formation, storage, and modification (Asok, Leroy, Rayman, and Kandel, 2019; Johansen, Cain, Ostroff, and LeDoux, 2011; Kandel, 2012; Park and Kaang, 2019). For example, short-term memories for learned associations are susceptible to disruption until completion of a time-dependent process known as consolidation (McGaugh, 2015), which requires *de novo* protein synthesis as broad-spectrum manipulation of this or upstream intracellular signaling cascades often impairs long-term memory (Bailey et al., 1999; Bekinschtein et al., 2007; Schafe et al., 1999; Shrestha et al., 2020). Furthermore, following retrieval, memories destabilize and are temporarily rendered labile, requiring new protein synthesis in order for the memory to be restabilized, a process referred to as reconsolidation (Nader and Hardt, 2009; Nader, Schafe, and Le Doux, 2000; Roesler, 2017). A number of transcriptional control pathways upstream of mRNA and protein synthesis have been implicated in the consolidation and reconsolidation processes, including PKA, PKC, ERK/MAP, CREB, CaMKII and mTOR (Cho et al., 2018; Giese and Mizuno, 2013; Hoeffler and Klann, 2010; Kandel, 2012; Medina and Viola, 2018; Park et al., 2020; Peng et al., 2010; Silva, Kogan, Frankland, and Kida, 1998; Zalcman, Federman, and Romano, 2018). As a result, it is clear that the memory storage process is complicated, requiring a large number of individual and interacting processes that ultimately converge on increased regulation of transcriptional and translational processes necessary for memory formation and modification.

While traditional models of activity- and learning-dependent synaptic plasticity had focused on the need for mRNA and protein synthesis (Johansen et al., 2011), current models now take into account the potential role for protein degradation in these processes (Hegde, 2017; Hegde, Haynes, Bach, and Beckelman, 2014; Jarome and Helmstetter, 2013; 2014; Park and Kaang, 2019). The ubiquitin-proteasome system (UPS) controls the majority of protein degradation in cells and is involved in a number of molecular processes through proteolytic and nonproteolytic mechanisms. However, the focus in eukaryotes has remained on the canonical protein degradation function that has been shown to be involved in cell cycle progression, transcription, and synaptic plasticity. For example, protein degradation has been shown to control homeostatic scaling, new dendritic spine growth *in vitro*, long-term depression (LTD) and long-term potentiation (LTP), the proposed cellular analog of memory (Bingol et al., 2010; Cai, Frey, Sanna, and Behnisch, 2010; Djakovic et al., 2012; Dong et al., 2008; Dorrbaum et al., 2020; Ehlers, 2003; Hamilton et al., 2012; Li, Korte, and Sajikumar, 2016). Furthermore, recent evidence suggests that protein degradation is a critical regulator of memory formation and stability. Within cells of multiple brain regions, learning increases the amount of degradation-specific polyubiquitination and proteasome catalytic activity and administration of catalytic inhibitors of the proteasome result in memory impairments for a variety of behavioral tasks (Artinian et al., 2008; Cullen, Ferrara, Pullins, and Helmstetter, 2017; Figueiredo et al., 2015; Jarome, Werner, Kwapis, and Helmstetter, 2011; Lopez-Salon et al., 2001; Reis, Jarome, and Helmstetter, 2013; Rodriguez-Ortiz et al., 2011) and disease models, such as drug addiction (Werner et al., 2019; Werner et al., 2018).

Furthermore, protein degradation has been shown to control the destabilization of memory after retrieval, suggesting a critical role for it in the reconsolidation process (Ferrara et al., 2019; Jarome et al., 2011; Lee, 2008; Lee et al., 2008), and has been widely implicated in the reconsolidation of drug-associated behaviors (Massaly et al., 2013; Werner et al., 2015). Learning-induced NMDA receptor activation results in changes in the activity of several intracellular signaling pathways responsible for memory formation. Interestingly, activation of NMDA receptors also controls learning-dependent increases in ubiquitin-proteasome activity (Jarome et al., 2011; Rosenberg, Elkobi, Dieterich, and Rosenblum, 2016). Downstream of NMDA receptors, CaMKII has been shown to target the proteasome and increase its catalytic activity via phosphorylation of the regulatory subunit RPT6 at serine-120 (Jarome, Ferrara, Kwapis, and Helmstetter, 2016; Jarome, Kwapis, Ruenzel, and Helmstetter, 2013), indicating that the protein degradation process is intricately connected to intracellular signaling mechanisms previously reported to be critical for memory formation.

While strong evidence supports that ubiquitin-proteasome mediated protein degradation is a critical regulator of synaptic plasticity and memory formation, very little attention has been paid to the degradation-independent functions of this system in these processes. Importantly, while protein degradation is thought of as the canonical process, it is only one of many functions of protein ubiquitination as this modification can result in many other functions and cellular fates for the target substrate. In fact, strong evidence indicates that protein ubiquitination can regulate a wide variety of processes independent of the proteasome, including kinase activation, endocytosis, epigenetic processes, intracellular trafficking, and mRNA stability (Akutsu, Dikic, and Bremm, 2016; Komander and Rape, 2012; Swatek and Komander, 2016). Additionally, some ubiquitin modifications can even impede the protein degradation process. These diverse functions are a result of there being eight different linkage-specific polyubiquitin chains that a target protein can acquire, resulting in a complex “ubiquitin code.” In this review, we will identify the different types of ubiquitin modifications and discuss the known functions of them outside of the brain. Next, we review what is currently known about the role of these ubiquitin modifications in synaptic plasticity and memory formation. We conclude by discussing the technical issues that have limited the identification and manipulation of these diverse ubiquitin modifications in neurons and theorize about how these nonproteolytic functions of protein ubiquitination contribute to activity-and learning-dependent synaptic plasticity.

2. The Ubiquitin-Proteasome System

The UPS is a central component of eukaryotic cells responsible for controlling intracellular protein homeostasis via degradation (Cromm and Crews, 2017; Gaczynska and Osmulski, 2018; Hershko and Ciechanover, 1998) of misfolded proteins due to heat or oxidation stresses, worn-out long-lived proteins, or short-lived transcription factors are among the many UPS substrates (Eisele and Wolf, 2008). In this system, the multi-subunit 26S proteasome complex recognizes and degrades target proteins that are “tagged” by ubiquitin and this degradation process is one of the most versatile mechanisms in the cell as it can regulate a myriad of intracellular biological and pathological functions (Hegde, 2017; Mabb and Ehlers, 2010; Shaid, Brandts, Serve, and Dikic, 2013). As the proteasome complex has been extensively reviewed elsewhere (Bard et al., 2018; Bedford et al., 2010), this section

will focus primarily on the protein ubiquitination process, though we will briefly discuss the proteasome structure.

2.1. The ubiquitination process

Ubiquitin protein tagging is catalyzed by an enzymatic cascade involving three families of enzymes, a ubiquitin activating enzyme (E1), ubiquitin conjugating enzymes (E2), and ubiquitin ligases (E3) (Gropper et al., 1991; Jentsch, 1992). The first step begins with the E1 enzyme forming a thioester bond between its active site cysteine residue and the C-terminus of the glycine residue of a free ubiquitin molecule using ATP. Prior to the binding of ATP, the E1 has a low affinity for the ubiquitin, however, the ubiquitin binding site may be more accessible after ATP-dependent conformational changes occur (Pickart, 2001). Each E1 carries two activated ubiquitin molecules, one as an adenylate and the other as a thiolester, the latter of which is then transferred to a cysteine residue of the E2 conjugating enzymes (Cromm and Crews, 2017; Eletr and Kuhlman, 2007; Kumari, Lee, and Jha, 2018). The E1 concentration is often less than the E2 concentration, but due to its high efficiency, the E1 produces an adequate amount of activated ubiquitin for the downstream conjugation reactions (Stewart, Ritterhoff, Klevit, and Brzovic, 2016). There are about 40 different E2 enzymes in humans, which share a conserved core domain and perform a wide range of functions in the cell. However, in general, E2s are mostly responsible for carrying the ubiquitin molecule to the next enzyme, the E3 ligase (Stewart et al., 2016).

The E3 ligase recognizes the target substrate and binds to it, transferring the E2-bound ubiquitin molecule to a lysine residue of the target substrate through an isopeptide bond (Stewart et al., 2016; Zheng and Shabek, 2017). E3 ligases can only interact with a limited number of E2 enzymes, while E2 enzymes can interact with several E3 ligases, which increases the specificity of the ubiquitination process. The E3 category of enzymes, with more than 600 species identified thus far, have been classified into three different families of proteins based on their mechanism to transfer the ubiquitin: Really Interesting New Gene (RING), Homologous to the E6AP Carboxyl Terminus (HECT), and RING-between-RING (RBR). E3 ligases containing the HECT domain and RBRs act as intermediates by accepting the ubiquitin from the E2 and forming a covalent E3-ubiquitin thioester bond before transferring it directly to the substrate. In contrast, the largest family among the three, RING domain ligases, function as a scaffold for the E2 by bringing it to the proximity of the substrate and catalyzing the ubiquitin transfer directly from the E2 enzyme to the substrate (Eletr and Kuhlman, 2007; Kumari et al., 2018; Metzger, Hristova, and Weissman, 2012). As a result, the ubiquitination is complex and requires the coordinated actions of hundreds of different protein ligases.

2.2. The proteasome

Once proteins are tagged by specific ubiquitin modifications, such as lysine 48 linkage (K48), the 26S proteasome complex can identify and degrade them. The complete 26S proteasome complex, which is named after its sedimentation coefficient (Tanaka, 2009), is made up of two subcomplexes: A 20S catalytic core particle (CP) with a 19S regulatory particle (RP) linked on either or both ends of it, which regulates its activity. The RP consists of about 20 different subunits that can be categorized into two groups, the Regulatory

particle of non-ATPase (RPN) subunits and the Regulatory particle of triple-ATPase (RPT) subunits. At least nine RPN subunits form the lid subcomplex, and six RPT along with four RPN subunits form the base subcomplex of the 19S cap (Tanaka, 2009). The 20S complex is a highly conserved structure among eukaryotic species, consisting of two outer rings of α subunits and three inner catalytic rings formed by β subunits facing the lumen of the barrel (Kunjappu and Hochstrasser, 2014; Latham, Sekhar, and Kay, 2014). The center of the outer rings, called the α -annulus, is in an almost closed state, forming a gate to keep unwanted proteins from penetrating the catalytic barrel of the complex. Polyubiquitinated proteins of specific chain linkage are recognized by the 19S base and deubiquitinating enzymes on the lid remove the chain and recycle the ubiquitin molecules. Then, the base unfolds the protein via the mechanical pulling force its heterohexameric ATPase motor generates, opens the 20S channel via the α ring and shuttles the unfolded substrates into the core for degradation by the β subunits (Bard et al., 2018; Finley, Chen, and Walters, 2016; Liu and Jacobson, 2013; Tanaka, 2009). The target substrate is cut into oligopeptides ranging from 3–15 amino acid residues, which may be hydrolyzed to monomers by oligopeptidases later (Kloetzel and Ossendorp, 2004; Rabl et al., 2008; Tanaka, 2009).

3. The different types of protein ubiquitination chains

Ubiquitin is a highly conserved 76 amino acid protein that is ubiquitously expressed in cells. Four genes code for ubiquitin, *Ubb*, *Ubc*, *Uba52*, and *Rps27a* (Figure 1A). *Ubb* and *Ubc* are unique poly-cistronic genes in which the coding region is repeated 3 or 8 times, respectively, resulting in them coding for multiple ubiquitin that are linked together in a head-to-tail manner (Wiborg et al., 1985). Conversely, the N-terminal of *Uba52* and *Rps27a* each code for a single ubiquitin that is fused at the C-terminal to the ribosomal proteins L40 and S27a, respectively (Redman and Rechsteiner, 1989; Webb, Baker, Coggan, and Board, 1994). These precursors are usually processed post-translationally into single ubiquitin molecules by deubiquitinating enzymes, though processing during translation has also been reported for *Ubb* and *Ubc* (Grou et al., 2015). While it is clear all the ubiquitin genes code for the same protein and are critical for cell viability (Kobayashi et al., 2016; Park and Ryu, 2014; Ryu et al., 2008), their functions are not necessarily redundant, and the importance of these four genes to ubiquitin homeostasis remains largely unknown. The ubiquitin protein contains eight different sites at which polyubiquitin chains can form: The first methionine on the N-terminal (M1) or lysine (K) 6, 11, 27, 29, 33, 48, and 63 (Figure 1B). While some proteins will acquire only a single ubiquitin modification, termed monoubiquitination, most substrates acquire large polyubiquitin chains (Figure 1C). The linkage for these polyubiquitin chains can be homogeneous or mixed, with each ubiquitin molecule being modified at only one site by the next ubiquitin. However, in some cases, a target substrate can acquire a branched polyubiquitin chain, which is when ubiquitin is modified at multiple sites by additional ubiquitin molecules (Akutsu et al., 2016; Komander and Rape, 2012; Swatek and Komander, 2016). As a result, the ubiquitin code is highly complex, and a given polyubiquitin linkage site may result in different cell fates or functions depending on whether the chain is homogeneous, mixed, or branched. In this section, we discuss the known biological functions of the seven homogeneous polyubiquitin chains. Additionally, we also discuss the cellular functions of mixed and branched polyubiquitin chains and

monoubiquitination. A summary of the various functions regulated by different polyubiquitin chains is presented in Table 1.

3.1. The canonical degradation chain: K48

The most abundant and well-studied polyubiquitin linkage site is K48, largely due to evidence that it is the canonical degradation chain (Chau et al., 1989). The proteasome can target proteins with at least four K48-linked ubiquitin molecules for degradation, and binding occurs at 19S subunits RPN10 and RPN13 (Grice and Nathan, 2016; Peth, Uchiki, and Goldberg, 2010). Importantly, the proteasome's affinity for K48-linked ubiquitin chains is up to 100-fold greater than for mono- or di-ubiquitinated proteins (Clague and Urbe, 2010; Thrower, Hoffman, Rechsteiner, and Pickart, 2000). Additionally, the application of proteasome inhibitors results in a rapid accumulation of K48 polyubiquitinated proteins (Xu et al., 2009), furthering emphasizing the importance of this linkage site in the protein degradation process. To date, functions of K48-linked chains that are independent of the proteasome remain equivocal, and those that have been identified are usually associated with mixed or branched chains, as described in a later section.

3.2. The canonical degradation-independent chain: K63

K63 linkage sites have been the second most studied polyubiquitin chains and are considered to be the canonical protein degradation-independent chain. This designation resulted from studies showing that levels of K63 polyubiquitin conjugates remain largely unchanged following proteasome inhibition and, in general, the proteasome has low affinity for this ubiquitin chain (Jacobson et al., 2009; Nathan et al., 2013; Xu et al., 2009). Instead, this chain generally targets substrates for other cellular fates, and while the potential functions of K63 polyubiquitination in cells are diverse, the focus has generally been on control of protein kinase activation (Chen and Sun, 2009). For example, there is strong evidence for K63 chain involvement in the activation of the NF- κ B transcriptional pathway (Wang et al., 2012), a well described regulator of synaptic plasticity and memory formation (Merlo, Freudenthal, Maldonado, and Romano, 2005; Yeh, Lin, Lee, and Gean, 2002). In the canonical pathway, protein K63 polyubiquitination can act as docking sites for other factors necessary for NF- κ B activation, such as TAB2 (Kanayama et al., 2004; Kulathu et al., 2009), or can lead to the oligomerization of proteins such as RICK that also influences the activation of this transcriptional pathway (Hasegawa et al., 2008). Activation of NF- κ B can also occur via K63 polyubiquitination of other proteins, such as TRAF2 and RIP1 (Bertrand et al., 2011; Skaug, Jiang, and Chen, 2009). Additionally, strong evidence suggests a role for K63 polyubiquitination in the activation of other protein kinases, such as AKT (Yang et al., 2009; Yang, Zhang, and Lin, 2010), further emphasizing the role of the K63 chain in kinase activation. K63 polyubiquitination has also been shown to be involved in the DNA damage response where K63 chains on histone proteins serve as recognition sites for downstream repair proteins (Lee et al., 2017). Furthermore, a large number of plasma membrane proteins have been identified as targets of K63 polyubiquitination, suggesting a role in intracellular trafficking (Erpapazoglou, Walker, and Hagenauer-Tsapis, 2014). This chain has also been shown to be involved in endocytosis, autophagy, and mitophagy (Cunningham et al., 2015; Shaid et al., 2013; Tanno and Komada, 2013). Thus, while the potential functions of the K48

chain are minimal, the K63 linkage site can target substrates for a variety of different cellular fates.

Despite the notion that K63 polyubiquitination is independent of the protein degradation process, some evidence suggests that the proteasome can target this ubiquitin chain. *In vitro* models have shown that K63 linked tetra-ubiquitin can be targeted by the proteasome for degradation (Hofmann and Pickart, 2001), an effect that has also been reported *in vivo* (Saeki et al., 2009). While the reason for this discrepancy is unclear, some recent evidence suggests that it may be due to branched ubiquitin chains. For example, K63 ubiquitination can act as a seed to recruit a K48/K63 branched ubiquitin chain, leading to degradation of the substrate by the proteasome (Ohtake, Tsuchiya, Saeki, and Tanaka, 2018). We discuss these branched chains in more detail in a later section; however, this does suggest that K63 polyubiquitination can lead to degradation of the substrate under certain conditions.

3.3. Noncanonical lysine chains: K6, K11, K27, K29, K33

K6 polyubiquitin chains do not accumulate following proteasome inhibition (Kim et al., 2011), thus are considered to be independent of the protein degradation process. Instead, this linkage site has been implicated in several cellular processes. Similar to K63, the K6 linkage site has been widely implicated in the DNA damage response (Morris and Solomon, 2004; Nishikawa et al., 2004; Wagner et al., 2011). Additionally, strong evidence suggests a role for K6 polyubiquitination in mitochondria homeostasis as this chain typically accumulates on depolarized mitochondria (Durcan et al., 2014). Consistent with this, mutations of K6 delayed mitophagy, similar to what was observed with K63 chains (Cunningham et al., 2015; Ordureau et al., 2015). Interestingly, some evidence indicates that K6 polyubiquitination can also inhibit the protein degradation process (Shang et al., 2005), suggesting a potentially unique function of this linkage site relative to many of the other ubiquitin chains. However, due to difficulties in its detection, much still remains unknown about the physiological roles of K6 polyubiquitin chains.

Unlike the K6 chain, K11 polyubiquitination has been well studied, and, in general, there is a strong consensus that this linkage site is involved in the protein degradation process (Matsumoto et al., 2010; Qin et al., 2014). However, this chain is unique relative to the K48 degradation signal in that proteasome affinity for K11 linkage is poor. Instead, K11 branched chains are generally a better signal for degradation (Grice et al., 2015; Meyer and Rape, 2014). This has led to questions regarding the actual function of homogeneous K11 chains as a lack of proteasome affinity suggests a role independent of protein degradation. Though indirect evidence points to a role in kinase activation and receptor trafficking (Hu et al., 2013; Swatek and Komander, 2016), to date, the functions of homogeneous K11 polyubiquitination remain unknown.

The K27 chain has been implicated in a variety of cellular processes. Similar to K11 and K48, K27 ubiquitin chains are associated with degradation of the substrate by the proteasome; however, this linkage site actually slows the degradation process (Birsa et al., 2014). Additionally, other studies suggest potential functions that are independent of the protein degradation process (Arimoto et al., 2010). For example, K27 polyubiquitination of NEMO serves as a docking site for Rbhd3, a member of the rhomboid family of proteases,

which also undergoes K27 polyubiquitination. This results in the recruitment of deubiquitinating enzymes that prevent K63 polyubiquitination of NEMO and the subsequent activation of NF- κ B during the autoimmune response (Liu et al., 2014). Similar to K6 and K63 chains, K27 polyubiquitination has been implicated in the DNA damage response where it recruits DNA damage repair factors such as the p53 binding protein 1 (Gatti et al., 2015), suggesting a protein recruitment role of this polyubiquitin chain. Interestingly, some evidence indicates that K27 chains are not cleaved by most deubiquitinating enzymes, a unique characteristic relative to other linkage sites (Castaneda et al., 2016). This has made it difficult to identify the interacting proteins of the K27 linkage site, limiting our understanding of this noncanonical ubiquitin chain.

K29 chains have been largely implicated in functions independent of the protein degradation process, though some exceptions have been noted. Proteasome inhibition results in an accumulation of K29 polyubiquitinated proteins (Kim et al., 2011), suggesting that this mark could be targeted for substrate degradation by the proteasome. However, this relationship is complicated by evidence indicating that proteasome subunit RPN13 is ubiquitinated with a K29 chain in response to proteasomal stress (Besche et al., 2014), which prevents further substrate engagement with the proteasome, indicating that accumulation of this polyubiquitin chain following proteasome inhibition may occur as a result of mechanisms independent of K29-targeted substrate degradation. Independent of the protein degradation process, K29 chains have been implicated in the regulation of Wnt/ β -catenin signaling (Fei et al., 2013), a critical regulator of memory formation (Maguschak and Ressler, 2011; Tan et al., 2013). This polyubiquitin chain has also been implicated in mRNA stability and epigenetic regulation via its targeting of the histone lysine demethylase KDM4D (Jin et al., 2016; Zhou, Geng, Luo, and Lou, 2013), the latter of which targets histone H3 lysine 9, a well described regulator of synaptic plasticity and memory formation (Gupta-Agarwal et al., 2012; Gupta-Agarwal, Jarome, Fernandez, and Lubin, 2014; Schaefer et al., 2009).

K33-linked polyubiquitin chains are considered to be independent of the protein degradation process, instead being primarily involved in intracellular trafficking. For example, this chain stabilizes Actin for post-Golgi transport and Coronin 7 for recruitment to the trans-Golgi network (Yuan et al., 2014). Additionally, K33 chains have also been shown to regulate T-cell receptor- ζ function by controlling its phosphorylation and protein binding abilities (Huang et al., 2010). It is important to note that some evidence does suggest that the K33 polyubiquitin mark can lead to protein degradation (Kim et al., 2013; Michel et al., 2015; Xu et al., 2009); however, this has never been directly proven.

3.4. Atypical, lysine-independent chain: M1

Unlike the polyubiquitin chains described above, linear (M1) chain linkage does not occur at a lysine site. Instead, in linear chains the linkage site is the first methionine on the N-terminal. Another unique characteristic of linear polyubiquitination is that only a single E3 ligase can conjugate this chain, which is known as LUBAC (Spit, Rieser, and Walczak, 2019). The physiological roles of linear polyubiquitination have been a hot topic in recent years, with most evidence suggesting functions independent of the protein degradation process. For example, LUBAC has been heavily studied in the activation of the NF- κ B

transcriptional pathway. LUBAC was shown to target NEMO, conjugating a linear polyubiquitin mark that was necessary for interaction with the TNF-R1 signaling complex. This resulted in the stabilization of the complex and activation of the NF- κ B pathway (Haas et al., 2009; Tokunaga et al., 2009). The linear chain then acts as a scaffold to recruit other essential factors for NF- κ B activation, including IKK, to the NEMO complex (Rahighi et al., 2009; Tokunaga, 2013; Tokunaga et al., 2011). Additionally, linear ubiquitination has been shown to activate NF- κ B via targeting of other proteins, including Optineurin (Nakazawa et al., 2016). Genetic loss of linear polyubiquitination partially impairs NF- κ B activation (Gerlach et al., 2011; Seymour et al., 2007), though other studies have reported no change or increases in NF- κ B activity following manipulations of LUBAC (Zak et al., 2011). While the reasons for these conflicting results are unclear, it is possible that this could be due to the redundant role of K63 polyubiquitination in NF- κ B activation or evidence that linear polyubiquitination can be involved in integrin activation (Rantala et al., 2011). Regardless, it is clear that the linear linkage site at least partially contributes to the regulation of NF- κ B activation.

Considering its role in TNF signaling, the linear chain has also been shown to regulate apoptosis via targeting of cFLIP, a master anti-apoptotic regulator and resistance factor, protecting it from proteasome-mediated degradation (Tang et al., 2018). Interestingly, unlike K6, K27, and K63 polyubiquitin chains, evidence suggests linear linkage is not involved in the DNA damage response (Zhao and Ulrich, 2010). It should also be noted that while generally considered to be independent of the protein degradation process, some evidence suggests that linear polyubiquitination can target substrates for degradation by the proteasome (Zhao and Ulrich, 2010), though this has not yet been confirmed by other studies.

3.5. Branched/mixed ubiquitin chains

Complicating our understanding of the diverse functions of specific polyubiquitin chains is evidence that some substrates can carry multiple ubiquitin of mixed linkage. Adding another layer of complexity, these mixed (or homeogenous) chains can be also be branched, whereby two separate polyubiquitin chains are formed on the same substrate. In general, these mixed and branched chains have been challenging to study as the current methodology cannot distinguish a homogeneous chain from one that is mixed or branched, essentially making them “invisible” to detection (Swatek and Komander, 2016). Despite this, we have been able to learn about the physiological role of at least a few mixed and branched polyubiquitin chains (Figure 2). For example, strong evidence suggests that K63 chains can be modified by M1 chains in a mixed or branched structure (Emmerich et al., 2013). The basis for this is that LUBAC has been shown to bind to polyubiquitin chains of different compositions, and one of the main components of this complex, HOIP (RNF31), can bind ubiquitin that is part of an existing chain (Stieglitz et al., 2013). This mixed K63/M1 chain could explain the apparently redundant role of each of these linkage sites in NF- κ B activation via NEMO. A branched chain of K48/K63/K11 has been shown to impede while a branched K11 chain facilitates degradation of the substrate by the proteasome (Kim et al., 2007; Meyer and Rape, 2014). Conversely, despite the well-known role of K48 homogeneous chains in the protein degradation process (Ohtake et al., 2016), branched K48/K63 chains have been

shown to facilitate NF- κ B activation or lead to substrate degradation, as described in Section 3.2 (Ohtake et al., 2018). Mixed chains of K11/K48 are primarily a degradation signal (Grice et al., 2015; Meyer and Rape, 2014). The K11/K63 mixed polyubiquitin chains can lead to endocytosis (Boname et al., 2010). Some other identified mixed polyubiquitin chains include K11/K29, K6/K48, K27/K48, K29/K48, and K11/K33, though the functions of these chains are unknown.

3.6. Monoubiquitination

In general, there is a strong consensus that monoubiquitination is involved in substrate localization, endocytosis, transcriptional control, and epigenetic regulation (Hicke, 2001). For example, the transcription factor FOXO4 can be monoubiquitinated, which results in re-localization to the nucleus and an increase in its activity (van der Horst et al., 2006). Some of the more well-described monoubiquitin targets are histones H2A and H2B, the latter which regulates the chromatin structure via recruitment of other epigenetic modifiers such as histone H3 methylation (Chandrasekharan, Huang, and Sun, 2009; Sun and Allis, 2002). While monoubiquitin modifications typically do not target proteins for degradation, some recent evidence suggests that it can under certain circumstances. In particular, some substrates that acquire multiple monoubiquitin marks can be degraded by the proteasome (Dimova et al., 2012; Lu et al., 2015), though this is complicated by evidence that not all multi-monoubiquitinated proteins undergo protein degradation (Lu et al., 2015). Why the reason for this sparing of some but not other multi-monoubiquitinated proteins remains equivocal, some evidence suggests that it may be related to the position of unfolded regions within the target substrate (Prakash et al., 2004; Shabek et al., 2012).

4. The role of protein ubiquitination in synaptic plasticity and memory formation

Despite the diversity of ubiquitin chains and the various functions they serve in cellular signaling, few studies have directly examined the role of polyubiquitin modifications in synaptic plasticity and memory formation (Table 2). As the primary focus has been on protein degradation, K48-linked chains have received the most attention, though some recent evidence implicates K63-linked and linear polyubiquitin chains in synaptic plasticity and/or memory storage processes. However, to date, no study has examined if K6, K11, K27, K29, and K33 polyubiquitin chains are altered as a result of or involved in activity- and learning-dependent synaptic plasticity. Furthermore, the potential involvement of mixed or branched polyubiquitin chains in any cellular process in the brain remains unknown. In this section, we will review the known roles of K48, K63, and linear polyubiquitination in synaptic plasticity and memory formation. Additionally, we will briefly discuss evidence implicating protein monoubiquitination in these processes.

4.1. K48 polyubiquitination

Considering the focus on protein degradation in activity- and learning-dependent synaptic plasticity, several studies have examined changes in K48 polyubiquitination in the brain. Stimulation of cultured hippocampal neurons with NMDA decreased K48 polyubiquitination in dendritic spines, consistent with increased proteasome activity and

protein degradation (Bingol et al., 2010). Furthermore, auditory and contextual fear learning transiently increased K48 polyubiquitination in the amygdala in a learning-dependent manner (Jarome et al., 2013; Jarome et al., 2011), which is largely localized to the nucleus and cytoplasm (Orsi et al., 2019). A similar increase in K48-linked polyubiquitination has been observed in the medial prefrontal cortex following trace fear conditioning (Reis et al., 2013). Additionally, retrieval of contextual and auditory fear memories increases K48 polyubiquitination in the amygdala (Ferrara et al., 2019; Jarome et al., 2011), which is largely localized to synapses (Orsi et al., 2019). While no study has directly manipulated K48 ubiquitin chains in the brain, which is due to the diversity of ubiquitin ligases involved in conjugation of this linkage site, indirect evidence from proteasome inhibition strongly implicates K48 polyubiquitination in synaptic plasticity and memory formation and modification (Dong, Bach, Haynes, and Hegde, 2014; Dong et al., 2008; Ehlers, 2003; Ferrara et al., 2019; Jarome, Ferrara, Kwapis, and Helmstetter, 2015; Jarome et al., 2011; Lee, 2008; Lee et al., 2008). Furthermore, while the protein targets of K48 polyubiquitination during memory formation and storage have yet to be identified, a number of strong candidates exist based off of proteasome targeting including the synaptic scaffolds SHANK and GKAP, the RNAi-induced silencing complex (RISC) factor MOV10 and the epigenetic modifier HDAC7 (Jarome et al., 2011; Jing et al., 2017; Lee et al., 2008).

4.2. K63 polyubiquitination

Unlike K48-linked chains, K63 polyubiquitination has seldom been examined in the brain. One study found that K63 polyubiquitination is abundant in the mouse forebrain and hippocampus and that in cultured hippocampal neurons, PSD95 can undergo K63 polyubiquitination (Ma et al., 2017). This modification of PSD95 occurred via the coordinated actions of the E2 complex UBC13/UEV1a and E3 ligase TRAF6 and could be reversed by the deubiquitinating enzyme CYLD. Neuronal stimulation resulted in a rapid decrease in K63 conjugates globally and locally at synapses and silencing CYLD abolished chemically-induced long-term depression (LTD), suggesting that loss of K63 polyubiquitination may be related to K48-linked chain degradation during synaptic plasticity. Consistent with this relationship but with an inverse pattern, recently, our group found that learning in a contextual fear conditioning paradigm increased K63 polyubiquitination in the amygdala, which correlated with increased K48-linked polyubiquitination (Orsi et al., 2019). Interestingly, these changes in K63 polyubiquitin were present in the nucleus, but not in the cytoplasm or at synapses, suggesting that it could be involved in transcriptional control or the DNA damage response, as the latter has been shown to occur as a result of learning (Madabhushi et al., 2015). Additionally, we also found that in the amygdala, K63 polyubiquitination increased at synapses and in the nucleus, but not in the cytoplasm, following retrieval (Orsi et al., 2019). Collectively, these results suggest potentially unique and contradicting functions for K63 polyubiquitination in synaptic plasticity and memory formation. However, these remain the only investigations of how K63-linked ubiquitin chains may be involved in activity-and learning-dependent synaptic plasticity. Furthermore, the protein targets and functional significance of K63 polyubiquitination to memory formation or other forms of synaptic plasticity has yet to be elucidated.

4.3. Linear polyubiquitination

Similar to K63 polyubiquitination, linear ubiquitination has been largely unexplored in the brain, and, to date, only one study has examined the role of this polyubiquitin chain in activity- or learning-dependent synaptic plasticity. Our group found that following contextual fear conditioning linear polyubiquitination increased in the nucleus, but not cytoplasm or at synapses, in the amygdala (Orsi et al., 2019). These results support a potential role of linear polyubiquitination in transcriptional control during memory formation, though this has never been directly tested. We also found that memory retrieval again increased linear polyubiquitination, though this was selective to synapses, suggesting a potential role for this ubiquitin modification in the reconsolidation process. However, similar to K63-linked chains, the protein targets and functional significance of linear polyubiquitination to synaptic plasticity and memory formation have yet to be elucidated.

4.4. Monoubiquitination

While less studied than polyubiquitination, some evidence suggests that protein monoubiquitination is also critical for synaptic plasticity and memory formation. The first study to investigate this modification in the brain found that neuronal stimulation increased nonproteolytic monoubiquitination of CPEB3, which occurred via the E3 ligase Neuralized1, and this was critical for the consolidation of hippocampus-dependent spatial memories (Pavlopoulos et al., 2011). Additionally, one recent study found that TrkB, the receptor for BDNF, carried a multimonomubiquitin modification, which was subsequently deubiquitinated (removed) in stimulated hippocampal neurons (Guo et al., 2017). Furthermore, inhibiting removal of the multimonomubiquitin modification from TrkB impaired hippocampus-dependent spatial memory, suggesting that multimonomubiquitin modifications may act to limit memory formation. However, beyond this, little is known about the role of protein single or multi monoubiquitination in activity- and learning-dependent synaptic plasticity.

5. Technical limitations and future directions

Despite recent evidence that some degradation-independent polyubiquitin chains are likely involved in synaptic plasticity and memory formation, much still remains unknown about the ubiquitin code during these processes. This largely results from several technical limitations, which have hindered identifying and manipulating diverse polyubiquitin chains in the brain. However, as technology has started to advance in this field, it is becoming increasingly more likely that many different polyubiquitin linkage sites will be revealed to play a significant role in various cellular processes during activity- and learning-dependent synaptic plasticity. In this section, we review the technical limitations that have slowed the study of the ubiquitin code in brain tissue and what the next steps the field should take to remedy the resulting lack in knowledge. Additionally, we speculate on the potential functions of diverse polyubiquitin chains in synaptic plasticity and memory formation.

5.1. Technological limitations in identifying and manipulating diverse polyubiquitin chains in the brain

While recent technological advancements have increased our understanding of the ubiquitination process in various cell and tissue types (Choi et al., 2019; Michel, Swatek, Hospenhal, and Komander, 2017), a number of limitations have hindered our understanding of diverse polyubiquitin chains in the brain. One of the main technological limitations is in identifying different polyubiquitin linkage sites. For example, one of the most widely published polyubiquitin antibodies, the FK2 clone, cannot distinguish between the many different linkage sites, resulting in the quantification of overall polyubiquitination irrespective of the type of chain a target protein has acquired (Fujimuro, Sawada, and Yokosawa, 1994). However, recent developments of linkage-specific polyubiquitin antibodies have helped to bridge this technology gap (Newton et al., 2008) and, to date, commercially available antibodies are now available that can detect K11, K27, K48, K63 and M1/linear in a broad range of species and tissue types. Additionally, the development of Tandem Ubiquitin Binding Entities (TUBEs), when combined with mass spectrometry, now allow for the identification of K48, K63, and M1-targeted protein substrates (Hjerpe et al., 2009). Despite this, the spectrum of commercially available antibodies and TUBE reagents remains limited, and some of the less studied linkage sites, such as K6, K29, and K33, remain difficult to quantify. Different polyubiquitin chains can also be identified via electrophoretic mobility as each linkage site migrates differently during SDS-PAGE (Emmerich and Cohen, 2015; Komander et al., 2009). Furthermore, linkage-specific ubiquitin chains on a protein of interest can be determined by using immobilized ubiquitin-binding domains (UBD) of defined ubiquitin chain specificity, followed by immunoblotting. This method is capable of detecting M1, K29, K33, and K63 chains on a target protein (Scott et al., 2015). Finally, polyubiquitin chains can also be identified by treatment of the tissue with specific deubiquitinating enzymes, as some have a high affinity for only one type of linkage site (Mevisen et al., 2013). Despite this, a majority of these methods, except for the simultaneous use of multiple UBDs and TUBES, are incapable of deciphering between homogeneous and mixed polyubiquitin chains and no method is able to identify whether the linkage type is branched. Consequently, while these technological developments have aided in the recent study of linkage-specific polyubiquitin chains in synaptic plasticity and memory formation (Jarome et al., 2011; Orsi et al., 2019), a number of methodological barriers still exist.

Even when identified, a major hurdle in understanding the role of a specific polyubiquitin chain in synaptic plasticity comes from the inability to specifically manipulate that linkage site in a given brain region. A common strategy is to target the E3 ligase responsible for conjugating that specific polyubiquitin chain to the target substrate (Tokunaga et al., 2011), which could be achieved via controlled knockout or RNAi, and has been effective at elucidating the role of specific ubiquitin conjugating enzymes to various behavioral processes and disease states (e.g., Pick, Malumbres, and Klann, 2013; Pick, Wang, Mayfield, and Klann, 2013; Werner et al., 2018; Yao et al., 2011). However, this is complicated by the diversity and promiscuous nature of E3 ligases as, with the exception of linear polyubiquitination, multiple E3 ligases are capable of conjugating specific ubiquitin chains to a target substrate. Thus, this approach often provides very little information about the role

of a specific polyubiquitin linkage site in the biological process examined. This issue potentially could be partially overcome by combining ubiquitin E3 ligase manipulation with mass spectrometry, which could identify the specific type of polyubiquitin chains altered by loss of the protein. However, again it still remains likely that the E3 ligase could be regulating multiple polyubiquitin linkage sites. A better approach would be to make point mutations in specific linkage sites on ubiquitin, a method that has been widely used in yeast and various human cell lines but has yet to be applied to the brain (e.g., Meza Gutierrez et al., 2018; Petroski and Deshaies, 2005). A potential concern with these point mutations could be cell viability following mutations that lack temporal and spatial control as various polyubiquitin chains regulate cellular processes with critical roles in early brain development and loss of even a single ubiquitin coding gene can be lethal (Bowerman and Kurz, 2006; Chen et al., 2011; Hallengren, Chen, and Wilson, 2013; Kobayashi et al., 2016; Park and Ryu, 2014; Ryu et al., 2008). Thus, even if the levels of a specific polyubiquitin chain are shown to change in brain tissue during activity- or learning-dependent synaptic plasticity, specific manipulation of this linkage site can be difficult. However, it is possible that this issue could be circumvented by rapidly expanding technology, such as viral-mediated overexpression of ubiquitin dominant negative point mutations or via use of recently developed single base-pair DNA and RNA editing tools, including CRISPR-dCas9nuclease or CRISPR-Cas13 (Cox et al., 2017; Navabpour, Kwapis, and Jarome, 2020; Shen et al., 2014), which could control endogenous ubiquitin mutation expression in the adult brain. Consistent with this, recent evidence confirms that various CRISPR platforms can successfully control gene expression selectively in the adult brain via stereotaxic delivery (Butler, Johnston, Kaur, and Lubin, 2019; Kwapis et al., 2018; Savell et al., 2019), which would allow these point mutations to be expressed after the critical developmental period. Thus, while the current tools cannot manipulate specific polyubiquitin linkage sites in the brain, the technology is there for such approaches to be developed.

5.2. Furthering our understanding of linkage-specific polyubiquitin chains in synaptic plasticity and memory formation

Despite the difficulties in identifying and manipulating various polyubiquitin chains in the brain, increasing evidence suggests that they likely play a significant role in activity- and learning-dependent synaptic plasticity. The initial focus of future studies should be on quantifying changes in these different polyubiquitin chains following neuronal stimulation or behavioral training. As stated above, while K48, K63, and M1 chains have been reported to increase as a function of neuronal stimulation and/or learning (Bingol et al., 2010; Ma et al., 2017; Orsi et al., 2019), K11 and K27 have yet to be studied despite the availability of antibodies to detect them. Additionally, for those that have been reported to have altered expression, a majority of the target proteins have yet to be identified (Jarome et al., 2011; Lee et al., 2008; Ma et al., 2017). This information is essential, though, to fully understand the functional role of linkage-specific polyubiquitin modifications in synaptic plasticity and memory formation, especially for the chains that are known to have many functions (K63, M1). Furthermore, a better understanding of how these chains interact during these processes is particularly important since some linkage sites have been shown to regulate specific cellular processes via a mixed confirmation, such as K63/M1 (Emmerich et al., 2013). Finally, manipulation of specific polyubiquitin linkage sites will be critical to define

the functional significance of any ubiquitin chain to synaptic plasticity and memory formation, which may require the development of new technology, as discussed above.

5.3. Potential functions of linkage-specific polyubiquitin chains in synaptic plasticity and memory formation

Even as evidence has started to emerge supporting a role for diverse polyubiquitin chains in synaptic plasticity and memory formation, questions still remain regarding what the functional significance of these modifications are to these processes. However, based on work in cultures or the periphery and what is currently known about the molecular mechanisms of activity- and learning-dependent synaptic plasticity, a number of intriguing potential candidate functions exist. For example, although only studied outside the brain, to date, various polyubiquitin chains (K63, M1) can activate the NF- κ B transcriptional pathway, which plays a significant role in the long-term memory and synaptic plasticity (Kaltschmidt and Kaltschmidt, 2015). Also, considering the recently identified important role of double-stranded DNA breaks in chromatin remodeling during synaptic plasticity and memory formation (Madabhushi et al., 2015) and the well described role of various polyubiquitin chains in this process outside the brain (K6, K27, K63), there is a need for further studies to address the question of how specific polyubiquitin modifications regulate the DNA damage response during these processes (Merlo, Cuchillo-Ibañez, Parlato, and Rammes, 2016). Furthermore, mitochondria are a cellular organelle implicated in many psychological processes, especially ones that increase neuronal firing rates, such as learning and memory (Hebert-Chatelain et al., 2016) and synaptic plasticity (Todorova and Blokland, 2017), since it regulates cellular respiration and energy production. As we discussed earlier, various polyubiquitin chains have a role in mitochondria homeostasis (K6, K63). Based on this, there is a critical need to investigate the role of mitochondria polyubiquitination in synaptic plasticity and memory formation. Some ubiquitination marks also play a substantial role in receptor trafficking (mono, K33, K63), such as the deubiquitination of RTKs, membrane-bound receptors that facilitate the communication between cells and their environment, which triggers their endocytosis and recycling (Critchley et al., 2018). Interestingly, strong evidence indicates that RTKs have multiple functions in memory formation and synaptic plasticity (Giese and Mizuno, 2013); however, whether ubiquitination/deubiquitination of RTKs critical to these processes remains unknown. Thus, there are numerous ways in which diverse polyubiquitin modifications could contribute to activity- and learning-dependent synaptic plasticity, which should help guide future studies attempting to elucidate the role of the complex ubiquitin code in memory formation.

6. Conclusions

Strong support exists suggesting a role for ubiquitin-proteasome mediated protein degradation in synaptic plasticity and memory formation. While most protein substrates will be marked for degradation by a K48-linked polyubiquitin modification, there are at least seven other known linkage-specific polyubiquitin chains (M1, K6, K11, K27, K29, K33, K63) that a target protein can acquire, which can be independent of the proteasome and instead impede protein degradation or regulate kinase activation, endocytosis, the DNA damage response, mRNA stability, or apoptosis. Furthermore, in addition to homogenous

chains, mixed or branched polyubiquitin modifications can target substrates for cellular fates that are dependent or independent of the protein degradation process. However, as the focus has remained on protein degradation, much remains unknown about the role of these diverse polyubiquitin chains in the brain during activity and learning-dependent synaptic plasticity. Here, we reviewed the known functions of these diverse polyubiquitin chains and evidence implicating some of them in synaptic plasticity and memory formation. Additionally, we discussed the technical limitations and recent advancements in this field, which may help with the identification and study of linkage-specific polyubiquitination chains in the brain. Collectively, the studies outlined in this review suggest diverse polyubiquitin modifications are likely involved in various cellular processes that are independent of protein degradation and underlie memory formation.

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Abbreviations

AKT	Protein kinase B
BDNF	Brain-derived neurotrophic factor
CaMKII	Calcium/calmodulin-dependent protein kinase II
cFLIP	Cellular fllice-like inhibitor protein
CPEB3	Cytoplasmic polyadenylation element binding protein 3
CREB	Cyclic AMP response element binding protein
CYLD	Cylindromatosis tumor-suppressor protein
ERK/MAPK	Extracellular signal regulated kinase
FOX04	Forkhead box protein 04 peptide
GKAP	Disks large-associated protein 1
HDAC7	Histone deacetylase 7
HOIP (RNF31)	Ring Finger 31
IKK	The inhibitor of nuclear factor kappa B kinase
KDM4D	Histone lysine demethylase 4D
LUBAC	Linear ubiquitin chain assembly complex
MOV10	Mov10 RISC complex RNA helicase
mTOR	Mammalian target of rapamycin
NEMO	NF-kappa B essential modulator

NF-κB	Nuclear factor kappa B
PKA	Protein kinase A
PKC	Protein Kinase C
PSD95	Postsynaptic density protein 95
Rhbdd3	Rhomoid domain containing 3
RICK	RIP like interacting CLARP kinase
RIP1	Receptor interacting protein
RPN10	26S proteasome non-ATPase regulatory subunit 4
RPN13	Proteasomal ubiquitin receptor ADRM1
Rps27a	Ribosomal protein S27a
RPT6	Regulatory particle triple-A ATPase 6
RTK	Receptor Tyrosine Kinase
SHANK	SH3 and multiple ankyrin repeat domains
TAB2	TGF-beta activated kinase 1 MAP3K7 binding protein 2
TNF-R1	Tumor necrosis factor receptor 1
TRAF2	TNF receptor associated factor 2
TRAF6	TNF receptor associated factor 6
TrkB	Tropomyosin receptor kinase B
Uba52	Ubiquitin A-52 Residue Ribosomal Protein Fusion Product 1
Ubb	Ubiquitin B
Ubc	Ubiquitin C
Wnt	Wingless and Int-1

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Highlights

- Polyubiquitin chains occur at 8 different linkage sites and can be mixed or branched
- Many of these chains are involved in processes independent of the proteasome
- The K48, K63 and M1 chains have been implicated in memory formation
- K6, K11, K27, K29, K33 and mixed/branched chains have yet to be studied in the brain

Figure 1. The complexity of the ubiquitin code.

(A) Illustration of the four genes that code for ubiquitin and the number of ubiquitin molecules each code for. (B) The sequence of the 76 amino acid ubiquitin protein showing the 8 sites at which polyubiquitin chains can form. (C) The different structures of ubiquitin chains, including mono, homogeneous poly, mixed poly and branched poly forms.

Mixed or Branched Linkage Type	Function
NF-κB activation
NF-κB activation Protein degradation
Impede degradation
Protein degradation
Endocytosis

Figure 2. Known functions of mixed and branched polyubiquitin chains. Illustration of mixed and branched polyubiquitin chains (left) and their known functions (right). Mixed chains are indicated by a single ubiquitin (Ub) column attached to the base ubiquitin. Branched chains are indicated by multiple (2–3) ubiquitin columns attached to the base ubiquitin. Arrows denote the linkage type for that specific site.

Table 1:

The diverse functions of ubiquitin chains

Ubiquitin linkage	Promote degradation	Impede degradation	DNA damage response	Kinase in/activation*	mRNA stability	Epigenetics	Trafficking	Endocytosis	Apoptosis	Autophagy
Mono	X			X		X	X	X		
M1/Linear	X	X		X					X	
K6		X	X							
K11	X?									
K27		X	X	X						
K29				X	X	X				
K33	X?			X			X			
K48	X									
K63	X		X	X			X	X		X
Mixed M1/K63				X						
Branched K48/K63	X			X						
Branched K48/K63/K11		X								
Branched K11	X									

* This category includes protein binding activity

Table 2:

Ubiquitin modifications in synaptic plasticity and memory formation

Ubiquitin linkage	Neuronal stimulation	Learning	Critical for memory
Mono	Increase	Increase	Yes - positive regulator
Multimono	?	Decrease	Yes - negative regulator
M1/Linear	?	Increase	?
K6	?	?	?
K11	?	?	?
K27	?	?	?
K29	?	?	?
K33	?	?	?
K48	Decrease	Increase	Yes - positive regulator
K63	Decrease	Increase	?
Mixed	?	?	?
Branched	?	?	?