Neurodegeneration: From Hyperexcitability to Pathologic Tau, TDP-43 and α-Synuclein

Frank Lee
eMail: frank@geon.us
Website: http://www.geon.us

Posted on: March 30, 2017

Abstract

Amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam (ALS/PDC) is caused by misincorporation of β-N-methylamino-L-alanine (BMAA) into normal proteins, resulting in protein misfolding. Experiments have shown that protein misfolding may promote mTOR activation, leading to hyperexcitability (Paper 3). ALS/PDC exhibits Tau, TDP-43 and α-synuclein pathology, suggesting that these pathologies could originate from hyperexcitability. The underlying mechanisms are discussed in this paper. Although Ca^{2+} toxicity is a common consequence of hyperexcitability, the specific pathology depends on the neuronal compartment where Ca^{2+} enters the neuron. For Tau pathology, which occurs in the neurons containing "microtubule antennas" for long range synchronization (Paper 1), Ca^{2+} is likely to enter the neuron through T-type calcium channels at the axon initial segment, resulting in calpain activation and consequently leading to Tau hyperphosphorylation by GSK-3 or CDK5. The TDP-43 pathology may result mainly from over-stimulation of Ca^{2+}-permeable AMPA receptors at the dendritic spines that synapse with neurons participating in long range synchronization. In Parkinson's disease, the α-synuclein pathology occurs in the dopaminergic (DA) neurons of substantia nigra pars compacta (SNc), resulting from glutamate-mediated hyperexcitability which causes excessive Ca^{2+} influx through L-type calcium channels at axon terminals. The Ca^{2+} overload in DA neurons promotes α-synuclein aggregation, which inhibits tubulin polymerization into microtubules. As discussed in Paper 2, the highly negatively charged tubulin can regulate excitability. Elevated tubulin at the axon terminal should attenuate excitability, thereby reducing dopamine release.

Introduction

Hyperphosphorylated Tau (Grundke-Iqbal et al., 1986), phosphorylated and cleaved TDP-43 (Hasegawa et al., 2008; Yamashita et al., 2012), and oligomeric α-synuclein

(Roberts and Brown, 2015) are the pathological hallmarks for Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and Parkinson's disease, respectively. Frontotemporal dementia (FTD) is characterized by Tau and TDP-43 pathologies, with roughly equal prevalence (Seeley, 2008). Paper 5 suggests that they may all arise from neuronal hyperexcitability. This paper will discuss how hyperexcitability can lead to these pathologies.

Hyperexcitability results in excessive calcium ions (Ca^{2+}) inside the neuron. It has been well documented that calcium dysregulation may cause neurodegeneration (Mattson, 2007), but the underlying mechanism remains unclear. Previous works postulated that the aberrant Ca^{2+} influx might originate from the toxic forms of amyloid β-peptide (Aβ) (Bezprozvanny and Mattson, 2008; Zündorf and Reiser, 2011). This idea could be influenced by the Amyloid Cascade Hypothesis (Hardy and Higgins, 1992) which asserts that Aβ toxicity is the origin of Alzheimer's disease. Since its inception, numerous Aβ-targeting drugs have been developed, but to date none of them showed any reduction in neurofibrillary tangles or improvement in cognitive performance of patients with Alzheimer's disease (Dai et al., 2017).

In contrast to the Amyloid Cascade Hypothesis, the Wireless Communication Model centers on the Tau protein. Within this framework, the Ca^{2+} overload originates from hyperexcitability, rather than Aβ. The last two decades have revealed great insight into the pathogenic cascade following Ca^{2+} overload, including α-synuclein aggregation into oligomers. Interestingly, the oligomeric α-synuclein was found to inhibit tubulin polymerization (Chen et al., 2007). More than three decades ago, experiments have demonstrated that tubulin polymerization is linked to excitability (Sakai et al., 1985), consistent with the notion that tubulin and microtubule may play a critical role in regulating excitability (see Paper 2). This underappreciated function can readily explain why oligomeric α-synuclein impairs dopamine release.

Tau Pathology

From Ca^{2+} overload to Tau hyperphosphorylation

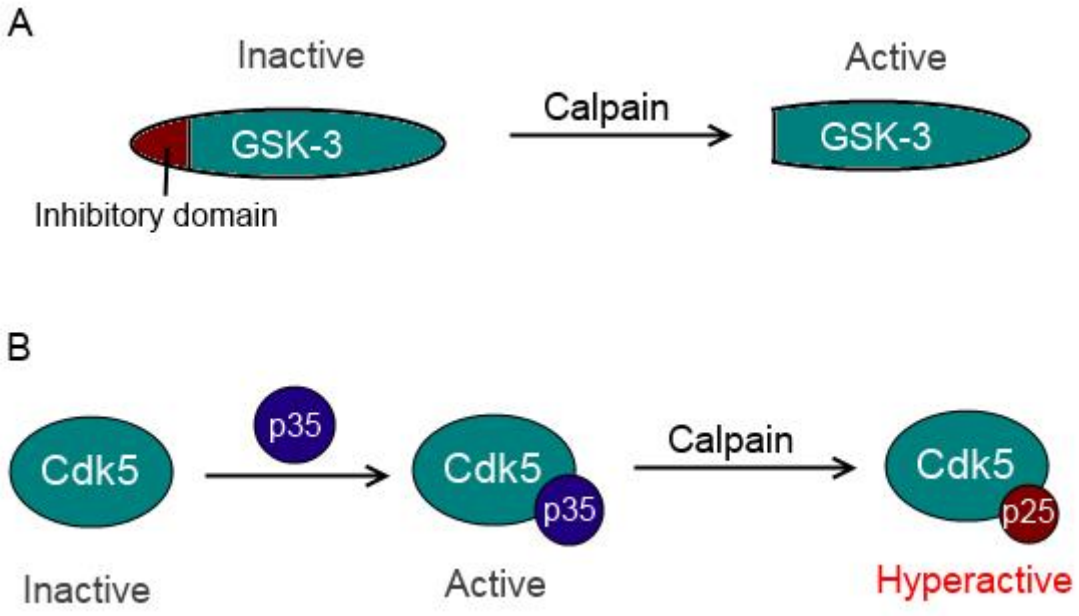

Figure 1. Regulation of GSK-3 and Cdk5 by calpain. (A) In the inactive form, GSK-3 contains an inhibitory domain. Calpain may remove this inhibitory domain to activate GSK-3 (Goñi-Oliver et al., 2007). (B) Cdk5 is normally activated by binding with the protein p35. Calpain may cleave p35 to p25, forming a hyperactive Cdk5/p25 complex.

Glycogen synthase kinase-3 (GSK-3) and cyclin dependent kinase 5 (Cdk5) are the major protein kinases capable of phosphorylating Tau at multiple sites (Lovestone et al., 1994; Kimura et al., 2014). GSK-3 has been demonstrated to be essential in the pathogenesis of Alzheimer's disease (Takashima, 2006). It is a promising therapeutic target for this devastating disease (Maqbool et al., 2016). Both GSK-3 and Cdk5 can be regulated by calpain (Figure 1) which is a Ca^{2+}-dependent protease that cleaves proteins (Ferreira, 2012). Hence, Ca^{2+} overload may stimulate calpain to activate GSK-3 and/or Cdk5, resulting in Tau hyperphosphorylation.

From Tau hyperphosphorylation to microtubule breakdown

The Tau protein is expressed only in neurons, and localized predominantly to the axon. Normal Tau binds with tubulin and promotes its assembly into a microtubule. Hyperphosphorylated Tau may aggregate with normal Tau to form neurofibrillary tangles. This will reduce the amount of normal Tau and result in microtubule disintegration at the axon (Alonso et al., 1996). Furthermore, the hyperphosphorylated Tau can pass the axon initial segment (AIS) (Li et al., 2011) and translocate to dendrites where they may trigger microtubule severing by TTLL6

and spastin (Zempel et al., 2013). The microtubule breakdown will eventually lead to neuronal death.

TDP-43 Pathology

From Ca^{2+} overload to abnormal TDP-43

TDP-43 can be cleaved by calpain (Yamashita et al., 2012). Its pathologic phosphorylation is catalyzed by casein kinase-1 (CK1) (Hasegawa et al., 2008). As described above, calpain is directly regulated by Ca^{2+}. CK1 also depends on Ca^{2+}, but indirectly. Ca^{2+} may stimulate protein phosphatase 2B (PP2B or calcineurin) to dephosphorylate CK1, resulting in its activation (Liu et al., 2002; Nakano et al., 2010).

AMPA receptors are implicated in both ALS (Kawahara et al., 2004) and FTD (Gascon et al., 2014). They are composed of four types of subunits, designated as GluR1 - GluR4. The presence of the GluR2 subunit renders the AMPA receptor impermeable to Ca^{2+}. Over-expression and/or over-stimulation of Ca^{2+} permeable AMPA receptors may cause excessive Ca^{2+} influx through dendritic spines, thereby resulting in neurodegeneration.

AMPA receptors belong to glutamate receptors which can be activated by the excitatory neurotransmitter glutamate. As discussed in Paper 5, ALS may begin in the motor cortex where a population of neurons oscillate at the beta band. These neurons will be referred to as "beta neurons". Hyperexcitable beta neurons may release glutamate to stimulate motor neurons in the motor cortex, i.e., Betz cells, leading to Ca^{2+} overload inside the Betz cells. In many types of cells, the toxicity of Ca^{2+} overload can be alleviated by Ca^{2+} buffering proteins, which are a special class of Ca^{2+} binding proteins that bind transiently with Ca^{2+} ions to prevent further Ca^{2+} signaling (Schwaller, 2010). Motor neurons express low level of Ca^{2+} buffering proteins (e.g., calbindin and parvalbumin) (Jaiswal, 2014), which makes them particularly vulnerable to Ca^{2+} toxicity.

Tau-positive vs.Tau-negative ALS

From the previous section, it is clear that neurons may display Tau pathology if their AIS is enriched with calcium channels, calpain, GSK-3 and Tau proteins. Generally speaking, they are the neurons whose AIS contains microtubule antennas for long range synchronization, which requires Ca^{2+} to regulate excitability via microtubule

dynamics (Paper 2). Motor neurons do not participate in beta synchronization. Hence Tau-negative ALS may occur if Ca^{2+} overload in Betz cells results mainly from over-expression of Ca^{2+}-permeable AMPA receptors, rather than over-stimulation by beta neurons, consequently leading to severe TDP-43 pathology in motor neurons, while beta neurons are spared.

In the Guam type ALS, neurofibrillary tangles are abundant not only in the primary motor cortex (Hof and Perl, 2002), but also in the entorhinal, frontal and temporal cortex (Hof et al., 1991; Hof et al., 1994). The Guam type ALS was originally discovered on the island of Guam in the Pacific (Arnold et al., 1953). It is caused by a non-protein amino acid, β-N-methylamino-L-alanine (BMAA), which can be misincorporated into normal proteins, resulting in protein misfolding (Dunlop et al., 2013). Misfolded proteins are known to enhance mTOR activity, thereby increasing the risk for Tau pathology (Paper 3). The BMAA-induced ALS also exhibits features of Parkinson's disease and Alzheimer disease (Miklossy et al., 2008). This demonstrates the common origin of these neurodegenerative disorders.

From abnormal TDP-43 to neurofilament dysregulation

Neurofilaments are a major component of the neuronal cytoskeleton, located primarily in the axon. Their dysregulation, which impairs axonal transport, has been shown to play an important role in the pathogenesis of ALS (Collard et al., 1995). In patients with ALS, the level of neurofilaments in the cerebrospinal fluid (CSF) is significantly elevated (Xu et al., 2016). A neurofilament is composed of polypeptide chains (subunits), which are classified into light, medium or heavy chains, on the basis of their molecular weight. The FTD, particularly its TDP-43 subtype, is associated with elevated neurofilament light chains in CSF (Landqvist-Waldö et al., 2013).

TDP-43 is involved in the biogenesis of microRNAs, including miR-132 and miR-9 (Paper 4). miR-132 regulates Tau expression while miR-9 targets neurofilaments. A single miR-9 binding site was observed on the mRNA of the neurofilament light chain, compared to nine miR-9 binding sites in the heavy chain mRNA (Haramati et al., 2010). Experiments have shown that TDP-43 mutations result in miR-9 down-regulation (Zhang et al., 2013), which may account for neurofilament dysregulation in TDP-43 pathology.

α-Synuclein Pathology

α-Synuclein localizes primarily to the presynaptic terminal (Iwai et al., 1995). Their aggregation into oligomers is the major cause of neurodegeneration. A plethora of possible mechanisms were proposed, but no consensus has been reached yet (Roberts and Brown, 2015). Several lines of evidence suggest that tubulin polymerization into microtubules plays a pivotal role.

1. α-Synuclein induces polymerization of purified tubulin into microtubules. Mutant forms of α-synuclein lose this potential. (Alim et al., 2004; Cartelli et al., 2016).
2. Oligomeric α-synuclein inhibits tubulin polymerization (Chen et al., 2007)
3. Parkin, whose mutations cause a familial form of Parkinson's disease, reduces microtubule depolymerization (Ren et al., 2009).
4. Leucine-rich repeat kinase 2 (LRRK2), whose mutations are the most common genetic cause of late-onset Parkinson's disease, regulates microtubule stability by tubulin phosphorylation and acetylation (Gillardon, 2009; Law et al., 2014).

From Ca^{2+} overload to α-synuclein aggregation

At the nerve terminal, Ca^{2+} entry is required to trigger the release of neurotransmitters, including dopamine (Okita et al., 2000). In most cases, Ca^{2+} enters the nerve terminal via L-type channels, whose antagonists are protective for Parkinson's disease (Ilijic et al., 2011; Swart and Hurley, 2016). This supports the view that Ca^{2+} overload at the nerve terminal leads to α-synuclein pathology. Mechanistically, the terminal Ca^{2+} overload, together with induced oxidative stress, promote α-synuclein aggregation (Nath et al., 2011; Goodwin et al., 2013; Rcom-H'cheo-Gauthier et al., 2014; Rcom-H'cheo-Gauthier et al., 2016).

From α-synuclein aggregation to microtubule deficiency

The toxicity of α-synuclein aggregation is a subject of intensive research. As described above, tubulin and microtubule may play a central role. A tubulin dimer contains about 50 more negatively than positively charged residues (Minoura and Muto, 2006). In a solution, tubulin dimers are surrounded by counterions and polar water molecules which may reduce the electrostatic interaction with external fields. The effective charge on a tubulin dimer was estimated to be 12 - 20 e^- (van den Heuvel et al., 2006; Minoura and Muto, 2006). Such negative charges can exert a hyperpolarizing field on voltage-activated ion channels, thereby regulating neuronal excitability (see Paper 1, Figure 1). Therefore, at the axon terminal, depolymerized free tubulin dimers may move close to the membrane to inhibit neuronal firing via

their hyperpolarizing fields. Tubulin polymerization into microtubules will reduce the amount of free tubulin dimers, thus enhancing excitability. Oligomeric α-synuclein has been shown to inhibit tubulin polymerization (Chen et al., 2007). This explains why α-synuclein oligomers reduce neuronal excitability (Kaufmann et al., 2016) and why dopamine release is impaired in Parkinson's disease.

In Tau pathology, hyperexcitability due to excessive Tau proteins eventually leads to hypoexcitability caused by Tau hyperphosphorylation (Paper 2). Similarly, in α-synuclein pathology, hyperexcitability due to excessive glutamate stimulation also leads to hypoexcitability, but in this case it is caused by high level of free tubulin dimers. Reduction in tubulin polymerization affects not only excitability, but also the amount of functional microtubules. Microtubule deficiency may impair axonal transport of essential cellular components (Pellegrini et al., 2017), eventually leading to neuronal death.

Summary

When a neuron fires, Ca^{2+} may enter the neuron through voltage-gated calcium channels, such as T-type calcium channels at AIS or L-type calcium channels at axon terminals. Moreover, the firing of glutamatergic neurons may release glutamate to excite postsynaptc neurons. Over-stimulation of Ca^{2+}-permeable glutamate receptors may lead to Ca^{2+} overload in the postsynaptc neurons. The Wireless Communication Model suggests that hyperexcitability of the neurons (theta or beta) whose AIS contains microtubule antennas for long range synchronization may cause Ca^{2+} overload via AIS, leading to Tau pathology. The TDP-43 pathology may result from over-stimulation of glutamate receptors (the AMPA type) at the dendritic spines that synapse with hyperexcitable theta or beta neurons. The α-synuclein pathology may arise from Tau-mediated hyperexcitability in the subthalamic nucleus (STN), resulting in glutamate-mediated hyperactive dopaminergic (DA) neurons in substantia nigra pars compacta (SNc). Subsequently, the hyperactive DA neurons may cause excessive Ca^{2+} influx through L-type calcium channels at axon terminals. The pathogenic cascade following Ca^{2+} overload is depicted in Figure 2.

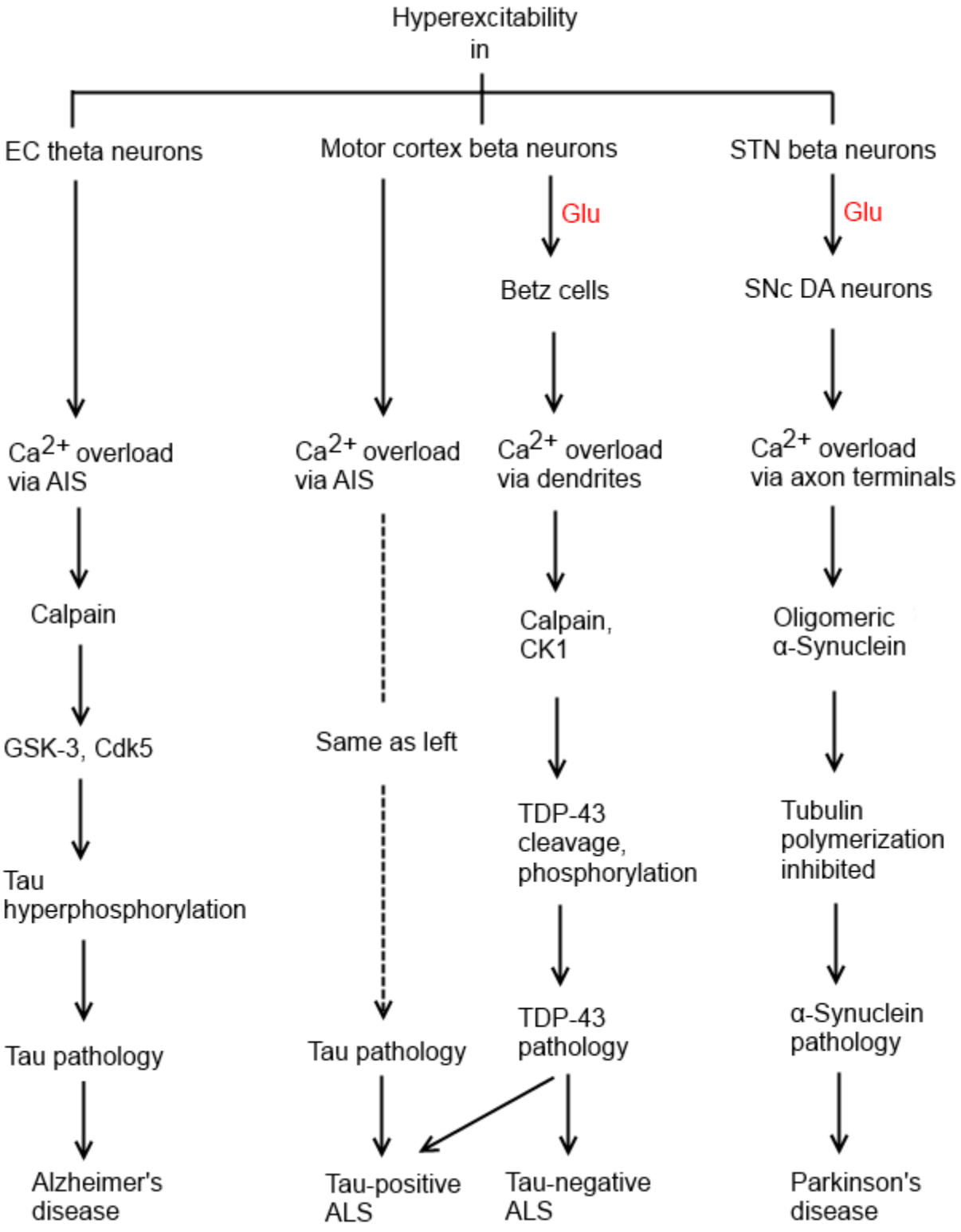

Figure 2. The proposed pathogenic cascade triggered by hyperexcitability of the neurons participating in long range synchronization. Theta and beta neurons refer to the neurons that oscillate at the theta and beta bands, respectively. Glu: glutamate; EC: entorhinal cortex; CK1: casein kinase-1.

The Guam type ALS, also known as ALS/parkinsonism-dementia complex of Guam (ALS/PDC), exhibits all three pathologies discussed above (Miklossy et al., 2008). This demonstrates their common origin. ALS/PDC is caused by the non-protein amino acid, BMAA, which can be misincorporated into normal proteins, resulting in protein misfolding (Dunlop et al., 2013). Experiments have shown that protein misfolding may promote mTOR activation, and consequently hyperexcitability (Paper 3). Figure 3 illustrates the BMAA-induced pathogenic cascade. This figure does not imply that activation of mTOR will definitely lead to all of these pathologies. Rather, mTOR activation simply increase the probability of developing pathologies. Many other factors are also involved in neurodegeneration, such as the expression level of miR-132, TDP-43, α-synuclein, GluR2, calpain, GSK-3, etc. The reason why BMAA induces all three pathologies is probably because mTOR activation by BMAA-containing proteins is overwhelming.

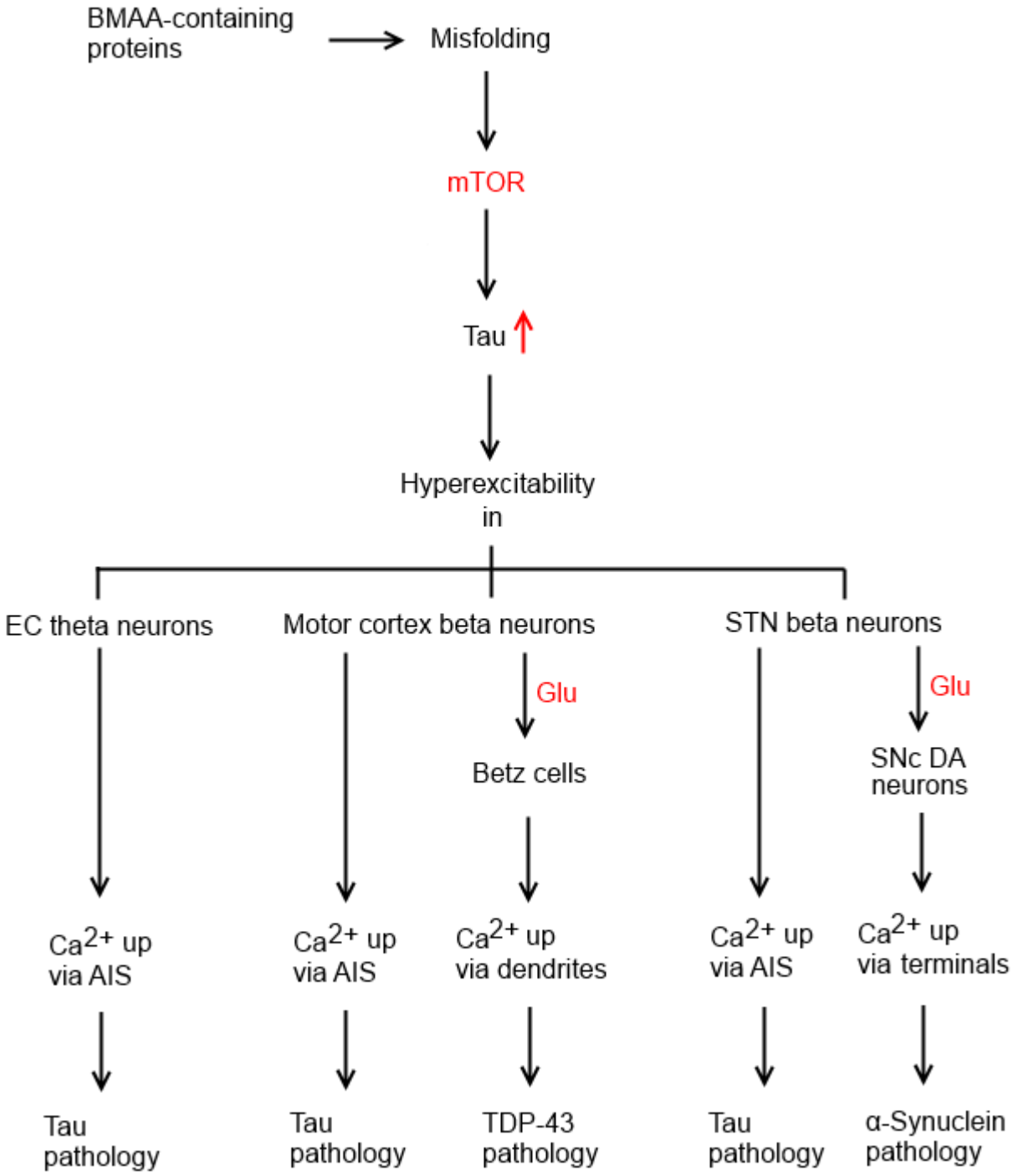

Figure 3. The proposed pathogenic cascade induced by misincorporation of β-N-methylamino-L-alanine (BMAA) into normal proteins.