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NMDAR-dependent LTP versus LTD induction: The role of Ca2+ influx amplitude

Literature Review

Long-term potentiation (LTP) and long-term depression (LTD) are two types of synaptic plasticity thought to be involved in learning and memory. Specifically, LTP refers to the strengthening of synapses while LTD refers to the weakening of synapses. Interestingly, both N-methyl-D-aspartate receptor-dependent LTP and LTD are induced by a calcium influx into the post-synaptic cell. This raises the question of how the cell selectively induces either LTP or LTD. Early research suggested the amplitude of calcium influx as a decisive mechanism. The essay critically evaluates this hypothesis by reviewing evidence and alternative candidates, namely the timing and location of calcium influx, N-methyl-D-aspartate receptor subunits, and the competition between α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor exocytosis and endocytosis. The essay concludes that the amplitude of calcium influx should be seen as only one of multiple components entailed in the complex decisive machinery for selective LTP versus LTD induction.

Keywords: neuroplasticity, long-term potentiation, long-term depression, calcium, NMDA receptors

INTRODUCTION

NMDAR-dependent LTP versus LTD induction: The role of Ca2+ influx amplitude

Synaptic plasticity, meaning activity-dependent changes in efficacy and strength of neuronal connections, is thought to underly learning and memory (Magee & Grienberger, 2020). Two types of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD). Specifically, the former refers to the strengthening of synapses, while the latter refers to the weakening of synapses (Pinar et al., 2017). The most prominent form of LTP and LTD in the central nervous system is N-methyl-D-aspartate receptor (NMDAR)-dependent, but there exist other NMDAR-independent forms of synaptic plasticity (e.g., glutamate metabotropic receptor-dependent LTP; Alkadhi, 2021). Interestingly, NMDAR-dependent LTP and LTD are both induced by the influx of calcium (Ca2+) into the post-synaptic cell. Thus, the question arises how the cell selectively chooses between LTP and LTD (Lüscher & Malenka, 2012; Alkadhi, 2021).

The post-synaptic membrane contains NMDARs and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). Both receptor types are ligand-gated ion channels that are activated by glutamate (Lüscher & Malenka, 2012). Upon glutamate binding, AMPARs allow the large influx of sodium (Na2+) with a simultaneous small efflux of potassium (K+) ions leading to the depolarization of the post-synapse (Chater & Goda, 2014). In contrast, activating NMDARs requires the binding of glutamate and the depolarization of the post-synapse. Only if

both requirements are met, a magnesium (Mg2+) ion is pulled outside the NMDAR pore, allowing the influx of Ca2+. Upon Ca2+ influx, kinases and phosphatases are activated which insert or remove synaptic AMPARs respectively, thereby, altering synaptic sensitivity in opposite directions (Malenka & Bear, 2004; Bartlett et al., 2007; Lüscher & Malenka, 2012).

As both types of NMDAR-dependent synaptic plasticity can be induced by Ca2+ influx, the cell must have a decisive mechanism to selectively induce LTP or LTD. Artola and Singer (1993) developed the differential threshold hypothesis, postulating that it is the amount of Ca2+ entering the post-synaptic cell that selectively induces LTP or LTD. Specifically, LTP in hippocampal cells can be experimentally induced by high-frequency stimulation (1s train at 100Hz) that causes a great rise in Ca2+, while LTD is induced by low-frequency stimulation (600-900 pulses at 1-5Hz), causing a lower rise in Ca2+ (Raymond, 2007; Massey & Bashir, 2007). Based on the differential threshold hypothesis, this essay critically evaluates whether the amount of Ca2+ influx can be seen as a decisive mechanism for selectively inducing NMDAR-dependent LTP or LTD.

Starting at the top – Amplitude, duration, location

The differential threshold hypothesis was supported by several blocking studies. As activating kinases requires a large Ca2+ influx and activating phosphatases requires a moderate Ca2+ influx, the idea was to block either of the two to investigate the subsequent effects on LTP and LTD induction, respectively. Specifically, inhibiting calcium-calmodulin kinase II (CaMKII) blocked LTP, while protein phosphatase inhibitors blocked LTD (Artola & Singer, 1993; Lisman, 1989). However, the Ca2+ influx is not only characterised by its amplitude, but also by its duration

and its location (Evans & Blackwell, 2015). By experimentally manipulating the amplitude of Ca2+ influx (e.g., by omitting the Mg2+ ion from the NMDAR pore or blocking NMDARs and/or AMPARs), a study revealed that the duration of stimulation is critical to induce either LTP or LTD (Mizuno et al., 2001). Several studies confirmed that LTP induction requires strong, brief stimuli, while LTD induction requires moderate, prolonged stimuli (e.g., Yang et al., 1999). Moreover, research suggests that the specific site of Ca2+ entry determines targets to which Ca2+ will bind and subsequently induces either LTP or LTD (Evans & Blackwell, 2015). For example, one study showed that activation of synaptic NMDARs induces LTP, while activation of extrasynaptic NDMARs induces LTD (Liu et al., 2013).

Taken together, numerous studies provided evidence in favour of post-synaptic Ca2+ influx being the decisive mechanism underlying selective LTP and LTD induction. However, LTP and LTD induction cannot be explained by the amplitude of the Ca2+ influx alone, but rather by a combination of the amplitude, duration, and location of the Ca2+ influx. Therefore, the differential threshold hypothesis should be complemented by a temporal and a spatial factor to increase its explanatory power (Evans & Blackwell, 2015). Even though, this conclusion is reasonable and in line with research findings, Ca2+ influx characteristics only pose a decisive candidate for inducing NMDAR-dependent LTP or LTD at the beginning of the synaptic plasticity cascade. Additionally, alternative candidates at later stages of the cascade should be considered.

Going one step further - NMDAR subunits

An alternative hypothesis suggests that it is the constellation of NMDAR subunits that selectively induces LTP or LTD, as NMDAR subunits confer different gating and pharmacological properties (Kash & Winder, 2007). NMDARs consist of two N1 and two N2 subunits. The constellation of N2 subunits can vary, as there are four subtypes (i.e., N2A, N2B, N2C, N2B) (Lüscher & Malenka, 2012). Support for the differential involvement of N2 subunits in LTP and LTD in hippocampal CA1 synapses of adult rats comes from Liu and colleagues (2004). They showed that administering a N2A antagonist (NVP-AAM077) resulted in the absence of LTP, while administering N2B antagonists (ifenprodil and R025-6981) prevented the induction of LTD (Liu et al., 2004). Similar findings were obtained for amygdalae NMDAR subunits in adult rats (Dalton et al., 2012).

However, there are contradictory findings. For example, a study has shown that administering a N2A antagonist (NVP-AAM077) inhibited LTD as well as LTP induction in hippocampal slices of two-week old rats (Bartlett et al., 2007). Additionally, this study found no effect of administering a N2B antagonist (R025-6981) on LTD, but on LTP induction (Bartlett et al., 2007). Even though, these contradictory findings might be explained by confounding factors (e.g., developmental stage, antagonist concentration, multiple drug targets), it becomes apparent that no definite conclusion can be drawn regarding the role of NR2A and NR2B subunits in selectively inducing LTP and LTD (Kash & Winder, 2007). Additionally, only few studies have investigated the potential role of the N2C or N2D subunits. Bartlett and colleagues (2007) highlighted that the N2A antagonist NVP-AAM077 may have had additional inhibitory effects on N2C and N2D subunits, but further

research is needed. In sum, research has not found distinct roles of NMDAR subunits in synaptic plasticity (yet). Therefore, they cannot (yet) be seen as a decisive mechanism for selectively inducing LTP or LTD. However, the synaptic plasticity cascade involves multiple other downstream candidates.

Going all in - Downstream mechanisms

It is widely accepted that the expression of LTP and LTD requires the insertion and removal of AMPARs from the post-synaptic membrane, respectively (Park, 2018). Specifically, post-synaptic AMPAR insertion is induced by the initial Ca2+ influx that activates kinases (e.g., CaMKII) that phosphorylates proteins. The exocytosis of AMPARs is mediated by the Ca2+ sensor synaptotagmin-1 (Syt1). In contrast, AMPAR removal is induced by Ca2+ influx activating phosphatases (e.g., calcineurin, protein phosphatase 1) which leads to dephosphorylation of proteins. The endocytosis of AMPARs is mediated by the Ca2+ sensor Protein Interacting with C Kinase 1 (PICK1) (Lüscher & Malenko, 2012; Chater & Goda, 2014). A recent study proposes the competition between endocytosis and exocytosis of AMPARs as the decisive mechanism underlying selective LTP versus LTD induction (Sumi & Harada, 2020).

Sumi and Harada (2020) built a network model to reproduce the bidirectional synaptic plasticity in hippocampal neurons. In their final model, endo- and exocytosis of AMPARs are induced to differential extents, depending on whether PICK1 or Syt1 is activated by the stimulation protocol. Interestingly, PICK1 (driving endocytosis) is activated by both LTP (i.e., strong and brief high-frequency) stimulation

and LTD (i.e., moderate and prolonged low-frequency) stimulation, while Syt1 (driving exocytosis) is activated mainly by LTP stimulation. Consequently, LTP stimulation activates both PICK1 and Syt1 leading to a competition between insertion and removal forces on AMPAR trafficking. However, as LTP stimulation creates a greater maximum exocytic drive than maximum endocytic drive, AMPAR exocytosis wins the competition and LTP is expressed. In contrast, LTD stimulation activates mainly PICK1, leading to a greater endocytic than exocytic drive. Thus, AMPARs are removed from the post-synaptic membrane, leading to LTD expression (Sumi & Harada, 2020).

The hypothesis by Sumi and Harada (2020) poses an interesting candidate for the selective induction of NMDAR-dependent LTP versus LTD. The model takes NMDAR-dependent Ca2+ influx as the input for LTP and LTD, which is in line with early hypotheses about the critical role of Ca2+ influx characteristics, namely its amplitude, duration, and location. Additionally, the model incorporates recent knowledge about downstream signalling pathways of LTP and LTD including (de)phosphorylation of proteins, AMPAR trafficking dynamics, Ca2+ sensors, and the recycling endosome. Lastly, it proposes a competition mechanism that allows for hypotheses that can be validated or refuted by future research (Sumi & Harada, 2020). However, research already examines other potential candidates and their roles in selectively inducing NMDAR-dependent LTP versus LTD (e.g., CaMKII holoenzyme; Cook et al., 2021).

CONCLUSION

Even though, more research is needed to investigate the selective mechanism underlying LTP and LTD induction, this essay argues in favour of a complex decisive machinery that entails multiple processes at different levels of the synaptic plasticity cascade. However, this essay is subject to several limitations. Selected research focused on NMDAR-dependent LTP and LTD occurring at excitatory synapses only. However, the picture is more complex, involving synaptic plasticity at inhibitory synapses, NMDAR-independent plasticity forms, and different mechanisms depending on cell types, brain regions, and the developmental state. Despite these limitations, several conclusions can be drawn regarding the role of Ca^{2+} influx amplitude in selectively inducing NMDAR-dependent LTP or LTD.

The search after a decisive mechanism for the selective induction of NMDAR-dependent LTP and LTD has yielded no comprehensive machinery yet. Early research proposed Ca^{2+} influx characteristics during induction, namely its amplitude, duration, and location, as potential candidates. However, it is reasonable that a decisive mechanism for bidirectional synaptic plasticity does not only consider characteristics present during the induction phase, but that it also includes components of later stages. While there are no conclusive results for the involvement of different NR2 subunits in the decisive machinery, a recent network model proposes the competition between AMPAR exocytosis and endocytosis to be involved in the selective induction of LTP versus LTD. However, future research needs to validate and test the suggested neural

network model. Taken together, Ca2+ influx amplitude should be seen as one of multiple components entailed in the complex decisive machinery for selectively inducing NMDAR-dependent LTP and LTD.

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