Title
Examination of Synaptic and Intrinsic Plasticity in Hippocampal CA1 Neurons

Author
Barragan, Eden Victoria

Publication Date
2021

Peer reviewed|Thesis/dissertation

Examination of Synaptic and Intrinsic Plasticity in Hippocampal CA1 Neurons

By

EDEN V. BARRAGAN

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

_____

John A. Gray, Chair

_____

Elva Diaz

_____

Johannes Hell

_____

Jon Sack

_____

Karen Zito

Committee in Charge

2021

Abstract

Within the brain, memories are generated during experience; learning activates a subpopulation of neurons – a neural ensemble, or engram – in the hippocampus that then encodes episodic, spatial, or contextual memories. It is thought that experience-dependent synaptic and intrinsic changes work in conjunction to form engrams; synaptic plasticity establishes neural ensembles via maintenance of connections between neurons with coincident activity, while intrinsic plasticity is responsible for the actual activation of engram. In the CA1 region of the hippocampus, functional and structural synaptic changes are typically dependent on the NMDA receptor, which have a unique requirement for concurrent glutamate and co-agonist (glycine or D-serine) binding. Despite the extensive characterization of the NMDAR's role in synaptic plasticity, it is still unclear what fundamental role co-agonism serves. Our current best guess, based on evidence of structural and functional plasticity in the absence of NMDAR-mediated ion-flux, is that the co-agonist site serves as a modulator of non-ionotropic NMDAR-mediated LTD (niLTD). Therefore, my first project investigated the role of the GluN1 co-agonist site in niLTD. In this study, we tested the effects of varying extracellular co-agonist availability on non-ionotropic plasticity. In-depth examination of this cellular phenomenon may reveal a novel role for non-ionotropic NMDAR plasticity in learning and memory. Indeed, under physiological conditions, synaptic activity drives changes in intrinsic plasticity, in an activity-dependent manner. My second project explored the relationship between learning-induced changes in CA1 pyramidal neurons and behavior following contextual fear conditioning. Future studies are needed to specifically pinpoint the interaction of these two phenomena and how exactly they work in combination to underlie learning and memory. By moving towards a more neurocentric view of learning mechanisms, we can appreciate the wide range of cellular tools employed for memory formation and storage. It should come as no surprise that the complexity of cellular processes underlying memory, mirrors the complexity of our own human experience.

Acknowledgements

To my old roommates – my mom, dad, and sister. Thank you for the phone calls, random visits, and care packages. You have been rooting for me since day one and I cannot express how much I appreciate your unwavering your love and support. Thank you for always being in my corner. Thank you for always encouraging my independence and my desire to learn. I owe all my success as a student and researcher to you.

To my closest friends – Patricia, Christina, Sam, and Taylor – you are the ultimate hype crew. You have stuck with me through all the highs and lows of grad school, and never let me doubt myself. Thank you for always lending an ear when I need to vent and for always keeping me grounded. You have kept me sane these past few years and I do not know how I would have made it to this point without your friendship.

To my fellow neurograds – especially Sasha, Lindsay, and Kyle – the people with which I can commiserate, share ideas, and be creative. It has been an honor to get to know and be stuck in grad school with you all. I cannot wait to see the amazing things you will go on to do.

To my undergrad mentor – Diane O'Dowd. Thank you for taking a chance and letting me into your lab. I am so grateful to have had the opportunity to work with you and be taught the value and joy of research.

To my dissertation committee – Elva Diaz, Johannes Hell, Jon Sack, and Karen Zito. Thank you for your scientific questions and advice. I greatly appreciate your support and guidance throughout the tangled web that is my thesis work.

To the Gray lab – Jon, Shekib, Raghava, Casey, Haley, Sunny. I am honored to have shared a lab space with you all. You made the daily torment that is electrophysiology somehow enjoyable. Thank you for being there to bounce around ideas, make solutions, and superstitiously wrap everything in foil.

And lastly, to my PI – John Gray. Thank you for all your mentorship and advice these past years. Thank you for letting me join your lab and giving me an opportunity to expand my scientific skillset. Thank you for always believing in me and (more importantly) my data, and for giving me the chance to learn and grow as a scientist. I cannot express how honored I am to have worked with you.

Thank you all for believing in me and sticking by my side.

I'm happy to say… we did it, Joe!

TABLE OF CONTENTS

Chapter 1

Introduction

Memory is central to who we are and how we behave – learning from our past to interact with the present and create our futures. Our memories are our most cherished and personal possessions. While the learning and memory field has made abundant advances in uncovering the neurobiology of memory over the past few decades, many questions remain to be answered before we fully understand the intricacies of memory formation, storage, maintenance, and recall. The quest to understand memory spans the breadth of both ancient and modern science – dating back to the time of Plato and Aristotle. The persistence of memory following an experience implies a physical, internal representation of the memory, which not only stores the memory but allows it to be recalled upon presentation of context pertaining to the original experience. By the middle of the 20th century, two main 'approaches' to studying learning and memory had emerged. The first – the *aggregate field approach* – was popularized by the likes of Karl Lashley and Ross Adey and stated that information regarding a learned experience or memory was stored in a bioelectric field generated by the combined activity of a population of neurons. The second, seemingly conflicting, approach predated the first and was originally proposed by Santiago Ramon y Cajal. This *cellular connectionist approach* posited that learning results from changes in the functional strength of synapses. This latter approach was later renamed *synaptic plasticity* by Jerzy Konorski and incorporated as a foundational aspect of Donald Hebb's more refined models of learning that are familiar to most modern neuroscientists. Consistent with these models, there is substantial evidence implicating synaptic plasticity in memory acquisition, retention, and extinction. However, it has become increasingly clear in recent years that synaptic plasticity is not the only cellular mechanism contributing to memory formation. After all, learning can result from single experiences, whereas most synaptic plasticity is induced by some form of repetitive stimulation. Unlike synaptic plasticity, which relies on changes in synaptic neurotransmitter receptors, intrinsic plasticity can be influenced by changes in a variety of ion channel subtypes. Therefore, Intrinsic cellular changes are another promising form of plasticity that have been observed following learning. Synaptic plasticity is known to drive intrinsic plasticity induction – potentiated synapses

are more likely to increase the likelihood of a cell firing. Although these intrinsic changes are fleeting, the learning-induced synaptic changes are much longer lasting and will persist, allowing the memory to be retrieved when cued. This suggests that these two cellular processes work in combination as the mechanistic underpinnings of memory.

While the two projects covered in this dissertation are seemingly disparate, they are united in their aim of gaining a deeper understanding of the cellular processes that underlie memory. In this chapter, I will outline important studies on intrinsic and synaptic plasticity – two well-established cellular substrates of learning and memory. I will start with an overview of the hippocampus, including basic hippocampal anatomy (which is important for the techniques employed in my research) and its role in learning and memory. Then, I will cover the role of NMDA receptors in synaptic plasticity – touching on receptor properties as well as ionotropic and non-ionotropic signaling – and their putative involvement in memory. Lastly, I will review the potential mechanisms of intrinsic excitability in memory and then briefly review the growing body of work that suggests cellular learning mechanisms extend past the scope of the synapse. I will conclude with a summary of my own research aims and goals for this dissertation.

The Hippocampus and Memory

The hippocampus is one of the most popular model systems for the study of information-storage processes in the brain. This subcortical structure's function, connectivity, and organization have been extensively explored and characterized, from the cellular to circuit level, to better understand its contribution to behavior, especially regarding learning and memory. Studies have consistently demonstrated that hippocampal involvement is required for episodic memory formation, but it is uncertain whether the hippocampus is capable of long-term memory storage or if it purely serves as more of an indexing locale for temporary storage during consolidation –

the process by which recent experiences become long-term memories. The ways in which neural networks represent and encode experiences relies heavily on single neuron learning mechanisms. Thus, mechanistic studies at the cellular and synaptic scale are crucial for a holistic understanding of the role of the hippocampus in memory. Additionally, dysfunction in the hippocampus has also been implicated in a wide array of neurobiological disorders – making a thorough understanding of the region also imperative from a therapeutic perspective.

Hippocampal Anatomy

The hippocampus proper is a part of a larger structure called the hippocampal formation which includes the dentate gyrus (DG), subicular complex, and the entorhinal cortex. Known for its seahorse-like appearance, for which it was named, the hippocampus proper is comprised of three major subfields – CA1, CA2, and CA3 – each with its own distinct connectivity (Figure 1A-B). These three subfields each have essentially one cell layer – the pyramidal cell layer or *stratum pyramidale* – unlike the more common six-layer structure of the neocortex. The border of the hippocampus proper is formed by axons of pyramidal cells and is called the alveus. Between the alveus and the pyramidal cell layer lies the *stratum oriens* which contains the basal dendrites of pyramidal cells and several interneuron types. The area superficial to the pyramidal cell layer contains the apical dendrites of pyramidal neurons in addition to a rich diversity of interneurons. This region is divided into the *stratum lucidum*, *stratum radiatum*, and the *stratum lacunosum-moleculare* (Figure 1C) (Schultz and Engelhardt, 2014). The role of interneurons in the functional circuitry of the hippocampus is less well characterized than that of its excitatory neighbors.

The primary forward projecting connections within the hippocampus are typically described as the hippocampal trisynaptic circuit. The main inputs to the hippocampus are axons from the entorhinal cortex to the DG, called the perforant pathway. These inputs make excitatory synapses onto the dendrites of DG granule cells, which in turn project, through their axons (mossy

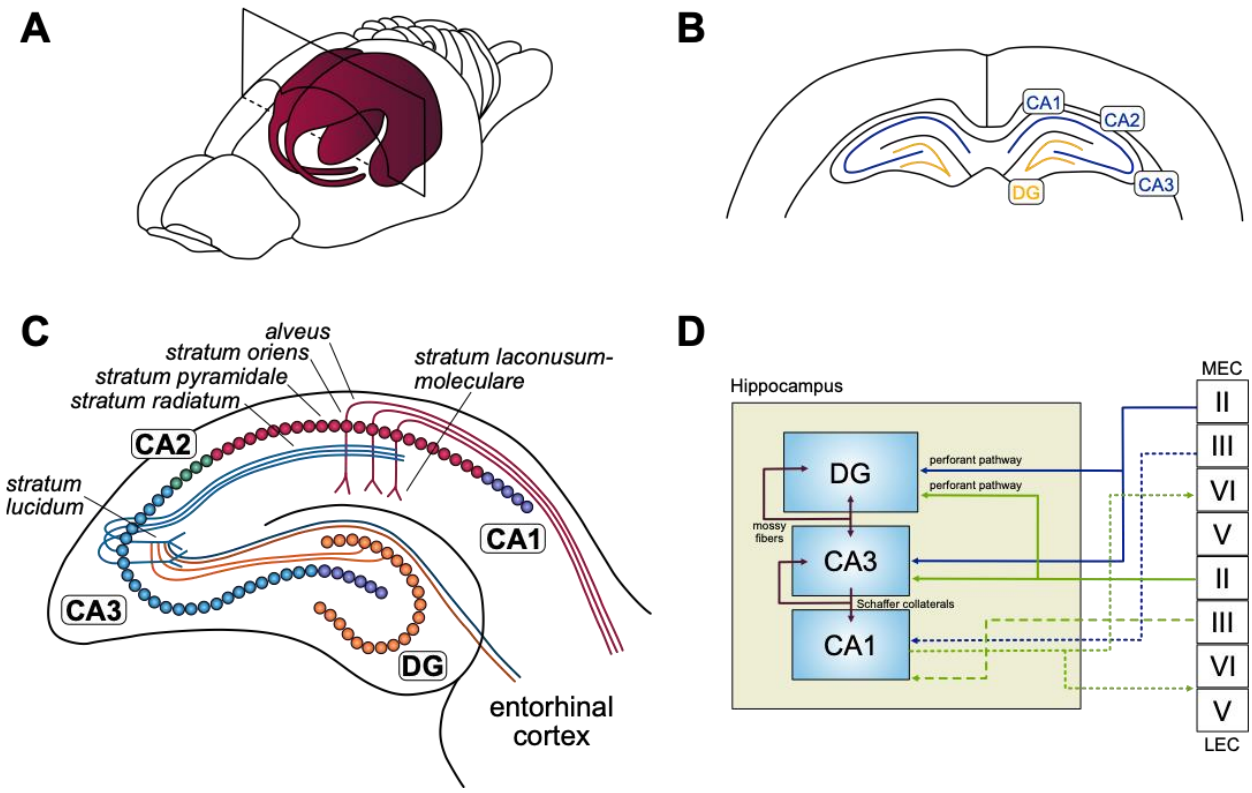

Figure 1. Hippocampal anatomy and organization. A. Schematic rodent brain with the hippocampus highlighted in maroon. **B.** Schematic coronal hippocampal slice showing the orientation and organization of the hippocampus proper (CA1-CA3) and dentate gyrus (DG). **C.** Representative hippocampal slice depicting different subfields and layers. CA1 neurons (red) receive input from CA3 Schaffer collaterals (light blue) in the stratum radiatum. **D.** Schematic of the hippocampal trisynaptic circuit. DG granule neurons receive afferent inputs, via the performant path, from the layer II of the lateral and medial entorhinal cortex. Next, granule neurons project to the CA3 pyramidal neurons via mossy fibers. CA1 neurons receive inputs from the CA3 by the Schaffer collaterals, by the contralateral hippocampus through associational/commissural fibers or direct inputs from the performant path. To close the hippocampal synaptic loop, CA1 pyramidal neurons project back to the entorhinal cortex.

fibers), to the proximal apical dendrites of CA3 pyramidal cells. CA3 pyramidal cells synapse onto ipsilateral CA1 through the Schaffer collaterals and contralateral CA3 and CA1 pyramidal cells via commissural connections. In the hippocampus, the Schaffer collaterals are the primary input system to CA1 where they heavily innervate the apical dendrites of pyramidal cells in the *stratum radiatum* bordering CA1 (Figure 1C) (Ishizuka et al., 1990). The characteristic laminated organization of the hippocampus makes the CA3-CA1 connections an ideal experimental system for electrophysiological stimulation and recordings. In the CA1 subfield, placement of a stimulating electrode in the Schaffer collaterals allows for selective stimulation of the CA3 inputs to CA1, yielding robust evoked synaptic responses from the apical dendrites of CA1 neurons that can be experimentally observed for extended periods of time.

Role of the Hippocampus in Memory

The hippocampus' unique anatomy coupled with its demonstrated roles in memory formation, have made it one of the most widely used model systems for studying learning and memory at the cellular and molecular level. Animal lesion and human studies have confirmed that the hippocampus plays critical roles in the formation and storage of episodic memory (Neves et al., 2008; Scoville and Milner, 1957). Additionally, animal behavioral studies with more specific manipulations like pharmacological inactivation or molecular knockouts show disruption of hippocampal function prevents spatial memory formation (Martin et al., 2005; Morris et al., 1986; Pastalkova et al., 2006; Tsien et al., 1996). These results have been further supported by correlative evidence in electrophysiological, molecular and MRI studies that hippocampal activity is involved in episodic memory (Berger et al., 1983; Gabrieli et al., 1997; Guzowski et al., 2001; Neves et al., 2008; Vazdarjanova, 2004).

Within the hippocampus, cell type and connectivity (Figure 1D) vary with region. The distinctive composition of each region can underlie differential functions in learning and memory.

One example is in the dentate gyrus (DG). DG granule cells can perform robust pattern separation of overlapping representations transmitted from the entorhinal cortex. The DG being ideally suited for this process is attributed to the large number of granule cells, their lack of recurrent connections, and sparse downstream connectivity to other hippocampal regions (Bernier et al., 2017; Forrest et al., 1994; O'Reilly and McClelland, 1994; Yassa and Stark, 2011). Thus, this region plays a more predominant role in memory retrieval and recall. This contrasts with CA1 pyramidal cells which undergo repetitive hippocampal replay during sleep and wakefulness following learning, a process that is thought to play a role in memory consolidation (Carr et al., 2011). These are just two examples of the strong relationship between structure and function in hippocampus, rendering each subfield with its own discrete role to play.

The hippocampus is a well-organized structure with many demonstrated roles in episodic memory in both rodents and humans. Early hippocampal lesion studies proved that loss of this structure can be truly devastating to memory. Since these pioneering works, the field has been focused on investigations of the neural basis of learning and memory. A deeper understanding of cellular learning mechanisms is necessary before the neural circuitry responsible for memory acquisition, encoding, and retrieval can be fully elucidated.

Synaptic and Intrinsic Plasticity are Cellular Correlates of Memory

Within the brain, memories are generated during experience; learning activates a subpopulation of neurons – a neural ensemble – that encodes the memory in the hippocampus (Tonegawa et al., 2015). Many neurological disorders have been linked to dysfunction in the hippocampus and long-term memory, such as depression, schizophrenia, dementia, stroke, and PTSD, meaning a systematic understanding of memory is needed to create more effective therapeutics. A major goal in neuroscience is to untangle the mechanisms by which the hippocampus stores and retrieves information. While some might argue that investigating memory

at the network or circuit level will contribute the most to our knowledge of the subject, it is just as important to research the molecular and cellular processes that underlie the creation of these neural networks. Historically, the discussion of cellular correlates of memory has been predominated by the idea that synaptic plasticity is the critical cellular substrate for the encoding of learning and memory. Given that a single excitatory neuron can receive thousands of inputs, each able to undergo bidirectional synaptic plasticity (strengthening and weakening of that connection's functional strength and size), it is easy to envision why synaptic plasticity is an attractive candidate for storing the vast repertoire of experiences one may encounter in a single day, let alone a lifetime.

Indeed, learning-induced synaptic changes are input specific, rapidly induced, and long-lasting – optimal for information storage (Abraham et al., 2002). And while the cellular underpinnings of learning and memory are most likely much more complicated than just one synaptic process (bidirectional plasticity), it is hard to deny that long-lasting synaptic changes are highly correlated to learning – there is far too much demonstrated evidence. Synapses do functionally contribute to learning; specifically, they convey distinctive information contents and input patterns that are to be memorized, their plasticity shapes connectivity maps via establishment of connection patterns and assignment of synaptic weights, and their activity induces intrinsic plasticity and drives the activation/reactivation of memory engrams albeit without the need for accompanying changes in synaptic weight. However, the assumptions underlying most current synaptic learning theories are that LTP alone can enable synapses to efficiently contribute to spike generation and that cells incorporated into the engram must show suprathreshold activity. For example. the amplitudes of locally evoked EPSPs are strongly attenuated as they travel from the synapse to the soma, making it difficult to envision even several summated EPSPs sufficiently depolarizing a neuron enough to reach spike threshold. Therefore, another cellular mechanism likely exists to amplify responses – even from potentiated synapses

– to drive neuronal firing. Learning has been shown to produce intrinsic cellular changes, like increases in neuronal excitability, whose role in memory is not yet well understood. These changes most likely work in conjunction with synaptic plasticity to properly learn and encode memories.

The Role of NMDARs in Synaptic Plasticity

The NMDA receptor is a ligand- and voltage-gated ion channel that plays a crucial role in synapse development and the regulation of synaptic plasticity. NMDARs are arguably the most complex subfamily of ionotropic glutamate receptors. They are expressed throughout the brain and spinal cord, in both neurons and glia, (Hollmann and Heinemann, 1994; Johnson and Ascher, 1987; Schneggenburger et al., 1999) and are found as heterotetrameric complexes composed of two obligatory GluN1 subunits and any combination of GluN2 (GluN2A-D) or GluN3 (GluN3A-B) subunits. The receptor itself functions as a dimer of dimers, most commonly composed of a pair of GluN1 and GluN2 subunits (Figure 2). The wide array of interchangeable subunits allows for a variety of configurations, which results in an assortment of NMDAR subtypes in the central nervous system (CNS), each with their own distinct properties and signaling capabilities. At a prototypical glutamatergic synapse, the α-amino-3-hyddroxy-5-methyl-4-isoxazoleproprionic acid receptors (AMPARs) generate the fast response to neurotransmitter release – opening quickly and allowing cations to flow into the postsynaptic compartment – which produces a local change in membrane potential. This depolarization allows NMDAR channels to open and conduct calcium ions into the postsynaptic neuron. Ca^{2+} then acts as a second messenger to elicit long-term synaptic changes based on the frequency and intensity of the presynaptic stimuli.

Emphasizing their role as key modulators of synaptic strength, NMDARs have long been implicated in major forms of Hebbian synaptic plasticity, such as long-term potentiation (LTP)

(Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973; Collingridge et al., 1983) and long-term depression (LTD) (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Numerous studies (Cummings et al., 1996; Mulkey et al., 1994; Mulkey and Malenka, 1992; Yang et al., 1999) have demonstrated that the magnitude and duration of Ca^{2+} influx through NMDARs are the principal determinants of the directionality of synaptic plasticity. Following LTP and LTD induction, the NMDAR-mediated Ca^{2+} influx triggers downstream signaling pathways. Both LTP and LTD have their own distinct signaling cascades which have also been extensively characterized. To fully understand how these receptors are capable of bidirectionally modifying synaptic strength, it is important to understand NMDAR composition and properties, as well as the intracellular molecular signaling pathways that are activated during LTP and LTD.

NMDAR Properties

NMDARs have many characteristic properties that distinguish them from other ionotropic glutamate receptors (Cull-Candy and Leszkiewicz, 2004; Paoletti, 2011; Traynelis et al., 2010). First, NMDARs are unique among most (if not all) other receptors in the brain, in that, in addition to glutamate binding to the GluN2 subunit, they have an absolute requirement for co-agonist – either glycine or D-serine – binding to the GluN1 subunit (Kleckner and Dingledine, 1988). Concurrent glutamate and co-agonist binding causes closure of the receptor's ligand binding domains (LBDs) and initiates opening of the channel pore, allowing cation flux (Karakas and Furukawa, 2014; Sobolevsky et al., 2009). The NMDAR is also subject to a voltage dependent Mg^{2+} block that prohibits most of the current flow through the channel pore at more negative membrane potentials (Mayer et al., 1984; Nowak et al., 1984). However, once open, NMDAR channels are highly Ca^{2+} permeable. Ca^{2+} permeability, Mg^{2+} blockade, and single-channel conductance are all greatly influenced by the receptor's subunit composition. Differences in these properties affect the relative contribution of specific NMDAR subtypes to synaptic integration and

plasticity (Siegler Retchless et al., 2012). Lastly, NMDARs have a host of modulatory sites on the receptor, most located on the intracellular C-terminal domain (CTD), which will vary depending on GluN2 subunit subtype or GluN1 isoform. Taken together, these properties render NMDARs with an incredible sensitivity to their surrounding intracellular and extracellular environments, with each receptor subtype equipped with its own signature charge transfer capabilities and temporal signaling profiles.

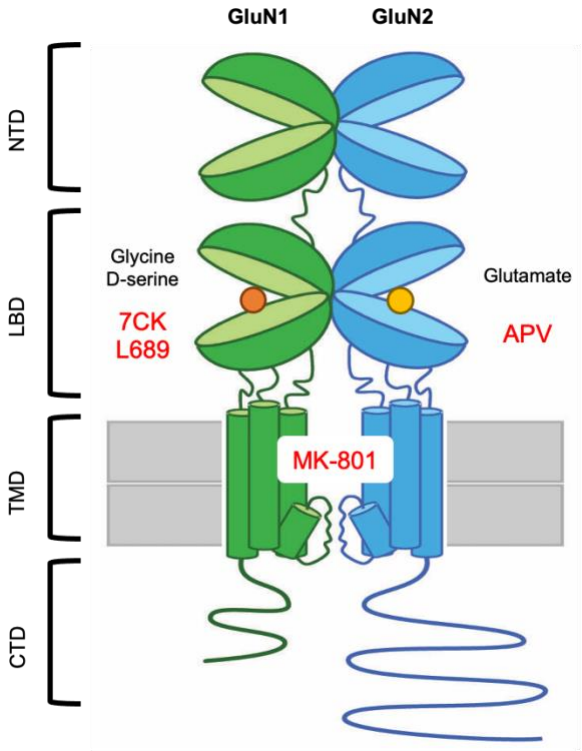

Figure 2. NMDA receptor topology and expression. A. Schematic of the NMDAR. Functional NMDARs are tetramers, where the NTDs and LBDs assemble as dimers, and the full receptor operates as a dimer of dimers. The NMDAR is shown here as a dimer for simplicity. Each dimer contains one GluN1 subunit and GluN2 subunit; these subunits uniquely bind co-agonist and glutamate, respectively. Agonists bind to the LBD on their respective subunits. Commonly used antagonists of each site listed in red.

Within the CA1 region of the hippocampus, by the second week of postnatal development, the predominant GluN2 subunits are GluN2A and GluN2B (Gray et al., 2011), meaning most NMDARs in CA1 are either diheteromers (GluN1/2A- or GluN1/2B-containing) or triheteromers (GluN1/2A/2B-containing). Between these two subunits, there is still the potential for distinct functional properties, current kinetics and associated intracellular signaling partners (Cull-Candy and Leszkiewicz, 2004; Monyer et al., 1994; Vicini et al., 1998). The NMDAR subunits' intracellular CTDs are the least conserved segments of each subunit. Each contains distinctive modulatory sites that can influence receptor trafficking, localization, and signaling (Martel et al., 2012; Sanz-Clemente et al., 2013; Sprengel et al., 1998). GluN2A and GluN2B CTDs, in particular, each include subunit-specific binding motifs which control both intracellular and surface trafficking of the receptor, which result in different rates of endocytosis (Lau and Zukin, 2007; Lavezzari et al., 2004). Therefore, it is easy to envision how subunit composition can have substantial effects on a particular synapse's functioning and ability to undergo bidirectional plasticity.

NMDAR Expression and Distribution

The four GluN2 (2A-2D) subunits exhibit drastically different spatiotemporal expression profiles (Akazawa et al., 1994; Monyer et al., 1992; Sheng et al., 1994). As previously mentioned, GluN2A and GluN2B are the predominant GluN2 subtypes contributing to synaptic NMDAR currents in CA1 pyramidal neurons in the hippocampus (Gray et al., 2011), and therefore will be the only ones discussed in the rest of this introduction. Adding another layer of complexity, NMDAR subunit composition is not static; it is dynamic and is modified throughout development and, at mature synapses, in response to neural activity. These changes occur rapidly, on the timescale of minutes, and – depending on which particular subunit is being trafficked to the synapse – can have a significant effect on basal synaptic functioning (Barria and Malinow, 2002).

At most excitatory synapses in the forebrain, NMDAR subunit composition changes during development. NMDARs are primarily GluN2B-containing early in development and are gradually replaced or supplemented by “mature” GluN2A-containing NMDARs (Flint et al., 1997; Roberts and Ramoa, 1999; Sheng et al., 1994). Electron microscope immunogold analyses of postnatal CA1 demonstrate that synaptic labeling for GluN2B peak at postnatal day 2 (P2) and shows a gradual decrease to about half as much by P35, while GluN2A labeling at the synapse is very low at P2 and increases twelve-fold by P35 (Petralia et al., 2005). These findings are in agreement with western blot analyses of both GluN2A and GluN2B expression at the same developmental time points (Sans et al., 2000). Interestingly, this shift, or switch, in the ratio of GluN2A/GluN2B may alter the threshold for NMDAR-mediated synaptic plasticity induction (Yashiro and Philpot, 2008). The postnatal upregulation of GluN2A shortens the deactivation time constant of synaptic NMDAR EPSCs in the hippocampus, and similar developmental shifts in subunit composition occur in other brain regions (Barth and Malenka, 2001; Flint et al., 1997; Kirson and Yaari, 1996; Lu et al., 2001; Stocca and Vicini, 1998). From a physiological standpoint, the shift from GluN2B- to GluN2A-containing receptors is thought to serve as a refinement system that reduces plasticity processes that are not conducive to proper information processing.

Additionally, NMDARs are expressed in both neurons and glia (Káradóttir et al., 2005; Lalo, 2006; Salter and Fern, 2005). In neurons, NMDARs have been detected at both pre- and postsynaptic sites (Tzingounis and Nicoll, 2008), and distribution of receptors varies based on neuron type, even within a specific brain region. For example, in cortical interneurons, NMDARs are evenly distributed along the dendritic shaft (Goldberg et al., 2003), whereas in excitatory neurons, NMDARs have zones of high density, within the postsynaptic density (PSD), and low density, on the dendritic shaft and somatic membrane. NMDARs within the PSD are considered synaptic (they are near presynaptic release sites) while NMDARs in spines, next to the PSD, are considered perisynaptic (also sometimes referred to as extrasynaptic). Perisynaptic NMDAR

activation requires access to glutamate spillover (Huang and Bergles, 2004; Kullmann and Asztely, 1998) which is highly dependent on the location and activity of neuronal and glial glutamate transporters. NMDARs located on dendrites outside spines and on the soma are distinct from perisynaptic NMDARs and are considered extrasynaptic – these complexes are difficult to activate via endogenous glutamate release but are thought to be tonically active (Sah et al., 1989). GluN2B often localizes at extrasynaptic sites in adult neurons (in cortex, dentate gyrus, CA1, and cerebellum) and can be specifically activated, but this is neither restricted nor due to increased GluN2A expression during development (Brickley et al., 2003; Dalby and Mody, 2003; Scimemi et al., 2004; Stocca and Vicini, 1998; Tovar and Westbrook, 1999). In fact, GluN2B remains in synapses of neurons co-expressing GluN2A and recruits molecules important for downstream signaling or internalization (Bayer et al., 2001; Fujisawa and Aoki, 2003; Janssen et al., 2005; Köhr et al., 2003; Krapivinsky et al., 2003; Roche et al., 2001). GluN2A is also not exclusively localized to synapses and has been found extrasynaptically, indicating that separation of GluN2A and GluN2B into extrasynaptic and synaptic sites is not absolute (Köhr, 2006; Li et al., 1998; Mohrmann et al., 2002; Thomas et al., 2006).

The subcellular localization of NMDARs, and therefore the location of Ca^{2+} influx into the cell, is known to activate various signaling pathways that can differ between extrasynaptic and synaptic sites. The activity of synaptic and extrasynaptic NMDARs is additionally regulated by region-specific signal transduction mechanisms, which can also determine signaling downstream of the NMDAR. Distinct functions for synaptic or extrasynaptic NMDARs are often difficult to definitively demonstrate, unless the two populations' roles are found to be in opposition (Ivanov et al., 2006; Kim et al., 2005; Vanhoutte and Bading, 2003). However, differential roles for synaptic and extrasynaptic receptor populations does suggest that the cellular localization of scaffolding and signaling complexes is as critical as the subunit composition of NMDARs for activating the full gambit of NMDAR signal transduction pathways. Interestingly, demarcation of synaptic and

extrasynaptic space seems closely related to areas of D-serine and glycine abundance, respectively (Papouin and Oliet, 2014).

NMDAR Co-agonism

The NMDAR is unique in its absolute requirement of co-agonist binding at the co-agonist site (Kleckner and Dingledine, 1988), although the fundamental role for this distinctive property is still unknown. The co-agonist site is located on GluN1, the obligatory subunit of the NMDAR, and deletion of this subunit is a postnatally lethal manipulation (Forrest et al., 1994; Li et al., 1994). Even a reduction of GluN1 co-agonist affinity via a point mutation results in postnatal lethality (Kew et al., 2000), further highlighting the importance of NMDAR co-agonism in development. In the brain, both co-agonists are present in the extracellular space (although total brain D-serine levels are only 40% of glycine levels), but surprisingly, under physiological conditions, the co-agonist site is thought to be unsaturated (Bergeron et al., 1998). Changes in co-agonist availability, regulation, and levels in the brain over development all influence the saturation levels of the co-agonist site. Additionally, GluN2 subunit identity can also influence the receptor's co-agonist affinity (Paoletti, 2011). Overall affinity for both co-agonists are similar (EC_{50} ~0.1-1 μM) (Priestley and Kemp, 1994), although D-serine has been shown to be a more potent agonist of the co-agonist site and also essential for NMDAR-dependent LTP in the hippocampus and other forebrain regions (Balu et al., 2013; Basu et al., 2009; Fossat et al., 2012; Le Bail et al., 2015; Papouin et al., 2012). Nonetheless, the identity of the endogenous ligand of the co-agonist site varies across developmental timepoints, brain regions, and cellular localization of the receptors (i.e. synaptic vs. extrasynaptic) (Balu and Coyle, 2015; Le Bail et al., 2015; Mothet et al., 2015; Papouin et al., 2012).

Unlike AMPARs which can activate while partially liganded, NMDAR activation entails concurrent occupancy of all four LBDs on the receptors (two co-agonist molecules and two

glutamate molecules) (Benveniste and Mayer, 1991; Clements et al., 1998; Clements and Westbrook, 1991). One of the complicating factors in understanding the role of the obligatory co-agonist site in NMDAR function is the negative cooperativity between co-agonist and glutamate binding. This process decreases the affinity of the receptor for glycine upon glutamate binding and vice versa and it was originally described as a 'glycine-dependent desensitization' and was observed as a potentiation of NMDAR responses at subsaturating concentrations of glycine, which causes the initial glycine- and glutamate-mediated peak currents to decrease as glycine dissociates from the receptor (Mayer et al., 1989). However, this 'desensitization' is due to allosteric interactions between the GluN1 and GluN2 subunits, which results in negative cooperativity for the binding of both agonists (Durham et al., 2020; Leeson and Iversen, 1994). Other evidence suggests the observed negative cooperativity results from the GluN2 subunit reducing the dynamics of the GluN1 LBD upon glutamate binding (Rajab et al., 2021). Why the two agonists are required for receptor activation despite this negative interaction upon binding remains unknown.

Glycine

In the mammalian brain, glycine is a nonessential amino acid that, primarily in brain stem regions, acts as an inhibitory neurotransmitter through the activation of strychnine-sensitive pentameric glycine receptors (GlyRs) (Traynelis et al., 2010). Surprisingly. glycine also binds to several ionotropic glutamate receptor subunits: GluN1, GluN3A, GluN3B, GluD1, and GluD2. Among these subunits, its action on GluN1, as an obligatory NMDAR co-agonist, is probably the most well described (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). It is glycine, not D-serine, that is the predominant co-agonist during development (Le Bail et al., 2015) and at extrasynaptic NMDARs (Papouin et al., 2012).

The concentration of glycine in the synaptic cleft is strictly regulated by reuptake via specific high-affinity transporters located in neuronal and glial plasma membranes (Neal and

Pickles, 1969). Indeed, the extracellular concentration of glycine is approximately 10 µM which would be sufficient to saturate the NMDAR co-agonist site (EC_{50} 0.4-3 µM) if not for perisynaptic glycine transporters. The primary regulators of intracellular and extracellular glycine levels are the sodium-dependent glycine transporters, GlyT1 and GlyT2. The reuptake mechanism can also be reversed upon depolarization of the synaptic terminal, pumping glycine out of the cell in a Ca^{2+}-independent manner (Adam-Vizi, 1992; Attwell et al., 1993). The balance between reuptake and release of glycine could serve as a means of activity-dependent regulation of NMDAR activity. For this type of regulation to be effective, baseline glycine concentration in the synaptic cleft should be kept below saturating levels for NMDARs by the glycine transporters.

Evidence that the NMDAR co-agonist site is unsaturated at baseline activity levels has been demonstrated in hippocampal slices, as well as other experimental systems (Bergeron et al., 1998). However, it is difficult to conclusively say whether this is also true in vivo, since co-agonist levels are known to fluctuate with activity, developmental age, synapse type and brain region (Henneberger et al., 2013; Papouin and Oliet, 2014; Schell, 2004). In cases when the co-agonist site may be saturated, it is important to consider the effects of glycine binding in the absence of glutamate. Accumulating evidence demonstrates types of metabotropic NMDAR signaling that are dependent on glycine binding independent of ion flux (Danysz and Parsons, 1998; Ferreira et al., 2017; Han et al., 2013; Nong et al., 2003; Papouin et al., 2012). These studies will be discussed at length later in this chapter (see *Non-ionotropic NMDAR Signaling and Function*).

D-serine

In contrast to glycine, the co-agonist D-serine activates NMDARs without affecting overall circuit excitability. However, it has been shown that D-serine binds to other ionotropic glutamate receptor subunits (GluN3 and GluD). This interaction is much less well characterized than binding to NMDARs and it is unknown whether D-serine alone functions as the endogenous ligand for

GluN3 or GluD receptors. In the mammalian brain D-amino acids are thought to minimally contribute to proper CNS function; one notable exception is D-serine. High amounts of D-serine and the enzyme that synthesizes it from L-serine – serine racemase (SR) – are found in both excitatory and inhibitory neurons. Distribution of endogenous D-serine in the brain is similar to that of NMDARs (Schell et al., 1997). (Stroebel et al., 2021). D-serine is also a more potent and efficient ligand of the co-agonist binding site, due to its tighter binding within the co-agonist site (Le Bail et al., 2015; Mothet et al., 2015).

D-serine is thought to be the main co-agonist site ligand at synaptic NMDARs in the mature, adult forebrain. Historically, the origin of D-serine in the past has been quite controversial; evidence for both neuronal (Balu et al., 2012; Benneyworth et al., 2012; Kartvelishvily et al., 2006; Miya et al., 2008) and astrocytic (Fossat et al., 2012; Mothet et al., 2006; Panatier et al., 2006) production have been demonstrated. However, definitive evidence has shown that, unlike classical neurotransmitters which are released from the presynaptic locations, D-serine appears to be mainly released from postsynaptic neuronal sites (Balan et al., 2009; Lin et al., 2016; Wong et al., 2020). The proximity of SR to postsynaptic sites allows for local activation of synaptic NMDARs, such that partial saturation of synaptic NMDARs by tonic D-serine release could permit immediate activation of NMDARs upon glutamate binding, “priming” the receptors. Neuron-specific deletion of SR has shown that neurons are the main sites of D-serine synthesis. This neuronal D-serine (not astrocytic D-serine) plays an instrumental role in hippocampal NMDAR-dependent synaptic plasticity (Benneyworth et al., 2012; Neame et al., 2019). Crosstalk between AMPAR and NMDAR activity may also potentially play a role in homeostatic regulation of synaptic strength (Ma et al., 2014).

Considering the localization of D-serine has only recently been elucidated, it is perhaps unsurprising that our understanding of the mechanisms of D-serine transport and release are limited. Studies suggest that reuptake and/or release of D-serine may be mediated by the amino

acid transporter, Asc-1 (Fukasawa et al., 2000; Rosenberg et al., 2013), which is expressed exclusively in neurons, not astrocytes. Indeed, manipulation of Asc-1 expression or function affects extracellular D-serine levels and NMDAR-mediated plasticity and synaptic activation (Rosenberg et al., 2013; Sakimura et al., 2016; Sason et al., 2016). These transporters are also capable of moving glycine molecules across the cell membrane. Compared to the more powerful glycine transporters which limit glycine exposure to synaptic NMDARs, Asc-1 is relatively less effective, potentially allowing D-serine to remain in the synaptic cleft for much longer periods of time than glycine, rendering it functionally more effective (Bergeron et al., 1998).

There is also evidence that endogenous D-serine may be critical for proper network development, synaptic development, dendritic arborization, and spine density (Balu et al., 2014, 2013, 2012; Miya et al., 2008; Van Horn et al., 2017), which are imperative for efficient CNS performance. Altered D-serine levels are thought to contribute to several neurological conditions, including the etiology of schizophrenia. Patients with schizophrenia display lower D- and L-serine levels in the brain and CSF compared to control patients (Kumashiro et al., 1995). Indeed, SR-knockout mice are often used as a model for studying schizophrenia, as these mice – similar to humans with schizophrenia – exhibit decreased D-serine levels, decreased spine densities and schizophrenia-like cognitive phenotypes (Balu et al., 2013; Basu et al., 2009). This dysfunction also provides a unique target for therapeutic treatment of neurological disorders: the co-agonist site on the GluN1 subunit. The importance of this binding site and its full functional and translational potential have yet to be fully understood and utilized.

Ionotropic Plasticity: LTP and LTD

In 1949, Donald Hebb postulated that if two interconnected neurons are activated within a close temporal window of each other, their connection will strengthen; the concept has since been condensed into “cells that fire together, wire together”. This strengthening of connections

was thought to underlie the long-lasting neuronal changes that encoded learning and memory. However, empirical evidence to support this proposed phenomenon was not reported until 1973, when high frequency stimulation of the perforant pathway was shown to evoke a persistent enhancement of the synaptic responses in the rabbit dentate gyrus. This formative Bliss and Lomo study characterized the synaptic phenomenon of LTP, a functional increase in synaptic strength (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973), and at least partially supported Hebb's postulate. A decade later, an NMDAR-dependent form of LTP was observed at the Schaffer collateral CA3–CA1 synapses (Collingridge et al., 1983). Because the NMDAR is effectively a biological coincidence detector – both ligand and voltage-gated – its demonstrated necessity for LTP induction (Artola and Singer, 1993; Bourne and Nicoll, 1993; Herron et al., 1986; Kauer et al., 1988; Morris et al., 1986) only reaffirms that this is a form of Hebbian plasticity.

However, LTP alone is not sufficient to fully account for the complex processes of memory formation and storage. If LTP represented the lone cellular correlate of memory – without a mechanism for reversing or weakening this process – synapses would grow limitlessly in strength. With synapses in a persistently potentiated state, the overall tuning capabilities of the neuron would be lost and as a result, that cell's network would suffer without discrete communication between neurons. Before any reliably reproducible empirical evidence emerged, it was widely accepted that a process must exist to either weaken naïve synapses or reverse previously potentiated synapses. In the years that followed the discovery of LTP (Bliss and Lomo, 1973), it was shown that synaptic potentiation could be disrupted and reversed by synaptic activity (Barrionuevo et al., 1980; Fujii et al., 1991; Staubli and Lynch, 1990), but reliable induction protocols remained elusive. In 1982, the Bienenstock Cooper and Munro (BCM) model proposed that a synaptic input below a threshold value unique to an individual synapse could lead to synaptic depression (Bienenstock et al., 1982); this model led to an intense search for evidence of activity-dependent synaptic depression.

Nearly two decades later, the first reliable protocol to elicit synaptic depression from Schaffer collateral–CA1 synapses in rodent hippocampal slices was found (Dudek and Bear, 1992). Although long-term depression (LTD) with identical properties has been observed in the neocortex of several species (Kirkwood et al., 1993; Kirkwood and Bear, 1994), this particular form of LTD has specific characteristics that distinguish it from most other forms of LTD – either in different brain regions or evoked by different stimulation paradigms. While the term LTD encompasses several forms of synaptic plasticity, this project focused on homosynaptic low-frequency (LFS)-induced NMDAR-mediated LTD. This form of LTD requires NMDAR activation, a rise in postsynaptic calcium concentration, and activation of a specific phosphatase cascade (Dudek and Bear, 1992; Mulkey et al., 1994; Mulkey and Malenka, 1992). LTD is a subject of intense exploration, as it is a neuronal process that has a potentially expansive behavioral scope, being implicated in both learning and memory, cognitive effects of acute stress, addictive potential of some drugs of abuse, and the elimination of synapses in neurodegenerative disorders (Collingridge et al., 2010).

Like NMDAR-dependent LTP, LTD of excitatory synaptic transmission was first observed at the Schaffer collateral synapses in the hippocampus (Bear, 1995; Dudek and Bear, 1992). The most widely used protocol for LFS-induced LTD in the hippocampus is 900 pulses at 1Hz – this is sufficient to induce robust LTD in CA1 pyramidal cells (Dudek and Bear, 1993, 1992). This form of LTD was observed to require both NMDAR activation and an increase in postsynaptic Ca^{2+} (Dudek and Bear, 1993). Prolonged stimulation is essential to evoke a significant change in synaptic response, although a shorter stimulation is necessary to induce a change if the postsynaptic neuron is modestly depolarized (Selig et al., 1995) – this relieves the Mg^{2+} block that is present in NMDARs at more hyperpolarized potentials, or in the presence of specific neuromodulators (Kirkwood et al., 1999; Scheiderer et al., 2004). While this specific protocol has been reliably effective in inducing robust LTD (Dudek and Bear, 1992; Mulkey and Malenka,

1992), it is important to acknowledge that induction of LTD (and LTP) is ultimately influenced by the recent history of synaptic and cellular activity (Abraham and Bear, 1996; Ngezahayo et al., 2000).

The LTD requirements for NMDAR activation and an increase in postsynaptic Ca^{2+} (Bear, 1995; Dudek and Bear, 1993), presented somewhat of a paradox, given that both are also necessary for NMDAR-dependent LTP. How could two of the most universal elements of synaptic signaling be involved in mechanisms that yield opposing synaptic changes? One immediate clue was the evidence that both LTD and LTP can be evoked with the same number of stimuli simply by varying the frequency of stimulation – with low frequencies evoking LTD and high frequencies triggering LTP (Dudek and Bear, 1992). However, stimulation frequency alone is not the determining factor, considering a high frequency induction protocol typically used to trigger LTP can reliably induce LTD in the presence of a partial NMDAR blockade (Cummings et al., 1996). Instead, it is posited that the postsynaptic Ca^{2+} level, especially the Ca^{2+} dynamics within the postsynaptic compartment – which can vary according to strength, duration, and frequency of stimulation – are crucial determinants of the directionality of synaptic plasticity. Modest but prolonged elevations of postsynaptic Ca^{2+} in the submicromolar range induce LTD, while a brief Ca^{2+} elevation to ~10 μM is required for LTP induction (Lee et al., 1998). Additional evidence also suggests that unlike LTP induction, presynaptic activity is not necessary for LTD induction, as both uncaging of glutamate by flash photolysis or bath application of NMDA is sufficient to induce robust LTD (Cummings et al., 1996; Dodt et al., 1999; Kandler et al., 1998).

Expression Mechanisms

There is myriad evidence that inhibition of NMDARs blocks LTD, activation of NMDARs induces LTD, and buffering the rise in intracellular Ca^{2+} concentration prevents LTD (Cummings et al., 1996; Dudek and Bear, 1992; Kamal, 1999; Kandler et al., 1998; Lee et al., 1998; Li et al., 2004; Mulkey and Malenka, 1992; Yang et al., 1999). Intracellular Ca^{2+} stores contribute little to

LTD when NMDARs are optimally activated (Nakano et al., 2004). Based on this, a model emerged that the key signal for LTD induction is Ca^{2+} entering the PSD through NMDARs, but the threshold at which the magnitude and duration of the Ca^{2+} concentration induce LTD is undetermined. This subject is further complicated when considering that GluN2 subtype may affect LTD induction capabilities. There are differential effects of antagonists with varying affinities for GluN2A/B and GluN2C/D on LTD induction and, additionally, subunit composition at the synapse is regulated by activity – perhaps to further influence the directionality of plasticity at that synapse (Barria and Malinow, 2002; Hrabetova et al., 2000; Quinlan et al., 1999; Williams et al., 2003, 1998). However, it has more recently been demonstrated that genetic deletion of GluN2A or GluN2B does not affect LTD induction in the hippocampus (Wong and Gray, 2018).

Expression of homosynaptic NMDAR-mediated LTD requires the activation of a variety of phosphatases (Mulkey et al., 1994, 1993). During LTD induction, when NMDARs are activated, Ca^{2+} enters the PSD and binds to calcineurin (protein phosphatase 2B, PP2B), which in turn dephosphorylates inhibitor-1 and this leads to activation of protein phosphatase 1 (PP1). Once activated, PP1 dephosphorylates its substrates, notably Ser-845 on the AMPAR subunit GluA1 (Figure 3A), which allows for receptor internalization and therefore a reduction in functional synaptic strength (Collingridge et al., 2004). Administration of either PP2B or PP1 inhibitors reliably prevents the induction of LTD (Kirkwood et al., 1999; Mulkey et al., 1994, 1993). In addition, protein kinases like protein kinase A (PKA), cyclin-dependent kinase 5, p38 mitogen-activated protein kinase (p38 MAPK), and glycogen synthase kinase-3 (GSK3) (Figure 3B) have also been implicated in the process (Brandon et al., 1995; Ohshima et al., 2005; Peineau et al., 2009, 2007; Zhu et al., 2005). Biochemical studies have established that LTD is correlated with the dephosphorylation of PKA and PKC substrates, but not with changes in CaMKII substrate phosphorylation (Hrabetova and Sacktor, 2001; Kameyama et al., 1998; Lee et al., 1998; van

Dam et al., 2002). Other forms of LTD do not require PP1 and PP2B for induction, which highlights these specific enzymes as key identifiers of LFS-induced homosynaptic LTD.

Once the LTD signaling cascade is triggered, the final step in LTD expression is the endocytosis of AMPARs – the receptors responsible for most of the synaptic response at resting membrane potentials. This results in an overall decrease in response to the presynaptic neurotransmitter release. During LTD, the N-ethylmaleimide-sensitive factor (NSF) that usually stabilizes AMPARs at the synapse is replaced by the clathrin-adapter protein AP2 (Collingridge et al., 2004) (Figure 3C). Untethering AMPARs from the postsynaptic density also involves postsynaptic density protein 95 (PSD95), A-kinase anchor protein-150 (AKAP-150), and hippocalcin (HPC), a member of the neuronal calcium sensor family. The C-tail of the GluA2 subunit also specifically interacts with glutamate receptor interacting protein (GRIP), AMPAR-binding protein (ABP), and protein interacting with C-kinase (PICK1) (Figure 3D), all of which are rich in PDZ domains – which enable stabilization of AMPARs in both the synaptic membrane and intracellular sites (Collingridge et al., 2004; Sheng and Kim, 2002). Once freed, AMPARs are thought to diffuse laterally from their synaptic sites to sites of endocytosis, where they are internalized via a clathrin- and dynamin-dependent process. The steps of expression thus far outlined occur in the time frame of a typical electrophysiological recording, about one hour after induction. For maintenance of longer lasting LTD, protein synthesis is required (Manahan-Vaughan et al., 2000), although this is outside of the time frame of most acute slice recordings.

It is important to note that at least two fundamentally different forms of NMDAR-dependent LTD are thought to exist: *de novo* synaptic depression, found at naïve synapses which have not been exposed to potentiating stimuli, and depotentiation, or depression of recently potentiated synapses. An intracellular rise in postsynaptic Ca^{2+} concentration has been shown to be critical for both forms of LTD. Evidence also now suggests that the type of LTD is determined by the phosphorylation state of the AMPAR subunit, GluA1. Over 80% of synaptic receptors are thought

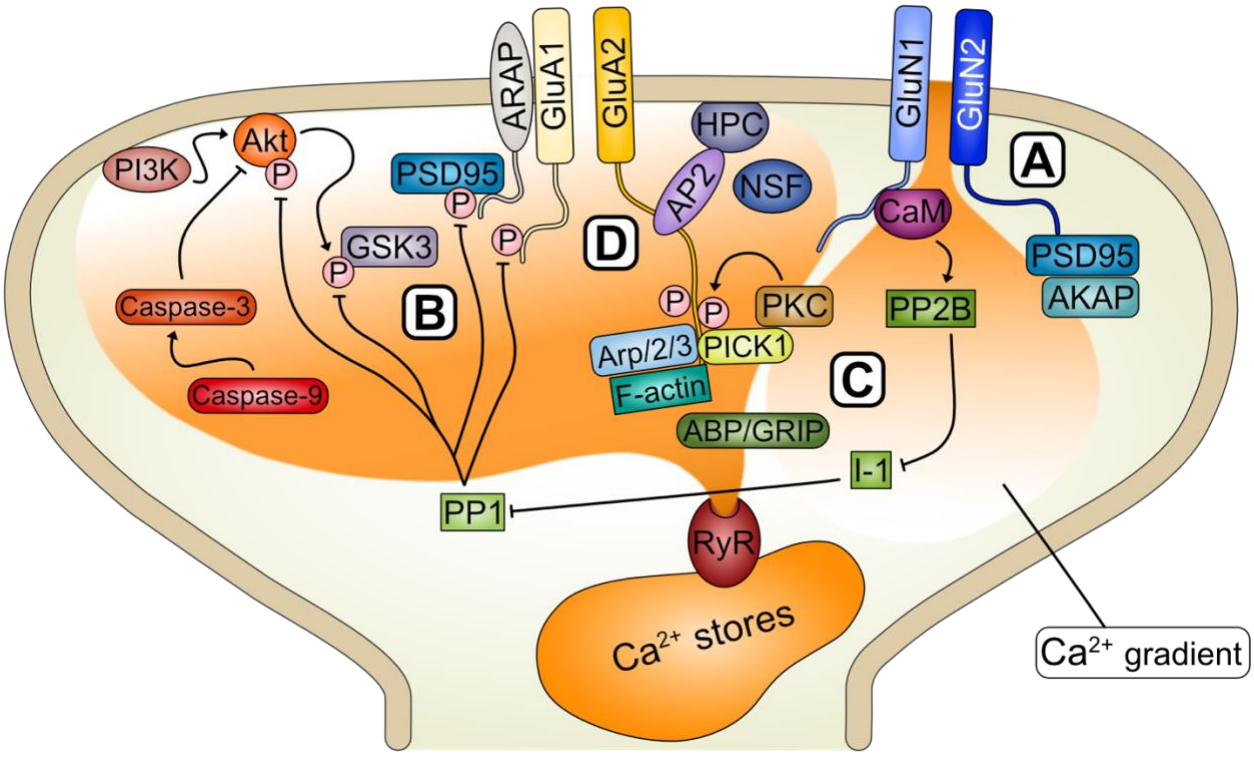

Figure 3. NMDAR-dependent LTD downstream signaling mechanisms. A. Calmodulin (CaM) detects Ca^{2+} (orange gradient) that enters through NMDARs, which activates protein phosphatase 1 (PP1). Once activated, PP1 can dephosphorylate many targets, most importantly Ser-845 on the AMPAR GluA1 subunit. **B.** PP1 also dephosphorylates glycogen synthase kinase-3ß (GSK3ß) and its upstream regulators, PI3K and Akt, leading to activation of GSK3ß during LTD. Caspase-9 and caspase-3 can also cleave Akt, which will also result in GSK3ß activation. **C.** Protein interacting with C kinase 1 (PICK1) may facilitate the disassociation of AMPARs from AMPAR-binding protein–glutamate receptor interacting protein (ABP–GRIP), potentially via the targeted phosphorylation of ser880 of GluA2 by protein kinase Cα (PKCα). PICK1 also acts as a negative regulator of Arp2/3-mediated actin polymerization via direct interaction with Arp2/3 and F-action. **D.** GluA2-containing AMPARs are stabilized at synapses by an interaction with *N*-ethylmaleimide-sensitive factor (NSF). The calcium sensor protein hippocalcin (HPC) is a high-affinity Ca^{2+} sensor that can target adaptor protein 2 (AP2) to GluA2 and therefore displace NSF and initiate clathrin-mediated endocytosis of AMPARs.

to be GluA1-containing, which is the subunit containing the key dephosphorylation sites that are important for triggering AMPAR removal from the synapse (Diering and Huganir, 2018; Henley and Wilkinson, 2016; Lu et al., 2009; Wenthold et al., 1996). Dephosphorylation of Ser-845 was first described in a chemically induced model of synaptic depression, but it is interesting that this form of LTD was not associated with dephosphorylation of Ser-831, a phosphorylation site implicated in NMDAR-dependent LTP (Barria, 1997; Dodt et al., 1999). This highlights that while LTP and LTD have inverse effects on functional synaptic strengths, they are not precisely opposite processes. Later studies provided more evidence involving the dephosphorylation of Ser-845 of synaptic GluA1-containing AMPARs after LFS-induced LTD (Lee et al., 2000). Additionally knock-in mice which replaced Ser-831 and Ser-845 with alanine residues exhibited both reduced LTP and a lack of NMDAR-dependent LTD (Lee et al., 2003). This data suggests that at naïve synapses *de novo* LTD leads to a dephosphorylation of Ser-845, while at previously potentiated synapses, depotentiation, which may represent a more accurate inverse of LTP, results from a dephosphorylation of the Ser-831 residue. It is now widely accepted that the key mechanism of NMDAR-mediated plasticity involves the highly regulated trafficking and internalization of AMPARs via involvement of several other postsynaptic molecules (Collingridge et al., 2004; Sheng and Kim, 2002).

At a typical CA1 synapse, bidirectional plasticity (both LTP and LTD) has been shown to require Ca^{2+} influx through NMDARs, but there is no straightforward answer as to whether NMDAR subunit composition – which is known to influence Ca^{2+} permeability and conductance of the channel – dictates the directionality of synaptic plasticity. Indeed, the induction of synaptic plasticity is highly complex and perhaps more nuanced than the classical model suggests, in which all the onus of determining the directionality of synaptic change is placed on the magnitude and duration of the Ca^{2+} influx through the NMDAR. Further interrogation of these complex molecular signaling devices is needed to fully understand their role in plasticity induction.

Non-ionotropic NMDAR Signaling and Function

As discussed in previous sections, NMDARs are ionotropic glutamate receptors, meaning ion conductance through the receptor channel is the primary mechanism of stimulating downstream signaling molecules/pathways. Activation of the NMDAR and opening of the channel pore is only possible upon binding of both glutamate and co-agonist and sufficient postsynaptic depolarization which ejects the Mg^{2+} block from the pore. These requirements are often all satisfied during synaptic transmission when pre- and postsynaptic activity occur simultaneously, which is why NMDARs are sometimes referred to as synaptic coincidence detectors. This traditional view of NMDAR activation operates under the assumption that NMDAR conformational signaling is minimal, an idea which has been challenged by several studies demonstrating multiple instances of non-ionotropic NMDAR function, including evidence of non-ionotropic synaptic plasticity.

Non-ionotropic NMDAR signaling

In direct contrast to the classical model of synaptic plasticity, evidence of functional and structural NMDAR-mediated synaptic depression has been shown in the absence of ion flux through the NMDAR (niLTD) (Nabavi et al., 2013; Stein et al., 2020, 2015; Wong and Gray, 2018). However, it is worth noting that this finding has been repeatedly challenged for functional niLTD (although there has not been any evidence refuting structural niLTD) (Babiec et al., 2014; Sanderson et al., 2016; Volianskis et al., 2015). Studies supporting non-ionotropic action of NMDARs show that in the presence of ligand binding – without full receptor activation or Ca^{2+} influx – conformational movement of the NMDAR CTDs occur, initiating downstream signaling pathways that result in LTD. The physiological significance and precise conditions in which this form of plasticity can arise are still unclear, but what we do know about ion flux-independent synaptic depression will be discussed at length later in this chapter (see *Non-ionotropic NMDAR-mediated LTD*).

While the concept of Ca^{2+} influx-independent NMDAR-mediated LTD is controversial, non-ionotropic (also referred to as metabotropic) action of NMDARs is not (Dore et al., 2017; Montes de Oca Balderas, 2018; Rajani et al., 2020). Growing evidence of non-ionotropic signaling of NMDARs is shifting the prevailing view that the receptor acts only as a ligand- and voltage-gated ion channel to that of a dynamic signaling complex capable of initiating lasting action through conformational changes as well. The non-ionotropic functions of NMDARs are thought to be caused by ligand binding alone, which causes conformational changes in the intracellular CTD. Movement of the CTD could then trigger downstream signaling cascades to cause receptor internalization. Postsynaptic NMDARs are found in close proximity to and directly interact with many important synaptic molecules including members of the membrane-associated guanylate kinase (MAGUK) family, calmodulin, and yotiao, a synaptic scaffolding protein (Ehlers et al., 1996; McBain and Mayer, 1994; Paoletti, 2011; Zukin and Bennett, 1995). It is therefore not surprising that conformational changes of the receptor, especially of the intracellular CTD which is rich with binding motifs, could affect the distribution and activity of neighboring proteins that contribute to the integrity of the PSD or have been implicated in synaptic plasticity. And in fact, studies have demonstrated multiple types of NMDAR signaling in the absence of ion flux through the receptor.

Glycine-primed internalization was the first confirmation of a transmembrane signaling process by which agonist binding alone to the NMDAR can induce intracellular conformational changes to initiate biochemical signaling, independent of ion flux (Nong et al., 2003). When using NMDA and glycine to stimulate isolated hippocampal neurons, a use-dependent decline in NMDAR EPSCs was exhibited when stimulation was preceded by a glycine conditioning stimulus. This reduction in current results from a decrease in NMDARs on the cell surface and was attenuated by inhibitors of dynamin-dependent endocytosis. Additionally, glycine stimulation causes an increased association of NMDARs with AP2, an intracellular endocytic adaptor protein, which is blocked by co-agonist site competitive antagonists. This result indicates that co-agonist

site stimulation alone is sufficient to prime the receptors for endocytosis (Nong et al., 2004, 2003). Subsequent glycine and glutamate stimulation triggers internalization in an ion flux-independent manner, as it is unaffected by MK-801 administration. Endogenous extracellular glycine and D-serine concentrations – which can vary by brain region – are usually between 5-10 µM, suggesting that basal co-agonist levels are just below the threshold to stimulate glycine priming for internalization. Thus, an increase in extracellular co-agonist concentration could easily trigger priming for NMDAR internalization. Glycine priming may be a key means of adjusting synaptic signaling in the presence of modulators that affect the potency of co-agonist binding (Danysz and Parsons, 1998) or important for functional changes in glycine and D-serine signaling, which may regulate NMDAR migration amongst synaptic and extrasynaptic compartments (Papouin et al., 2012) or the developmental switch from GluN2B- to GluN2A-containing (Ferreira et al., 2017). Lastly, in pathological conditions, like traumatic brain injury, ischemia, or epilepsy, glycine priming could potentially also serve as a homeostatic mechanism to prevent NMDAR-mediated excitotoxicity when glycine levels are increased (Danysz and Parsons, 1998).

Ion flux-independent NMDAR signaling has also been shown to dictate receptor trafficking upon glutamate binding (Coultrap et al., 2014; Malenka et al., 1989; Malinow et al., 1989) and tyrosine dephosphorylation of GluN2A (Vissel et al., 2001) – which leads to receptor endocytosis and decreased NMDAR EPSCs. However, it is important to note that these effects, particularly on NMDAR trafficking, require both agonist and co-agonist binding (Barria and Malinow, 2005; Nong et al., 2003; Vissel et al., 2001). The developmental subunit switch from GluN2B- to GluN2A-containing receptors has also been shown to require ligand binding but not ion flux (Barria and Malinow, 2002). Additionally, there are reports of non-ionotropic signaling in the regulation of structural plasticity (Stein et al., 2015), as well as in presynaptic control of spontaneous release (Abrahamsson et al., 2017). Clearly, the NMDAR possesses a great number of signaling capabilities in the absence of canonical receptor activation, which are in addition to

the receptor's already well characterized ionotropic capabilities, emphasizing its role as a highly sensitive and adaptable synaptic molecule.

Non-ionotropic NMDAR-mediated LTD (niLTD)

NMDARs have incredibly complex pharmacology due to their many requirements for activation. However, it is this complexity which allows their function to be modulated in a variety of ways. As previously discussed, there are many instances of downstream effects following ligand binding to the NMDAR in the absence of ion influx through the receptor. Most observed types of NMDAR non-ionotropic signaling are a result of co-agonist binding to the GluN1 subunit both in the presence or absence of glutamate. However, one of the most intriguing forms of non-ionotropic NMDAR signaling requires only glutamate binding and is an ion flux-independent form of LTD (Nabavi et al., 2013).

Over 20 years ago, data was published indicating that MK-801, an open channel "trapping" blocker, inhibited LTP but did not affect LTD (Mayford et al., 1995; Scanziani et al., 1996) – an observation that was not further discussed in either study. More recently, the formative non-ionotropic NMDAR-mediated LTD (niLTD) study used antagonists of both the glutamate and co-agonist sites, in addition to an open channel blocker, to thoroughly investigate this phenomenon (Nabavi et al., 2013). The observed synaptic depression was only blocked in the presence of a competitive glutamate site antagonist, which suggests that glutamate binding – not full receptor activation – is the sole requirement for LTD induction. Given that these results challenged the widely held tenet that LTD induction requires NMDAR activation and Ca^{2+} influx through the channel, it is not surprising that another study was immediately published in direct opposition to these results (Babiec et al., 2014). However, this refutation only tested the effects of MK-801 on LTD, and it is worth noting that experimental conditions used in the two studies in question were not identical. One putative explanation is that co-agonist site antagonism mimics low co-agonist availability, whereas application of MK-801 leaves the co-agonist site unoccupied and free to

interact with endogenous extracellular co-agonist. And while this may explain the discrepancy among groups, niLTD has been shown to be reliably inducible by many others in the presence of either MK-801 or the co-agonist site antagonists 7CK and L689 (Carter and Jahr, 2016; Dore et al., 2015; Krasteniakov et al., 2005; Mayford et al., 1995; Scanziani et al., 1996; Stein et al., 2020, 2015; Wong and Gray, 2018).

NiLTD occurs independent of Ca^{2+} influx through the NMDAR and GluN2 subunit identity (Wong and Gray, 2018), but the mechanisms underlying this phenomenon and conditions in which it can occur are still unclear. LTD was observed in experiments in which intracellular Ca^{2+} was clamped to basal levels, suggesting that while Ca^{2+} is indeed required for niLTD, a rise in intracellular Ca^{2+} is not. Therefore, glutamate binding and basal Ca^{2+} levels are the only receptor requirements that have been demonstrated thus far for niLTD. Interestingly, if these requirements are fulfilled in the presence of the open-channel blocker MK-801 while administering high-frequency stimulation (HFS) – an induction protocol normally used to evoke LTP – functional niLTD is observed (Nabavi et al., 2013). Similarly, high frequency uncaging (HFU), a structural LTP protocol, in the presence of the co-agonist site competitive antagonist, 7CK, converted spine growth into spine shrinkage (Stein et al., 2020, 2015). These results show that blocking the co-agonist site biases the NMDAR toward synaptic depression in the absence of ion flux through the NMDAR.

Most work examining subunit contribution to synaptic plasticity has focused on the GluN2 subunits and not the obligatory co-agonist binding GluN1 subunit. However, the GluN1 CTD contains a multitude of binding motifs for molecules that have been implicated in LTD, such as a calmodulin and PP1 (Mulkey et al., 1994, 1993). Infusing neurons with a GluN1 C-terminus antibody blocked LTD and the ligand-driven conformational change of the CTD that was observed in the presence of MK-801 or 7CK during glutamate stimulation (both bath application and uncaging) (Dore et al., 2015) – directly demonstrating glutamate-induced conformational

movement independent of channel opening. Surprisingly, the ligand-driven change in FRET, and therefore CTD conformation, did not require PP1 activity (Aow et al., 2015). This could be due to the target of PP1 being unavailable during basal conditions, despite the active site being exposed during changes in the GluN1 CTD. Indeed, the GluN1 subunit is a unique target of investigation for understanding the minimum receptor determinants for niLTD, as well as examining potential points of divergence between classical, ionotropic LTD and niLTD.

Downstream of the receptor, p38 MAPK has been the only protein implicated as a downstream component of functional and structural niLTD, while several other molecules – NOS1AP, nNOS, MK2, cofilin – have been shown to be required for only structural niLTD (Nabavi et al., 2013; Stein et al., 2020, 2015). Additionally, calcium-calmodulin dependent protein kinase II (CaMKII) is involved in both LTP and LTD (Coultrap et al., 2014; Malenka et al., 1989; Malinow et al., 1989). Its role as a potential target downstream of niLTD induction was demonstrated when a decrease in the interaction between FRET fluorophores on CaMKII and the NMDAR was observed during ion flux-independent LTD. This decrease was dependent on PP1 activity and dephosphorylation of CaMKII at Thr-286 (Aow et al., 2015). Interestingly, Thr-286 levels were reduced during and after niLTD induction, while total amount of CaMKII bound to the NMDAR was overall unchanged. These results are consistent with the model of niLTD in which glutamate binding to GluN2 stimulates a conformational movement of the GluN1 CTD that enables dephosphorylation of Thr-286 on CaMKII by PP1. Phosphorylation of Ser-567 on GluA1 by activated CaMKII does not require Ca^{2+} or calmodulin but has been shown to occur following LTD induction (Coultrap et al., 2014). This is consistent with the proposed mechanism for niLTD and ultimately could lead to increased AMPAR endocytosis (Kim et al., 2001; Lin et al., 2000; Shi et al., 2001) – a known means of decreasing postsynaptic functional strength. Further study of niLTD, and non-ionotropic NMDAR signaling in general, may lead to a greater understanding of the fundamental role of the obligatory GluN1 subunit.

The role of the NMDAR and the resultant cellular and synaptic changes caused by its activation have been extensively studied. As one of the most complex molecular signaling devices in the brain, it is no surprise that its synaptic proximity in combination with complex pharmacology and coincidence detection capability render it a master modulator of synaptic strength. As a regulator of both LTP and LTD induction, the NMDAR is one of the most popular synaptic molecules when discussing cellular correlates of learning and memory. There is also an extensive amount of empirical evidence demonstrating the importance of bidirectional functional plasticity in memory and the behavioral tasks used to test it. However, it is unlikely that synaptic plasticity can explain all types of learning or all types of memory and because of that, there has been a shift to look outside the synapse for the potential mechanisms that may work in conjunction with synaptic plasticity to underlie these behavioral phenomena at their most fundamental level – the cell.

The Role of Intrinsic Plasticity in Learning and Memory

The field of learning and memory has long searched for a physical representation of memory – the engram. The nature and location of the engram has proved elusive for decades, yet the development of more sophisticated molecular and transgenic tools has begun to shed light on the cellular processes underlying engram formation and activation.

The role of synaptic plasticity as a cellular substrate of memory is undeniable. Synaptic changes are activity-dependent and long-lasting, two properties which make it an ideal cellular mechanism for storing information. The idea that experience-induced modifications occurred in the connections between neurons was first proposed by Ramón y Cajal and in fact predated the term “synapse”. The century following this influential hypothesis was filled with evidence that demonstrated learning induced synaptic modifications such as increased levels of AMPA receptor

phosphorylation (Rumpel et al., 2005; Whitlock et al., 2006) and long-term potentiation (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997) – both processes that are known to be mediated by NMDARs. Therefore, following learning, populations of neurons that were activated are expected to have more potentiated synapses which will in turn lead to the formation of the engram, by strengthening the connections between cells that were coincidently active. Enhanced synaptic strength between neurons increases the probability that the same pattern of neural activity that occurred during learning can be reactivated. This outlines a direct link between experience-dependent NMDAR-mediated changes and formation of the engram.

Yet, these synaptic modifications alone do not comprise the engram, they only determine the neuronal ensemble that will encode the memory. LTP, and other forms of synaptic plasticity, have been challenged as cellular substrates of memory, as LTP does not match crucial properties of learning (Gallistel and Balsam, 2014; Gallistel and Matzel, 2013). For example, learning can occur following single experiences, while LTP and LTD induction usually entail repetitive stimulation. Additionally, experience has been shown to trigger changes in intrinsic cellular properties (Barth, 2004; Oh and Disterhoft, 2015; Titley et al., 2017). Intrinsic plasticity, which ultimately results in changes in neuronal excitability, is another potential cellular mechanism underlying learning and memory. While it has been shown that intrinsic plasticity may have region-specific roles in memory, it is thought to typically act in conjunction with various forms of synaptic plasticity (Daoudal and Debanne, 2003; Frick and Johnston, 2005; Marder et al., 1996; Mozzachiodi and Byrne, 2010; Zhang and Linden, 2003). It is postulated that synaptic plasticity is important for the *induction* of engram integration and establishment of connectivity maps, while intrinsic plasticity is responsible for the *expression* of the engram – incorporating a population of coincidently activated neurons into a neural ensemble (Ryan et al., 2015; Titley et al., 2017). It has become increasingly apparent that a purely synaptic model of learning is no longer enough to explain the process of information storage in neural networks.

Mechanisms of Intrinsic Plasticity

Intrinsic plasticity modulates a neuron's activity level and output efficacy – decisive factors for incorporation into an engram. Unlike synaptic plasticity, which is largely regulated by changes in neurotransmitter release and receptor modifications, intrinsic plasticity can be caused by changes in expression of a variety of voltage or calcium-dependent ion channels – for example, A-type K channels, BK channels, SK channels and HCN channels (Debanne and Poo, 2010; Titley et al., 2017). Current evidence also indicates that intrinsic plasticity can modulate essential cellular functions – like neuronal spike output – that are either irrespective of synaptic plasticity or, can differentially affect the likelihood of inducing synaptic plasticity. This is the case in the downregulation of SK channels, which can occur in an activity-dependent manner (Belmeguenai et al., 2010; Hammond et al., 2006; Ngo-Anh et al., 2005; Stackman et al., 2002). SK channel-mediated intrinsic plasticity is especially interesting because it can trigger effects that are both dependent and independent of synaptic plasticity (like when intrinsic changes amplify signals from potentiated synapses). Changes in SK channel expression have been shown to alter intrinsic neuronal properties, such that neurons are more excitable due to increased firing rate and decreased action potential threshold (Grasselli et al., 2016). These changes will also affect NMDAR activation and LTP induction (Babiec et al., 2017). This highlights an example of convergence of synaptic and intrinsic plasticity mechanisms. This is also an instance in which these two phenomena may work in complement in a way that allows for input-specific memory storage; it has been observed in layer 5 pyramidal neurons and Purkinje cells (Ohtsuki et al., 2012; Sourdet et al., 2003).

Under physiological conditions, changes in intrinsic plasticity are generally driven by synaptic activity; local intrinsic changes are often influenced most by responses from the largest, most potentiated synapses. Expression of many voltage-gated ion channels implicated in intrinsic excitability can be activity-dependent and therefore influenced by signals from synaptic inputs,

especially those which have been potentiated via NMDAR-dependent processes. The resulting changes in ion channel expression throughout the cell affect neuronal firing frequency, especially if these changes cause a shift in action potential threshold occur at or near the axon initial segment on the soma (Grubb et al., 2011; Grubb and Burrone, 2010). These modifications to neuronal output benefit all synapses in contact with the neuron, not just the most potentiated ones, and can be experimentally observed by comparing a neuron's responsiveness to various depolarizing currents before and after tetanization (administration of a high frequency stimuli) (Belmeguenai et al., 2010; Sourdet et al., 2003). Unlike changes in synaptic plasticity, intrinsic changes in ion channel expression are temporary, thus making it unclear their exact, defined role in memory formation or consolidation. However, it has been suggested that enhanced intrinsic excitability serves to enable action potential generation and promote burst firing. These increased firing capabilities alter the likelihood that a neuron will be integrated into a neural ensemble of synchronously active neurons. In addition, changes in intrinsic excitability will globally affect synaptic inputs, facilitating memory storage without altering synaptic weight ratios, which would conserve the memory's representation.

Previous studies examining changes in excitability in hippocampal CA1 neurons 24 hours after trace eyeblink conditioning in rabbits determined reductions in the medium, or peak, afterhyperpolarization (AHP) were the underlying cause of learning-induced increase in firing rate (Disterhoft et al., 2012; Matthews et al., 2008). The AHP is composed of a collection of potassium channel conductances, predominantly SK and BK channels. However, there are a multitude of other channels whose expression can influence membrane excitability. Hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels have been shown in CA1 pyramidal neurons to alter input resistance, EPSP summation, and firing rate in an activity-dependent manner (Gasselin et al., 2015). The potassium channels KCNQ2 and KCNQ3 have also been shown to contribute to the post-burst AHP, and deletion of these channels has been shown to

increase neuronal excitability (Brown and Adams, 1980; Delmas and Brown, 2005; Soh et al., 2014; Stocker et al., 1999; Tzingounis and Nicoll, 2008). A-type potassium channels have been shown to regulate neuronal firing rate by modulating action potential repolarization (Drion et al., 2015; Shibata et al., 2000; Simkin et al., 2015). Lastly, the inwardly-rectifying potassium channel Kir2.1, has recently been implicated in mediating learning-induced changes in intrinsic excitability in DG granule cells (Pignatelli et al., 2019). Following learning, the temporal window of experience-induced enhancement of intrinsic excitability varies by brain region, yet it is unclear whether the mechanisms underlying the learning-induced intrinsic changes are also varied.

Contributions of Intrinsic Plasticity to Engram Formation and Disease States

Increases in intrinsic excitability have been shown to occur in engram cells following learning (Titley et al., 2017). Yet endogenously increased excitability has also been shown to be important for engram allocation (the process by which neurons are recruited into a neural ensemble). Eligible neurons compete for incorporation into an engram; neurons with relatively high excitability states “win” and are more likely to be incorporated into the engram (Han et al., 2009; Rogerson et al., 2014; Yiu et al., 2014; Zhou et al., 2009). Excitability-based engram allocation has been shown to occur in different brain regions and in a variety of types of learning. In this case, it is thought that high excitability state is a means of priming cells for experience-dependent integration into the engram (Han et al., 2007; Josselyn and Tonegawa, 2020). Similarly, in CA1, neurons that are relatively more excitable before being placed in a novel environment, are more likely to become place cells than their comparatively silent neighboring cells (Cohen et al., 2017; Epsztein et al., 2011; Lee et al., 2012; Rich et al., 2014; Rickgauer et al., 2014). It is unknown what the thresholds for “high” or “silent” excitability state are; most likely they are relative to neighboring cells and vary with region and recent activity.

Disruption of intrinsic plasticity may play a role in disease states as well as difficulties with long-term memory formation. In epilepsy, networks of neurons become synchronously active, which in part may be mediated by pathologies of intrinsic plasticity. In chronic pain, changes in intrinsic plasticity can lead to an increased sensitivity to previously neutral stimuli (hyperalgesia) and to perception of pain after the noxious stimuli is removed (paresthesia). Normal aging subjects, including humans, have difficulty learning hippocampus-dependent tasks. An important cause is the reduced intrinsic excitability observed in hippocampal pyramidal neurons from normal aging subjects, as reflected by an enlarged after-hyperpolarization (AHP) and an increased spike-frequency adaptation (accommodation). Therefore, a more detailed, fundamental understanding of the role of intrinsic changes in memory formation and learning is needed as a foundation for development of disease treatments as well.

Dissertation Objective

The study of learning and memory is an age-old pursuit, and the field has made seemingly exponential progress in recent years (thanks to equally impressive progress in tool and technique development) in the observation and manipulation of engrams – the tangible traces of memories. Engrams arise through a combination of experience-dependent chemical and physical changes. It is posited that LTP and LTD (synaptic plasticity) enable formation of neuronal ensembles by establishing and regulating connections between cells with coincident activity, while changes in firing rate (intrinsic plasticity) are responsible for the actual encoding of the experience into that neuronal ensemble. Yet there are still many avenues of research open for exploration regarding both forms of plasticity. In this dissertation, I tackled two questions – each their own distinct project; one pertained to synaptic plasticity and the other, intrinsic plasticity. The results of these projects will strengthen the existing foundational knowledge of cellular and synaptic correlates of memory in the hippocampus.

Synaptic plasticity in the CA1 region of the hippocampus is largely NMDAR-mediated. Despite the NMDAR's role in plasticity being thoroughly characterized, it is still unclear why a requirement for co-agonist binding exists. Our current best guess, based on evidence of functional and structural plasticity in the absence of NMDAR-mediated ion flux (Carter and Jahr, 2016; Nabavi et al., 2013; Stein et al., 2020, 2015; Wong and Gray, 2018), is that the co-agonist serves as a modulator of non-ionotropic NMDAR-mediated plasticity. Studies have shown that glutamate binding alone is required to induce non-ionotropic LTD (Dore et al., 2017; Nabavi et al., 2013). It has also been shown that glutamate binding to the GluN2 subunit induces conformational changes in the NMDAR CTD (Aow et al., 2015; Dore et al., 2015). These conformational changes may induce a wide variety of downstream signaling pathways, sufficient to trigger synaptic plasticity. It is worth noting that these previous results were obtained mostly in the presence of a co-agonist site competitive antagonist, which is only a proxy for low co-agonist availability. Therefore, I sought to study the role of GluN1 in non-ionotropic plasticity by directly manipulating extracellular co-agonist concentration during both ionotropic and non-ionotropic LTD induction. The GluN1 subunit provides a unique target of investigation for understanding the determinants of niLTD, as well as examining potential points of divergence between LTD and niLTD. Further study of niLTD may lead to a greater understanding of the function of the co-agonist site and the obligatory GluN1 subunit.

As previously mentioned, intrinsic changes following learning are as common as synaptic changes. However, synaptic changes are long-lasting, while intrinsic changes have only been observed on a timescale of minutes to days following learning. This emphasizes the differential role the two cellular changes play in learning and memory. A causal relationship between engram cell excitability and context recognition capabilities has been demonstrated in dentate gyrus (Pignatelli et al., 2019), but no such link between neural ensemble activity and behavior has been shown in CA1. My second project aimed to further investigate the underlying mechanisms of

increased excitability state in CA1 neurons following contextual fear conditioning and potential relationship between engram activity and behavior. This project will contribute to the increasingly popular idea that modulation of intrinsic excitability plays a role in learning. By shifting away from exclusively synapse-based models of learning mechanisms, we can appreciate the wide range of cellular tools employed for memory formation and storage.

References

Abraham, W.C., Bear, M.F., 1996. Metaplasticity: The plasticity of synaptic plasticity. Trends in Neurosciences 19, 126–130. https://doi.org/10.1016/S0166-2236(96)80018-X

Abraham, W.C., Logan, B., Greenwood, J.M., Dragunow, M., 2002. Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 22, 9626–9634.

Abrahamsson, T., Chou, C.Y.C., Li, S.Y., Mancino, A., Costa, R.P., Brock, J.A., Nuro, E., Buchanan, K.A., Elgar, D., Blackman, A.V., Tudor-Jones, A., Oyrer, J., Farmer, W.T., Murai, K.K., Sjöström, P.J., 2017. Differential Regulation of Evoked and Spontaneous Release by Presynaptic NMDA Receptors. Neuron 96, 839-855.e5. https://doi.org/10.1016/j.neuron.2017.09.030

Adam-Vizi, V., 1992. External Ca^{2+} -Independent Release of Neurotransmitters. J Neurochem 58, 395–405. https://doi.org/10.1111/j.1471-4159.1992.tb09736.x

Akazawa, C., Shigemoto, R., Bessho, Y., Nakanishi, S., Mizuno, N., 1994. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. The Journal of comparative neurology 347, 150–60. https://doi.org/10.1002/cne.903470112

Aow, J., Dore, K., Malinow, R., 2015. Conformational signaling required for synaptic plasticity by the NMDA receptor complex. Proceedings of the National Academy of Sciences 112, 14711–14716. https://doi.org/10.1073/pnas.1520029112

Artola, A., Singer, W., 1993. Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. Trends in neurosciences 16, 480–7. https://doi.org/10.1016/0166-2236(93)90081-V

Attwell, D., Barbour, B., Szatkowski, M., 1993. Nonvesicular release of neurotransmitter. Neuron 11, 401–407. https://doi.org/10.1016/0896-6273(93)90145-H

Babiec, W.E., Guglietta, R., Jami, S.A., Morishita, W., Malenka, R.C., Dell, T.J.O., 2014. Ionotropic NMDA Receptor Signaling Is Required for the Induction of Long-Term Depression in the Mouse Hippocampal CA1 Region 34, 5285–5290. https://doi.org/10.1523/JNEUROSCI.5419-13.2014

Babiec, W.E., Jami, S.A., Guglietta, R., Chen, P.B., O'Dell, T.J., 2017. Differential Regulation of NMDA Receptor-Mediated Transmission by SK Channels Underlies Dorsal-Ventral Differences in Dynamics of Schaffer Collateral Synaptic Function. The Journal of Neuroscience 37, 1950–1964. https://doi.org/10.1523/JNEUROSCI.3196-16.2017

Balan, L., Foltyn, V.N., Zehl, M., Dumin, E., Dikopoltsev, E., Knoh, D., Ohno, Y., Kihara, A., Jensen, O.N., Radzishevsky, I.S., Wolosker, H., 2009. Feedback inactivation of D-serine synthesis by NMDA receptor-elicited translocation of serine racemase to the membrane. Proceedings of the National Academy of Sciences 106, 7589–7594. https://doi.org/10.1073/pnas.0809442106

Balu, D.T., Basu, A.C., Corradi, J.P., Cacace, A.M., Coyle, J.T., 2012. The NMDA receptor co-agonists, d-serine and glycine, regulate neuronal dendritic architecture in the somatosensory cortex. Neurobiology of Disease 45, 671–682. https://doi.org/10.1016/j.nbd.2011.10.006

Balu, D.T., Coyle, J.T., 2015. The NMDA receptor ‘ glycine modulatory site ’ in schizophrenia : D-serine, glycine , and beyond. Current Opinion in Pharmacology 20, 109–115. https://doi.org/10.1016/j.coph.2014.12.004

Balu, D.T., Li, Y., Puhl, M.D., Benneyworth, M.A., Basu, A.C., Takagi, S., Bolshakov, V.Y., Coyle, J.T., 2013. Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. Proceedings of the National Academy of Sciences of the United States of America 110, E2400-9. https://doi.org/10.1073/pnas.1304308110

Balu, D.T., Takagi, S., Puhl, M.D., Benneyworth, M.A., Coyle, J.T., 2014. d-Serine and Serine Racemase are Localized to Neurons in the Adult Mouse and Human Forebrain. Cell Mol Neurobiol 34, 419–435. https://doi.org/10.1007/s10571-014-0027-z

Barria, A., 1997. Regulatory Phosphorylation of AMPA-Type Glutamate Receptors by CaM-KII During Long-Term Potentiation. Science 276, 2042–2045. https://doi.org/10.1126/science.276.5321.2042

Barria, A., Malinow, R., 2005. NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48, 289–301. https://doi.org/10.1016/j.neuron.2005.08.034

Barria, A., Malinow, R., 2002. Subunit-Specific NMDA Receptor Trafficking to Synapses. Neuron 35, 203–221. https://doi.org/10.1016/B978-012369437-9/50015-3

Barrionuevo, G., Schottler, F., Lynch, G., 1980. The effects of repetitive low frequency stimulation on control and “potentiated” synaptic responses in the hippocampus. Life Sciences 27, 2385–2391. https://doi.org/10.1016/0024-3205(80)90509-3

Barth, A.L., 2004. Alteration of Neuronal Firing Properties after In Vivo Experience in a FosGFP Transgenic Mouse. Journal of Neuroscience 24, 6466–6475. https://doi.org/10.1523/JNEUROSCI.4737-03.2004

Barth, A.L., Malenka, R.C., 2001. NMDAR EPSC kinetics do not regulate the critical period for LTP at thalamocortical synapses. Nat Neurosci 4, 235–236. https://doi.org/10.1038/85070

Basu, A.C., Tsai, G.E., Ma, C.-L., Ehmsen, J.T., Mustafa, A.K., Han, L., Jiang, Z.I., Benneyworth, M.A., Froimowitz, M.P., Lange, N., Snyder, S.H., Bergeron, R., Coyle, J.T., 2009. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Molecular psychiatry 14, 719–27. https://doi.org/10.1038/mp.2008.130

Bayer, K.-U., De Koninck, P., Leonard, A.S., Hell, J.W., Schulman, H., 2001. Interaction with the NMDA receptor locks CaMKII in an active conformation. Nature 411, 801–805. https://doi.org/10.1038/35081080

Bear, M.F., 1995. Mechanism for a sliding synaptic modification threshold. Neuron 15, 1–4. https://doi.org/10.1016/0896-6273(95)90056-X

Belmeguenai, A., Hosy, E., Bengtsson, F., Pedroarena, C.M., Piochon, C., Teuling, E., He, Q., Ohtsuki, G., De Jeu, M.T.G., Elgersma, Y., De Zeeuw, C.I., Jorntell, H., Hansel, C., 2010. Intrinsic Plasticity Complements Long-Term Potentiation in Parallel Fiber Input Gain Control in Cerebellar Purkinje Cells. Journal of Neuroscience 30, 13630–13643. https://doi.org/10.1523/JNEUROSCI.3226-10.2010

Benneyworth, M.A., Li, Y., Basu, A.C., Bolshakov, V.Y., Coyle, J.T., 2012. Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in

cortical glutamatergic neurons. Cellular and Molecular Neurobiology 32, 613–624. https://doi.org/10.1007/s10571-012-9808-4

Benveniste, M., Mayer, M.L., 1991. Kinetic analysis of antagonist action at N-methyl-D-aspartic acid receptors. Two binding sites each for glutamate and glycine. Biophysical Journal 59, 560–573. https://doi.org/10.1016/S0006-3495(91)82272-X

Berger, T.W., Rinaldi, P.C., Weisz, D.J., Thompson, R.F., 1983. Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. Journal of Neurophysiology 50, 1197–1219. https://doi.org/10.1152/jn.1983.50.5.1197

Bergeron, R., Meyer, T., Coyle, J., Greene, R., 1998. Modulation of N-methyl-D-aspartate receptor function by glycine transport. Proceedings of the National Academy of Sciences 95, 15730–15734.

Bernier, B.E., Lacagnina, A.F., Ayoub, A., Shue, F., Zemelman, B.V., Krasne, F.B., Drew, M.R., 2017. Dentate Gyrus Contributes to Retrieval as well as Encoding: Evidence from Context Fear Conditioning, Recall, and Extinction. J. Neurosci. 37, 6359–6371. https://doi.org/10.1523/JNEUROSCI.3029-16.2017

Bienenstock, L., Cooper, N., Munro, W., 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex 17.

Bliss, T.V., Lomo, T., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaestetized rabbit following stimulation of the perforant path. The Journal of physiology 232, 357–374.

Bliss, T.V.P., Gardner-Medwin, A.R., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. The Journal of Physiology 232, 357–374. https://doi.org/10.1113/jphysiol.1973.sp010274

Bourne, H.R., Nicoll, R., 1993. Molecular machines integrate coincident synaptic signals. Cell 72, 65–75. https://doi.org/10.1016/S0092-8674(05)80029-7

Brandon, E.P., Zhuo, M., Huang, Y.Y., Qi, M., Gerhold, K.A., Burton, K.A., Kandel, E.R., McKnight, G.S., Idzerda, R.L., 1995. Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase. Proceedings of the National Academy of Sciences of the United States of America 92, 8851–5. https://doi.org/10.1073/pnas.92.19.8851

Brickley, S.G., Misra, C., Mok, M.H.S., Mishina, M., Cull-Candy, S.G., 2003. NR2B and NR2D Subunits Coassemble in Cerebellar Golgi Cells to Form a Distinct NMDA Receptor Subtype Restricted to Extrasynaptic Sites. J. Neurosci. 23, 4958–4966. https://doi.org/10.1523/JNEUROSCI.23-12-04958.2003

Brown, D.A., Adams, P.R., 1980. Muscarinic suppression of a novel voltage-sensitvie K+ current in a vertebrate neuron. Nature 283, 673–676. https://doi.org/10.2307/814290

Carr, M.F., Jadhav, S.P., Frank, L.M., 2011. Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. Nat Neurosci 14, 147–153. https://doi.org/10.1038/nn.2732

Carter, B.C., Jahr, C.E., 2016. Postsynaptic, not presynaptic NMDA receptors are required for spike-timing-dependent LTD induction. Nature Neuroscience 19, 1218–1224. https://doi.org/10.1038/nn.4343

Clements, J.D., Feltz, A., Sahara, Y., Westbrook, G.L., 1998. Activation Kinetics of AMPA Receptor Channels Reveal the Number of Functional Agonist Binding Sites. J. Neurosci. 18, 119–127. https://doi.org/10.1523/JNEUROSCI.18-01-00119.1998

Clements, J.D., Westbrook, G.L., 1991. Activation Kinetics Reveal the Number of Glutamate and Glycine Binding Sites on the N-Methyl-BAspartate Receptor. Neuron 7, 605–13.

Cohen, J.D., Bolstad, M., Lee, A.K., 2017. Experience-dependent shaping of hippocampal CA1 intracellular activity in novel and familiar environments. eLife 6, e23040. https://doi.org/10.7554/eLife.23040

Collingridge, G.L., Isaac, J.T.R., Wang, Y.T., 2004. Receptor trafficking and synaptic plasticity. Nature Reviews Neuroscience 5, 952–962. https://doi.org/10.1038/nrn1556

Collingridge, G.L., Kehl, S.J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. The Journal of Physiology 334, 33–46. https://doi.org/10.1113/jphysiol.1983.sp014478

Collingridge, G.L., Peineau, S., Howland, J.G., Wang, Y.T., 2010. Long-term depression in the CNS. Nature Reviews Neuroscience 11, 459–473. https://doi.org/10.1038/nrn2867

Coultrap, S.J., Freund, R.K., O'Leary, H., Sanderson, J.L., Roche, K.W., Dell'Acqua, M.L., Bayer, K.U., 2014. Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Reports 6, 431–437. https://doi.org/10.1016/j.celrep.2014.01.005

Cull-Candy, S.G., Leszkiewicz, D.N., 2004. Role of Distinct NMDA Receptor Subtypes at Central Synapses. Science Signaling 2004. https://doi.org/10.1126/stke.2552004re16

Cummings, J.A., Mulkey, R.M., Nicoll, R.A., Malenka, R.C., 1996. Ca2+ signaling requirements for long-term depression in the hippocampus. Neuron 16, 825–833. https://doi.org/10.1016/S0896-6273(00)80102-6

Dalby, N.O., Mody, I., 2003. Activation of NMDA Receptors in Rat Dentate Gyrus Granule Cells by Spontaneous and Evoked Transmitter Release. Journal of Neurophysiology 90, 786–797. https://doi.org/10.1152/jn.00118.2003

Danysz, W., Parsons, C.G., 1998. Glycine and N-Methyl-D-Aspartate Receptors: Physiological Significance and Possible Therapeutic Applications. Pharmalogical Reviews 50, 598–650.

Daoudal, G., Debanne, D., 2003. Long-Term Plasticity of Intrinsic Excitability: Learning Rules and Mechanisms. Learning & Memory 10, 456–465. https://doi.org/10.1101/lm.64103

Debanne, D., Poo, M.M., 2010. Spike-timing dependent plasticity beyond synapse - pre- and post-synaptic plasticity of intrinsic neuronal excitability. Frontiers in Synaptic Neuroscience 2, 1–6. https://doi.org/10.3389/fnsyn.2010.00021

Delmas, P., Brown, D.A., 2005. Pathways modulating neural KCNQ/M (Kv7) potassium channels. Nature Reviews Neuroscience 6, 850–862. https://doi.org/10.1038/nrn1785

Diering, G.H., Huganir, R.L., 2018. The AMPA Receptor Code of Synaptic Plasticity. Neuron 100, 314–329. https://doi.org/10.1016/j.neuron.2018.10.018

Disterhoft, J.F., Weiss, C., Burgdorf, J., Oh, M.M., McKay, B.M., Kroes, R.A., Galvez, R., Moskal, J.R., Adelman, J.P., 2012. Increasing SK2 channel activity impairs associative learning. Journal of Neurophysiology 108, 863–870. https://doi.org/10.1152/jn.00025.2012

Dodt, H., Eder, M., Frick, A., Zieglgansberger, W., 1999. Precisely Localized LTD in the Neocortex Revealed by Infrared-Guided Laser Stimulation. Science 286, 110–113. https://doi.org/10.1126/science.286.5437.110

Dore, K., Aow, J., Malinow, R., 2015. Agonist binding to the NMDA receptor drives movement of its cytoplasmic domain without ion flow. Proceedings of the National Academy of Sciences 112, 14705–14710. https://doi.org/10.1073/pnas.1520023112

Dore, K., Stein, I.S., Brock, J.A., Castillo, P.E., Zito, K., Sjöström, P.J., 2017. Unconventional NMDA Receptor Signaling. The Journal of neuroscience : the official journal of the Society for Neuroscience 37, 10800–10807. https://doi.org/10.1523/JNEUROSCI.1825-17.2017

Drion, G., O'Leary, T., Marder, E., 2015. Ion channel degeneracy enables robust and tunable neuronal firing rates. Proceedings of the National Academy of Sciences 112, E5361–E5370. https://doi.org/10.1073/pnas.1516400112

Dudek, S.M., Bear, M.F., 1993. Bidirectional Long-Term Modification of Synaptic Effectiveness in the Adult and Immature Hippocampus. The Journal of Neuroscience 13, 2910–2918.

Dudek, S.M., Bear, M.F., 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences of the United States of America 89, 4363–7. https://doi.org/10.1073/pnas.89.10.4363

Durham, R.J., Paudyal, N., Carrillo, E., Bhatia, N.K., Maclean, D.M., Berka, V., Dolino, D.M., Gorfe, A.A., Jayaraman, V., 2020. Conformational spread and dynamics in allostery of NMDA receptors. Proc Natl Acad Sci USA 117, 3839–3847. https://doi.org/10.1073/pnas.1910950117

Ehlers, M.D., Zhang, S., Bernhardt, J.P., Huganir, R.L., 1996. Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. Cell 84, 745–755. https://doi.org/10.1016/S0092-8674(00)81052-1

Epsztein, J., Brecht, M., Lee, A.K., 2011. Intracellular Determinants of Hippocampal CA1 Place and Silent Cell Activity in a Novel Environment. Neuron 70, 109–120. https://doi.org/10.1016/j.neuron.2011.03.006

Ferreira, J.S., Papouin, T., Ladépêche, L., Yao, A., Langlais, V.C., Bouchet, D., Dulong, J., Mothet, J.P., Sacchi, S., Pollegioni, L., Paoletti, P., Richard Oliet, S.H., Groc, L., 2017. Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses. eLife 6, 1–22. https://doi.org/10.7554/eLife.25492

Flint, A.C., Maisch, U.S., Weishaupt, J.H., Kriegstein, A.R., Monyer, H., 1997. NR2A Subunit Expression Shortens NMDA Receptor Synaptic Currents in Developing Neocortex. J. Neurosci. 17, 2469–2476. https://doi.org/10.1523/JNEUROSCI.17-07-02469.1997

Forrest, D., Yuzaki, M., Soares, H.D., Ng, L., Luk, D.C., Sheng, M., Stewart, C.L., Morgan, J.I., Connor, J.A., Curran, T., 1994. Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. Neuron 13, 325–338. https://doi.org/10.1016/0896-6273(94)90350-6

Fossat, P., Turpin, F.R., Sacchi, S., Dulong, J., Shi, T., Rivet, J.M., Sweedler, J.V., Pollegioni, L., Millan, M.J., Oliet, S.H.R., Mothet, J.P., 2012. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cerebral Cortex 22, 595–606. https://doi.org/10.1093/cercor/bhr130

Frick, A., Johnston, D., 2005. Plasticity of dendritic excitability. J. Neurobiol. 64, 100–115. https://doi.org/10.1002/neu.20148

Fujii, S., Saito, K., Miyakawa, H., Ito, K. ichi, Kato, H., 1991. Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig

hippocampal slices. Brain Research 555, 112–122. https://doi.org/10.1016/0006-8993(91)90867-U

Fujisawa, S., Aoki, C., 2003. In vivo blockade of N-methyl-d-aspartate receptors induces rapid trafficking of NR2B subunits away from synapses and out of spines and terminals in adult cortex. Neuroscience 121, 51–63. https://doi.org/10.1016/S0306-4522(03)00341-5

Fukasawa, Y., Segawa, H., Kim, J.Y., Chairoungdua, A., Kim, D.K., Matsuo, H., Cha, S.H., Endou, H., Kanai, Y., 2000. Identification and Characterization of a Na+-independent Neutral Amino Acid Transporter That Associates with the 4F2 Heavy Chain and Exhibits Substrate Selectivity for Small Neutral d- and l-Amino Acids. Journal of Biological Chemistry 275, 9690–9698. https://doi.org/10.1074/jbc.275.13.9690

Gabrieli, J.D.E., Brewer, J.B., Desmond, J.E., Glover, G.H., 1997. Separate Neural Bases of Two Fundamental Memory Processes in the Human Medial Temporal Lobe. Science 276, 264–266. https://doi.org/10.1126/science.276.5310.264

Gallistel, C.R., Balsam, P.D., 2014. Time to rethink the neural mechanisms of learning and memory. Neurobiology of Learning and Memory 108, 136–144. https://doi.org/10.1016/j.nlm.2013.11.019

Gallistel, C.R., Matzel, L.D., 2013. The Neuroscience of Learning: Beyond the Hebbian Synapse. Annu. Rev. Psychol. 64, 169–200. https://doi.org/10.1146/annurev-psych-113011-143807

Gasselin, C., Inglebert, Y., Debanne, D., 2015. Homeostatic regulation of h-conductance controls intrinsic excitability and stabilizes the threshold for synaptic modification in CA1 neurons. Journal of Physiology 593, 4855–4869. https://doi.org/10.1113/JP271369

Goldberg, J.H., Yuste, R., Tamas, G., 2003. Ca2+ imaging of mouse neocortical interneurone dendrites: Contribution of Ca2+-permeable AMPA and NMDA receptors to subthreshold Ca2+dynamics. The Journal of Physiology 551, 67–78. https://doi.org/10.1113/jphysiol.2003.042598

Grasselli, G., He, Q., Wan, V., Adelman, J.P., Ohtsuki, G., Hansel, C., 2016. Activity-Dependent Plasticity of Spike Pauses in Cerebellar Purkinje Cells. Cell Reports 14, 2546–2553. https://doi.org/10.1016/j.celrep.2016.02.054

Gray, J.A., Shi, Y., Usui, H., During, M.J., Sakimura, K., Nicoll, R.A., 2011. Distinct Modes of AMPA Receptor Suppression at Developing Synapses by GluN2A and GluN2B: Single-Cell NMDA Receptor Subunit Deletion In Vivo. Neuron 71, 1085–1101. https://doi.org/10.1016/j.neuron.2011.08.007

Grubb, M.S., Burrone, J., 2010. Building and maintaining the axon initial segment. Current Opinion in Neurobiology 20, 481–488. https://doi.org/10.1016/j.conb.2010.04.012

Grubb, M.S., Shu, Y., Kuba, H., Rasband, M.N., Wimmer, V.C., Bender, K.J., 2011. Short- and Long-Term Plasticity at the Axon Initial Segment. The Journal of Neuroscience 31, 16049–16055. https://doi.org/10.1523/JNEUROSCI.4064-11.2011

Guzowski, J.F., Setlow, B., Wagner, E.K., McGaugh, J.L., 2001. Experience-Dependent Gene Expression in the Rat Hippocampus after Spatial Learning: A Comparison of the Immediate-Early Genes *Arc* , c- *fos* , and *zif268*. J. Neurosci. 21, 5089–5098. https://doi.org/10.1523/JNEUROSCI.21-14-05089.2001

Hammond, R.S., Bond, C.T., Strassmaier, T., Jennifer Ngo-Anh, T., Adelman, J.P., Maylie, J., Stackman, R.W., 2006. Small-Conductance Ca $^{2+}$ -Activated K $^{+}$ Channel Type 2 (SK2)

Modulates Hippocampal Learning, Memory, and Synaptic Plasticity. J. Neurosci. 26, 1844–1853. https://doi.org/10.1523/JNEUROSCI.4106-05.2006
Han, J.-H., Kushner, S.A., Yiu, A.P., Cole, C.J., Matynia, A., Brown, R.A., Neve, R.L., Guzowski, J.F., Silva, A.J., Josselyn, S.A., 2007. Neuronal Competition and Selection During Memory Formation. Science 316, 457–460. https://doi.org/10.1126/science.1139438
Han, J.-H., Kushner, S.A., Yiu, A.P., Hsiang, H.-L. (Liz), Buch, T., Waisman, A., Bontempi, B., Neve, R.L., Frankland, P.W., Josselyn, S.A., 2009. Selective Erasure of a Fear Memory. Science 323, 1492–1496. https://doi.org/10.1126/science.1164139
Han, L., Campanucci, V.A., Cooke, J., Salter, M.W., 2013. Identification of a single amino acid in GluN1 that is critical for glycine-primed internalization of NMDA receptors. Mol Brain 6, 36. https://doi.org/10.1186/1756-6606-6-36
Henley, J.M., Wilkinson, K.A., 2016. Synaptic AMPA receptor composition in development, plasticity and disease. Nat Rev Neurosci 17, 337–350. https://doi.org/10.1038/nrn.2016.37
Henneberger, C., Bard, L., King, C., Jennings, A., Rusakov, D.A., 2013. NMDA receptor activation: Two targets for two co-agonists. Neurochemical Research 38, 1156–1162. https://doi.org/10.1007/s11064-013-0987-2
Herron, C.E., Lester, R.A.J., Coan, E.J., Collingridge, G.L., 1986. Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. Nature 322, 265–268. https://doi.org/10.1038/322265a0
Hollmann, M., Heinemann, S., 1994. Cloned Glutamate Receptors. Annual Review of Neuroscience 17, 31–108.
Hrabetova, S., Sacktor, T.C., 2001. Transient translocation of conventional protein kinase C isoforms and persistent downregulation of atypical protein kinase Mzeta in long-term depression. Brain research. Molecular brain research 95, 146–52. https://doi.org/10.1016/S0169-328X(01)00185-1
Hrabetova, S., Serrano, P., Blace, N., Tse, H.W., Skifter, D. a, Jane, D.E., Monaghan, D.T., Sacktor, T.C., 2000. Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, RC81–RC81.
Huang, Y.H., Bergles, D.E., 2004. Glutamate transporters bring competition to the synapse. Current Opinion in Neurobiology 14, 346–352. https://doi.org/10.1016/j.conb.2004.05.007
Ishizuka, N., Weber, J., Amaral, D.G., 1990. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. J. Comp. Neurol. 295, 580–623. https://doi.org/10.1002/cne.902950407
Ivanov, A., Pellegrino, C., Rama, S., Dumalska, I., Salyha, Y., Ben-Ari, Y., Medina, I., 2006. Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons: Synaptic and extrasynaptic NMDAR in ERK activation. The Journal of Physiology 572, 789–798. https://doi.org/10.1113/jphysiol.2006.105510
Janssen, W.G.M., Vissavajjhala, P., Andrews, G., Moran, T., Hof, P.R., Morrison, J.H., 2005. Cellular and synaptic distribution of NR2A and NR2B in macaque monkey and rat hippocampus as visualized with subunit-specific monoclonal antibodies. Experimental Neurology 191, S28–S44. https://doi.org/10.1016/j.expneurol.2004.08.020

Johnson, J.W., Ascher, P., 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325, 529–531. https://doi.org/10.1038/325529a0

Josselyn, S.A., Tonegawa, S., 2020. Memory engrams: Recalling the past and imagining the future. Science 367, eaaw4325. https://doi.org/10.1126/science.aaw4325

Kamal, A., 1999. Chemical LTD in the CA1 field of the hippocampus from young and mature rats. European Journal of Neuroscience 11, 3512–3516. https://doi.org/10.1046/j.1460-9568.1999.00769.x

Kameyama, K., Lee, H.K., Bear, M.F., Huganir, R.L., 1998. Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. Neuron 21, 1163–1175. https://doi.org/10.1016/S0896-6273(00)80633-9

Kandler, K., Katz, L.C., Kauer, J.A., 1998. Focal photolysis of caged glutamate produces long-term depression of hippocampal glutamate receptors. Nature Neuroscience 1, 119–123. https://doi.org/10.1038/368

Káradóttir, R., Cavelier, P., Bergersen, L.H., Attwell, D., 2005. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438, 1162–1166. https://doi.org/10.1038/nature04302

Karakas, E., Furukawa, H., 2014. Crystal structure of a heterotetrameric NMDA receptor ion channel. Science 344, 992–997.

Kartvelishvily, E., Shleper, M., Balan, L., Dumin, E., Wolosker, H., 2006. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. Journal of Biological Chemistry 281, 14151–14162. https://doi.org/10.1074/jbc.M512927200

Kauer, J.A., Malenka, R.C., Nicoll, R.A., 1988. NMDA application potentiates synaptic transmission in the hippocampus. Nature 334, 250–252. https://doi.org/10.1038/334250a0

Kew, J.N.C., Koester, A., Moreau, J., Jenck, F., Ouagazzal, A., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., Hundt, W., Reinscheid, R.K., Pauly-evers, M., Kemp, J.A., Bluethmann, H., 2000. Functional Consequences of Reduction in NMDA Receptor Glycine Affinity in Mice Carrying Targeted Point Mutations in the Glycine Binding Site. Journal of Neuroscience 20, 4037–4049.

Kim, C.H., Chung, H.J., Lee, H.K., Huganir, R.L., 2001. Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. Proceedings of the National Academy of Sciences of the United States of America 98, 11725–30. https://doi.org/10.1073/pnas.211132798

Kim, J., Wei, D.S., Hoffman, D.A., 2005. Kv4 potassium channel subunits control action potential repolarization and frequency-dependent broadening in rat hippocampal CA1 pyramidal neurones. Journal of Physiology 569, 41–57. https://doi.org/10.1113/jphysiol.2005.095042

Kirkwood, A., Bear, M.F.F., 1994. Homosynaptic long-term depression in the visual cortex. The Journal of neuroscience 14, 3404–3404.

Kirkwood, A., Dudek, S.M., Gold, J.T., Aizenman, C.D., Bear, M.F., 1993. Common Forms of Synaptic Plasticity in the Hippocampus and Neocortex in Vitro Published by : American Association for the Advancement of Science Stable URL : https://www.jstor.org/stable/2881527 JSTOR is a not-for-profit service that helps scholars , rese. Science 260, 1518–1521.

Kirkwood, A., Rozas, C., Kirkwood, J., Perez, F., Bear, M.F., 1999. Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. The Journal of neuroscience : the official journal of the Society for Neuroscience 19, 1599–1609.

Kirson, E.D., Yaari, Y., 1996. Synaptic NMDA receptors in developing mouse hippocampal neurones: functional properties and sensitivity to ifenprodil. The Journal of Physiology 497, 437–455. https://doi.org/10.1113/jphysiol.1996.sp021779

Kleckner, N.W., Dingledine, R., 1988. Requirement for Glycine in Activation of NMDA-Receptors Expressed in Xenopus Oocytes. Science 241, 835–837.

Köhr, G., 2006. NMDA receptor function: subunit composition versus spatial distribution. Cell Tissue Res 326, 439–446. https://doi.org/10.1007/s00441-006-0273-6

Köhr, G., Jensen, V., Koester, H.J., Mihaljevic, A.L.A., Utvik, J.K., Kvello, A., Ottersen, O.P., Seeburg, P.H., Sprengel, R., Hvalby, Ø., 2003. Intracellular Domains of NMDA Receptor Subtypes Are Determinants for Long-Term Potentiation Induction. J. Neurosci. 23, 10791–10799. https://doi.org/10.1523/JNEUROSCI.23-34-10791.2003

Krapivinsky, G., Krapivinsky, L., Manasian, Y., Ivanov, A., Tyzio, R., Pellegrino, C., Ben-Ari, Y., Clapham, D.E., Medina, I., 2003. The NMDA Receptor Is Coupled to the ERK Pathway by a Direct Interaction between NR2B and RasGRF1. Neuron 40, 775–784. https://doi.org/10.1016/S0896-6273(03)00645-7

Krasteniakov, N.V., Martina, M., Bergeron, R., 2005. Role of the glycine site of the N-methyl-D-aspartate receptor in synaptic plasticity induced by pairing. European Journal of Neuroscience 21, 2782–2792. https://doi.org/10.1111/j.1460-9568.2005.04099.x

Kullmann, D.M., Asztely, F., 1998. Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. Trends in Neurosciences 21, 8–14. https://doi.org/10.1016/S0166-2236(97)01150-8

Kumashiro, S., Hashimoto, A., Nishikawa, T., 1995. Free d-serine in post-mortem brains and spinal cords of individuals with and without neuropsychiatric diseases. Brain Research 681, 117–125. https://doi.org/10.1016/0006-8993(95)00307-C

Lalo, U., 2006. NMDA Receptors Mediate Neuron-to-Glia Signaling in Mouse Cortical Astrocytes. Journal of Neuroscience 26, 2673–2683. https://doi.org/10.1523/JNEUROSCI.4689-05.2006

Lau, C.G., Zukin, R.S., 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nature Reviews Neuroscience 8, 413–426. https://doi.org/10.1038/nrn2153

Lavezzari, G., MacCallum, J., Dewey, C.M., Roche, K.W., 2004. Subunit-Specific Regulation of NMDA Receptor Endocytosis. Journal of Neuroscience 24, 6383–6391. https://doi.org/10.1523/JNEUROSCI.1890-04.2004

Le Bail, M., Martineau, M., Sacchi, S., Yatsenko, N., Radzishevsky, I., Conrod, S., Ait Ouares, K., Wolosker, H., Pollegioni, L., Billard, J.-M., Mothet, J.-P., 2015. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 112, E204-13. https://doi.org/10.1073/pnas.1416668112

Lee, D., Lin, B.-J., Lee, A.K., 2012. Hippocampal Place Fields Emerge upon Single-Cell Manipulation of Excitability During Behavior. Science 337, 849–853. https://doi.org/10.1126/science.1221489

Lee, H.K., Barbarosie, M., Kameyama, K., Bear, M.F., Huganir, R.L., 2000. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405, 955–959. https://doi.org/10.1038/35016089

Lee, H.K., Kameyama, K., Huganir, R.L., Bear, M.F., 1998. NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21, 1151–1162. https://doi.org/10.1016/S0896-6273(00)80632-7

Lee, H.-K., Takamiya, K., Han, J.-S., Man, H., Kim, C.-H., Rumbaugh, G., Yu, S., Ding, L., He, C., Petralia, R.S., Wenthold, R.J., Gallagher, M., Huganir, R.L., 2003. Phosphorylation of the AMPA Receptor GluR1 Subunit Is Required for Synaptic Plasticity and Retention of Spatial Memory. Cell 112, 631–643. https://doi.org/10.1016/S0092-8674(03)00122-3

Leeson, P.D., Iversen, L.L., 1994. The Glycine Site on the NMDA Receptor: Structure-Activity Relationships and Therapeutic Potential. Journal of medicinal chemistry 37, 4053–4067. https://doi.org/10.1021/jm00050a001

Li, J.H., Wang, Y.H., Wolfe, B.B., Krueger, Karl.E., Corsi, L., Stocca, G., Vicini, S., 1998. Developmental changes in localization of NMDA receptor subunits in primary cultures of cortical neurons: NMDA receptor subtypes in developing cortical neurons. European Journal of Neuroscience 10, 1704–1715. https://doi.org/10.1046/j.1460-9568.1998.00169.x

Li, R., Dozmorov, M., Hellberg, F., Tian, Y., Jilderos, B., Wigström, H., 2004. Characterization of NMDA induced depression in rat hippocampus: Involvement of AMPA and NMDA receptors. Neuroscience Letters 357, 87–90. https://doi.org/10.1016/j.neulet.2003.11.004

Li, Y., Erzurumlu, R.S., Chen, C., Jhaveri, S., Tonegawa, S., 1994. Whisker-related neuronal patterns fail to develop in the trigeminal brainstem nuclei of NMDAR1 knockout mice. Cell 76, 427–437. https://doi.org/10.1016/0092-8674(94)90108-2

Lin, H., Jacobi, A.A., Anderson, S.A., Lynch, D.R., 2016. D-Serine and Serine Racemase Are Associated with PSD-95 and Glutamatergic Synapse Stability. Front Cell Neurosci 10, 34–34. https://doi.org/10.3389/fncel.2016.00034

Lin, J.W., Ju, W., Foster, K., Lee, S.H., Ahmadian, G., Wyszynski, M., Wang, Y.T., Sheng, M., 2000. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nature Neuroscience 3, 1282–1290. https://doi.org/10.1038/81814

Lu, H.-C., Gonzalez, E., Crair, M.C., 2001. Barrel Cortex Critical Period Plasticity Is Independent of Changes in NMDA Receptor Subunit Composition. Neuron 32, 619–634. https://doi.org/10.1016/S0896-6273(01)00501-3

Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R., Seeburg, P.H., Nicoll, R.A., 2009. Subunit Composition of Synaptic AMPA Receptors Revealed by a Single-Cell Genetic Approach. Neuron 62, 254–268. https://doi.org/10.1016/j.neuron.2009.02.027

Ma, T.M., Paul, B.D., Fu, C., Hu, S., Zhu, H., Blackshaw, S., Wolosker, H., Snyder, S.H., 2014. Serine racemase regulated by binding to stargazin and PSD-95: Potential N-methyl-D-aspartate-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (NMDA-AMPA) glutamate neurotransmission cross-talk. Journal of Biological Chemistry 289, 29631–29641. https://doi.org/10.1074/jbc.M114.571604

Malenka, R.C., Kauer, J.A., Perkel, D.J., Mauk, M.D., Kelly, P.T., Nicoll, R.A., Waxham, M.N., 1989. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340, 554–557. https://doi.org/10.1038/340554a0

Malinow, R., Schulman, H., Tsien, R.W., 1989. Inhibition of Postsynaptic PKC or CaMKII Blocks Induction But Not Expression of LTP. Science 245, 862–866. https://doi.org/10.1126/science.2549638

Manahan-Vaughan, D., Kulla, A., Frey, J.U., 2000. Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 8572–8576.

Marder, E., Abbott, L.F., Turrigiano, G.G., Liu, Z., Golowasch, J., 1996. Memory from the dynamics of intrinsic membrane currents. Proceedings of the National Academy of Sciences 93, 13481–13486. https://doi.org/10.1073/pnas.93.24.13481

Martel, M.A., Ryan, T.J., Bell, K.F.S., Fowler, J.H., McMahon, A., Al-Mubarak, B., Komiyama, N.H., Horsburgh, K., Kind, P.C., Grant, S.G.N., Wyllie, D.J.A., Hardingham, G.E., 2012. The Subtype of GluN2 C-terminal Domain Determines the Response to Excitotoxic Insults. Neuron 74, 543–556. https://doi.org/10.1016/j.neuron.2012.03.021

Martin, S.J., de Hoz, L., Morris, R.G.M., 2005. Retrograde amnesia: neither partial nor complete hippocampal lesions in rats result in preferential sparing of remote spatial memory, even after reminding. Neuropsychologia 43, 609–624. https://doi.org/10.1016/j.neuropsychologia.2004.07.007

Matthews, E.A., Weible, A.P., Shah, S., Disterhoft, J.F., 2008. The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. Proceedings of the National Academy of Sciences 105, 15154–15159. https://doi.org/10.1073/pnas.0805855105

Mayer, M.L., Vyklicky, L., Clements, J., 1989. Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. Nature 338, 425–427. https://doi.org/10.1038/338425a0

Mayer, M.L., Westbrook, G.L., Guthrie, P.B., 1984. Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 309, 261–263.

Mayford, M., Wang, J., Kandel, E.R., O'Dell, T.J., 1995. CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. Cell 81, 891–904. https://doi.org/10.1016/0092-8674(95)90009-8

McBain, C.J., Mayer, M.L., 1994. N-Methyl-D-Aspartic Acid Receptor Structure. Physiological Reviews 74, 723–753.

McKernan, M.G., Shinnick-Gallagher, P., 1997. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature 390, 607–611. https://doi.org/10.1038/37605

Miya, K., Inoue, R., Takata, Y., Abe, M., Natsume, R., Sakimura, K., Hongou, K., Miyawaki, T., Mori, H., 2008. Serine racemase is predominantly localized in neurons in mouse brain. Journal of Comparative Neurology 510, 641–654. https://doi.org/10.1002/cne.21822

Mohrmann, R., Köhr, G., Hatt, H., Sprengel, R., Gottmann, K., 2002. Deletion of the C-terminal domain of the NR2B subunit alters channel properties and synaptic targeting of N-methyl-D-aspartate receptors in nascent neocortical synapses: C-Terminal Truncation of NR2B Subunits. J. Neurosci. Res. 68, 265–275. https://doi.org/10.1002/jnr.10219

Montes de Oca Balderas, P., 2018. Flux-Independent NMDAR Signaling: Molecular Mediators, Cellular Functions, and Complexities. IJMS 19, 3800. https://doi.org/10.3390/ijms19123800

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., Seeburg, P.H., 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12, 529–540. https://doi.org/10.1016/0896-6273(94)90210-0

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., Seeburg, P.H., 1992. Heteromeric NMDA Receptors: Molecular and Functional Distinction of Subtypes. Science 256, 1217–1221.

Morris, R.G.M., Anderson, E., Lynch, G.S., Baudry, M., 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319, 774–776. https://doi.org/10.1038/319774a0

Mothet, J.P., Le Bail, M., Billard, J.M., 2015. Time and space profiling of NMDA receptor co-agonist functions. Journal of Neurochemistry 135, 210–225. https://doi.org/10.1111/jnc.13204

Mothet, J.P., Rouaud, E., Sinet, P.M., Potier, B., Jouvenceau, A., Dutar, P., Videau, C., Epelbaum, J., Billard, J.M., 2006. A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell 5, 267–274. https://doi.org/10.1111/j.1474-9726.2006.00216.x

Mozzachiodi, R., Byrne, J.H., 2010. More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends in Neurosciences 33, 17–26. https://doi.org/10.1016/j.tins.2009.10.001

Mulkey, R.M., Endo, S., Shenolikar, S., Malenka, R.C., 1994. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369, 486–488. https://doi.org/10.1038/369486a0

Mulkey, R.M., Herron, C.E., Malenka, R.C., 1993. An Essential Role for Protein Phosphatases in Hippocampal Long-Term Depression 261, 1051–1055.

Mulkey, R.M., Malenka, R.C., 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9, 967–975. https://doi.org/10.1016/0896-6273(92)90248-C

Nabavi, S., Kessels, H.W., Alfonso, S., Aow, J., Fox, R., Malinow, R., 2013. Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression 110. https://doi.org/10.1073/pnas.1219454110

Nakano, M., Yamada, S.I., Udagawa, R., Kato, N., 2004. Frequency-dependent requirement for calcium tore-operated mechanisms in induction of homosynaptic long-term depression at hippocampus CA1 synapses. European Journal of Neuroscience 19, 2881–2887. https://doi.org/10.1111/j.0953-816X.2004.03390.x

Neal, M.J., Pickles, H.G., 1969. Uptake of 14C Glycine by Spinal Cord. Nature 222, 679–680. https://doi.org/10.1038/222679a0

Neame, S., Safory, H., Radzishevsky, I., Touitou, A., Marchesani, F., Marchetti, M., Kellner, S., Berlin, S., Foltyn, V.N., Engelender, S., Billard, J.-M., Wolosker, H., 2019. The NMDA receptor activation by d-serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle. Proc Natl Acad Sci USA 116, 20736–20742. https://doi.org/10.1073/pnas.1909458116

Neves, G., Cooke, S.F., Bliss, T.V.P., 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat Rev Neurosci 9, 65–75. https://doi.org/10.1038/nrn2303

Ngezahayo, a, Schachner, M., Artola, a, 2000. Synaptic activity modulates the induction of bidirectional synaptic changes in adult mouse hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 2451–2458.

Ngo-Anh, T.J., Bloodgood, B.L., Lin, M., Sabatini, B.L., Maylie, J., Adelman, J.P., 2005. SK channels and NMDA receptors form a Ca2+-mediated feedback loop in dendritic spines. Nat Neurosci 8, 642–649. https://doi.org/10.1038/nn1449

Nong, Y., Huang, Y.-Q., Ju, W., Kalia, L.V., Ahmadian, G., Wang, Y.T., Salter, M.W., 2003. Glycine binding primes NMDA receptor internalization. Nature 422, 302–307. https://doi.org/10.1038/nature01497

Nong, Y., Huang, Y.-Q., Salter, M.W., 2004. NMDA receptors are movin' in. Current Opinion in Neurobiology 14, 353–361. https://doi.org/10.1016/j.conb.2004.05.001

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307, 462–465. https://doi.org/10.1038/307462a0

Oh, M.M., Disterhoft, J.F., 2015. Increased excitability of both principle neurons and interneurons during associative learning. Neuroscientist 21, 372–384. https://doi.org/10.1586/14737175.2015.1028369.Focused

Ohshima, T., Ogura, H., Tomizawa, K., Hayashi, K., Suzuki, H., Saito, T., Kamei, H., Nishi, A., Bibb, J.A., Hisanaga, S.I., Matsui, H., Mikoshiba, K., 2005. Impairment of hippocampal long-term depression and defective spatial learning and memory in p35-/- mice. Journal of Neurochemistry 94, 917–925. https://doi.org/10.1111/j.1471-4159.2005.03233.x

Ohtsuki, G., Piochon, C., Adelman, J.P., Hansel, C., 2012. SK2 Channel Modulation Contributes to Compartment-Specific Dendritic Plasticity in Cerebellar Purkinje Cells. Neuron 75, 108–120. https://doi.org/10.1016/j.neuron.2012.05.025

O'Reilly, R.C., McClelland, J.L., 1994. Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade-off. Hippocampus 4, 661–682. https://doi.org/10.1002/hipo.450040605

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H.R., 2006. Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. Cell 125, 775–784. https://doi.org/10.1016/j.cell.2006.02.051

Paoletti, P., 2011. Molecular basis of NMDA receptor functional diversity. European Journal of Neuroscience 33, 1351–1365. https://doi.org/10.1111/j.1460-9568.2011.07628.x

Papouin, T., Lad??p??che, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., Groc, L., Pollegioni, L., Mothet, J.P., Oliet, S.H.R., 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150, 633–646. https://doi.org/10.1016/j.cell.2012.06.029

Papouin, T., Oliet, S.H.R., 2014. Organization, control and function of extrasynaptic NMDA receptors. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20130601–20130601. https://doi.org/10.1098/rstb.2013.0601

Pastalkova, E., Serrano, P., Pinkhasova, D., Wallace, E., Fenton, A.A., Sacktor, T.C., 2006. Storage of Spatial Information by the Maintenance Mechanism of LTP. Science 313, 1141–1144. https://doi.org/10.1126/science.1128657

Peineau, S., Nicolas, C.S., Bortolotto, Z.A., Bhat, R.V., Ryves, W.J., Harwood, A.J., Dournaud, P., Fitzjohn, S.M., Collingridge, G.L., 2009. A systematic investigation of the protein kinases involved in NMDA receptor-dependent LTD: Evidence for a role of GSK-3 but not other serine/threonine kinases. Molecular Brain 2, 1–10. https://doi.org/10.1186/1756-6606-2-22

Peineau, S., Taghibiglou, C., Bradley, C., Wong, T.P., Liu, L., Lu, J., Lo, E., Wu, D., Saule, E., Bouschet, T., Matthews, P., Isaac, J.T.R., Bortolotto, Z.A.A., Wang, Y.T., Collingridge, G.L., 2007. LTP Inhibits LTD in the Hippocampus via Regulation of GSK3β. Neuron 53, 703–717. https://doi.org/10.1016/j.neuron.2007.01.029

Petralia, R.S., Sans, N., Wang, Y.-X., Wenthold, R.J., 2005. Ontogeny of postsynaptic density proteins at glutamatergic synapses. Molecular and Cellular Neuroscience 29, 436–452. https://doi.org/10.1016/j.mcn.2005.03.013

Pignatelli, M., Ryan, T.J., Roy, D.S., Lovett, C., Smith, L.M., Muralidhar, S., Tonegawa, S., 2019. Engram Cell Excitability State Determines the Efficacy of Memory Retrieval. Neuron 101, 1–11. https://doi.org/10.1016/j.neuron.2018.11.029

Priestley, T., Kemp, J.A., 1994. Kinetic Study of the Interactions Between the Glutamate and Glycine Recognition Sites on the N-Methyl-D-aspartic Acid Receptor Complex. Molecular Pharmacology 46, 1191–1196.

Quinlan, E.M., Olstein, D.H., Bear, M.F., 1999. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. Proceedings of the National Academy of Sciences of the United States of America 96, 12876–80. https://doi.org/10.1073/PNAS.96.22.12876

Rajab, S., Bismin, L., Schwarze, S., Pinggera, A., Greger, I.H., Neuweiler, H., 2021. Allosteric coupling of sub-millisecond clamshell motions in ionotropic glutamate receptor ligand-binding domains. Commun Biol 4, 1056. https://doi.org/10.1038/s42003-021-02605-0

Rajani, V., Sengar, A.S., Salter, M.W., 2020. Tripartite signalling by NMDA receptors. Mol Brain 13, 23. https://doi.org/10.1186/s13041-020-0563-z

Rich, P.D., Liaw, H.-P., Lee, A.K., 2014. Large environments reveal the statistical structure governing hippocampal representations. Science 345, 814–817. https://doi.org/10.1126/science.1255635

Rickgauer, J.P., Deisseroth, K., Tank, D.W., 2014. Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. Nat Neurosci 17, 1816–1824. https://doi.org/10.1038/nn.3866

Roberts, E.B., Ramoa, A.S., 1999. Enhanced NR2A Subunit Expression and Decreased NMDA Receptor Decay Time at the Onset of Ocular Dominance Plasticity in the Ferret. Journal of Neurophysiology 81, 2587–2591. https://doi.org/10.1152/jn.1999.81.5.2587

Roche, K.W., Standley, S., McCallum, J., Ly, C.D., Ehlers, M.D., Wenthold, R.J., 2001. Molecular determinants of NMDA receptor internalization. nature neuroscience 4, 9.

Rogan, M.T., Stäubli, U.V., LeDoux, J.E., 1997. Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390, 604–607. https://doi.org/10.1038/37601

Rogerson, T., Cai, D.J., Frank, A., Sano, Y., Shobe, J., Lopez-Aranda, M.F., Silva, A.J., 2014. Synaptic tagging during memory allocation. Nat Rev Neurosci 15, 157–169. https://doi.org/10.1038/nrn3667

Rosenberg, D., Artoul, S., Segal, A.C., Kolodney, G., Radzishevsky, I., Dikopoltsev, E., Foltyn, V.N., Inoue, R., Mori, H., Billard, J.-M., Wolosker, H., 2013. Neuronal D-Serine and Glycine Release Via the Asc-1 Transporter Regulates NMDA Receptor-Dependent Synaptic Activity. Journal of Neuroscience. https://doi.org/10.1523/JNEUROSCI.3836-12.2013

Rumpel, S., LeDoux, J., Zador, A., Malinow, R., 2005. Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning. Science 308, 83–88. https://doi.org/10.1126/science.1103944

Ryan, T.J., Roy, D.S., Pignatelli, M., Arons, A., Tonegawa, S., 2015. Engram cells retain memory under retrograde amnesia. Science 348, 1007–1014.

Sah, P., Hestrin, S., Nicoll, R.A., 1989. Tonic Activation of NMDA Receptors by Ambient Glutamate Enhances Excitability of Neurons. Science 246, 815–818.

Sakimura, K., Nakao, K., Yoshikawa, M., Suzuki, M., Kimura, H., 2016. A novel Na+-Independent alanine-serine-cysteine transporter 1 inhibitor inhibits both influx and efflux of D-Serine. Journal of Neuroscience Research 94, 888–895. https://doi.org/10.1002/jnr.23772

Salter, M.G., Fern, R., 2005. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438, 1167–1171. https://doi.org/10.1038/nature04301

Sanderson, J.L., Gorski, J.A., Dell'Acqua, M.L., 2016. NMDA Receptor-Dependent LTD Requires Transient Synaptic Incorporation of Ca2+-Permeable AMPARs Mediated by AKAP150-Anchored PKA and Calcineurin. Neuron 89, 1000–1015. https://doi.org/10.1016/j.neuron.2016.01.043

Sans, N., Petralia, R.S., Wang, Y.X., Blahos, J., Hell, J.W., Wenthold, R.J., 2000. A developmental change in NMDA receptor-associated proteins at hippocampal synapses. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 1260–1271. https://doi.org/10.1016/j.nlm.2011.01.004.Bidirectional

Sanz-Clemente, A., Nicoll, R.A., Roche, K.W., 2013. Diversity in NMDA receptor composition: many regulators, many consequences. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 19, 62–75. https://doi.org/10.1177/1073858411435129

Sason, H., Billard, J.M., Smith, G.P., Safory, H., Neame, S., Kaplan, E., Rosenberg, D., Zubedat, S., Foltyn, V.N., Christoffersen, C.T., Bundgaard, C., Thomsen, C., Avital, A., Christensen, K.V., Wolosker, H., 2016. Asc-1 Transporter Regulation of Synaptic Activity via the Tonic Release of d-Serine in the Forebrain. Cerebral Cortex bhv350–bhv350. https://doi.org/10.1093/cercor/bhv350

Scanziani, M., Malenka, R.C., Nicoll, R.A., 1996. Role of intercellular interactions in heterosynaptic long-term depression. Nature 380, 446–450. https://doi.org/10.1038/380446a0

Scheiderer, C.L., Dobrunz, L.E., McMahon, L.L., 2004. Novel Form of Long-Term Synaptic Depression in Rat Hippocampus Induced by Activation of α1 Adrenergic Receptors. Journal of Neurophysiology 91, 1071–1077. https://doi.org/10.1152/jn.00420.2003

Schell, M.J., 2004. The N-methyl D-aspartate receptor glycine site and D-serine metabolism: an evolutionary perspective. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 359, 943–964. https://doi.org/10.1098/rstb.2003.1399

Schell, M.J., Brady, R.O., Molliver, M.E., Snyder, S.H., 1997. D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 17, 1604–15.

Schneggenburger, R., Meyer, A.C., Neher, E., 1999. Released fraction and total size of a pool of immediately available transmitter quanta at a calyx synapse. Neuron 23, 399–409. https://doi.org/10.1016/S0896-6273(00)80789-8

Schultz, C., Engelhardt, M., 2014. Anatomy of the Hippocampal Formation, in: Szabo, K., Hennerici, M.G. (Eds.), Frontiers of Neurology and Neuroscience. S. KARGER AG, Basel, pp. 6–17. https://doi.org/10.1159/000360925

Scimemi, A., Fine, A., Kullmann, D.M., Rusakov, D.A., 2004. NR2B-Containing Receptors Mediate Cross Talk among Hippocampal Synapses. Journal of Neuroscience 24, 4767–4777. https://doi.org/10.1523/JNEUROSCI.0364-04.2004

Scoville, W.B., Milner, B., 1957. Loss of Recent Memory after Bilateral Hippocampal Lesions. Journal of Neurology, Neurosurgery & Psychiatry 20, 11–21. https://doi.org/10.1136/jnnp.20.1.11

Selig, K., Bear, F., Malenka, R.C., 1995. Reexamination of the Effects of MCPG on Hippocampal LTP, LTD, and Depotentiation 74.

Sheng, M., Cummings, J., Roldan, L. a, Jan, Y.N., Jan, L.Y., 1994. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368, 144–147. https://doi.org/10.1038/368144a0

Sheng, M., Kim, M.J., 2002. Postsynaptic Signaling and Plasticity Mechanisms. Science 298, 776–780. https://doi.org/10.1126/science.1075333

Shi, S.-H., Hayashi, Y., Esteban, J.A., Malinow, R., 2001. Subunit-Specific Rules Governing AMPA Receptor Trafficking to Synapses in Hippocampal Pyramidal Neurons. Cell 105, 331–343. https://doi.org/10.1016/S0092-8674(01)00321-X

Shibata, R., Nakahira, K., Shibasaki, K., Wakazono, Y., Imoto, K., Ikenaka, K., 2000. A-type K+ current mediated by the Kv4 channel regulates the generation of action potential in developing cerebellar granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 4145–55.

Siegler Retchless, B., Gao, W., Johnson, J.W., 2012. A single GluN2 subunit residue controls NMDA receptor channel properties via intersubunit interaction. Nature neuroscience 15, 406–13, S1-2. https://doi.org/10.1038/nn.3025

Simkin, D., Hattori, S., Ybarra, N., Musial, T.F., Buss, E.W., Richter, H., Oh, M.M., Nicholson, D.A., Disterhoft, J.F., 2015. Aging-Related Hyperexcitability in CA3 Pyramidal Neurons Is Mediated by Enhanced A-Type K+ Channel Function and Expression. Journal of Neuroscience 35, 13206–13218. https://doi.org/10.1523/jneurosci.0193-15.2015

Sobolevsky, A.I., Rosconi, M.P., Gouaux, E., 2009. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature 462, 745–56. https://doi.org/10.1038/nature08624

Soh, H., Pant, R., LoTurco, J.J., Tzingounis, A.V., 2014. Conditional Deletions of Epilepsy-Associated KCNQ2 and KCNQ3 Channels from Cerebral Cortex Cause Differential Effects on Neuronal Excitability. Journal of Neuroscience 34, 5311–5321. https://doi.org/10.1523/jneurosci.3919-13.2014

Sourdet, V., Russier, M., Daoudal, G., Ankri, N., Debanne, D., 2003. Long-Term Enhancement of Neuronal Excitability and Temporal Fidelity Mediated by Metabotropic Glutamate Receptor Subtype 5. J. Neurosci. 23, 10238–10248. https://doi.org/10.1523/JNEUROSCI.23-32-10238.2003

Sprengel, R., Suchanek, B., Amico, C., Brusa, R., Burnashev, N., Rozov, A., Hvalby, Oø., Jensen, V., Paulsen, O., Andersen, P., Kim, J.J., Thompson, R.F., Sun, W., Webster, L.C., Grant, S.G.N., Eilers, J., Konnerth, A., Li, J., McNamara, J.O., Seeburg, P.H., 1998. Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell 92, 279–289. https://doi.org/10.1016/S0092-8674(00)80921-6

Stackman, R.W., Hammond, R.S., Linardatos, E., Gerlach, A., Maylie, J., Adelman, J.P., Tzounopoulos, T., 2002. Small Conductance Ca $^{2+}$ -Activated K $^{+}$ Channels Modulate Synaptic Plasticity and Memory Encoding. J. Neurosci. 22, 10163–10171. https://doi.org/10.1523/JNEUROSCI.22-23-10163.2002

Staubli, U., Lynch, G., 1990. Stable depression of potentiated synaptic responses in the hippocampus with 1-5 Hz stimulation. Brain Research 513, 113–118. https://doi.org/10.1016/0006-8993(90)91096-Y

Stein, I.S., Gray, J.A., Zito, K., 2015. Non-Ionotropic NMDA Receptor Signaling Drives Activity-Induced Dendritic Spine Shrinkage 35, 12303–12308. https://doi.org/10.1523/JNEUROSCI.4289-14.2015

Stein, I.S., Park, D.K., Flores, J.C., Jahncke, J.N., Zito, K., 2020. Molecular Mechanisms of Non-ionotropic NMDA Receptor Signaling in Dendritic Spine Shrinkage. J. Neurosci. 40, 3741–3750. https://doi.org/10.1523/JNEUROSCI.0046-20.2020

Stocca, G., Vicini, S., 1998. Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. The Journal of Physiology 507, 13–24. https://doi.org/10.1111/j.1469-7793.1998.013bu.x

Stocker, M., Krause, M., Edarzani, P.P., 1999. An apamin-sensitive Ca2+-activated K+ current in hippocampal. Proceedings of the National Academy of Sciences 96, 4662–4667.

Stroebel, D., Mony, L., Paoletti, P., 2021. Glycine agonism in ionotropic glutamate receptors. Neuropharmacology 193, 108631. https://doi.org/10.1016/j.neuropharm.2021.108631

Thomas, C.G., Krupp, J.J., Bagley, E.E., Bauzon, R., Heinemann, S.F., Vissel, B., Westbrook, G.L., 2006. Probing N-methyl-D-aspartate Receptor Desensitization with the Substituted-Cysteine Accessibility Method. Mol Pharmacol 69, 1296–1303. https://doi.org/10.1124/mol.105.017350

Titley, H.K., Brunel, N., Hansel, C., 2017. Toward a Neurocentric View of Learning. Neuron 95, 19–32. https://doi.org/10.1016/j.neuron.2017.05.021

Tonegawa, S., Liu, X., Ramirez, S., Redondo, R., 2015. Memory Engram Cells Have Come of Age. Neuron 87, 918–931. https://doi.org/10.1016/j.neuron.2015.08.002

Tovar, K.R., Westbrook, G.L., 1999. The Incorporation of NMDA Receptors with a Distinct Subunit Composition at Nascent Hippocampal Synapses In Vitro. J. Neurosci. 19, 4180–4188. https://doi.org/10.1523/JNEUROSCI.19-10-04180.1999

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate Receptor Ion Channels: Structure, Regulation, and Function. Catheterization and cardiovascular diagnosis 62, 405–496. https://doi.org/10.1124/pr.109.002451.405

Tsien, J.Z., Huerta, P.T., Tonegawa, S., 1996. The Essential Role of Hippocampal CA1 NMDA Receptor–Dependent Synaptic Plasticity in Spatial Memory. Cell 87, 1327–1338. https://doi.org/10.1016/S0092-8674(00)81827-9

Tzingounis, A.V., Nicoll, R.A., 2008. Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. Proceedings of the National Academy of Sciences 105, 19974–19979. https://doi.org/10.1073/pnas.0810535105

van Dam, E.J.M., Ruiter, B., Kamal, A., Ramakers, G.M.J., Gispen, W.H., de Graan, P.N.E., 2002. N-methyl-D-aspartate-induced long-term depression is associated with a decrease in postsynaptic protein kinase C substrate phosphorylation in rat hippocampal slices. Neuroscience letters 320, 129–132. https://doi.org/10.1016/S0304-3940(02)00037-X

Van Horn, M.R., Strasser, A., Miraucourt, L.S., Pollegioni, L., Ruthazer, E.S., 2017. The Gliotransmitter d-Serine Promotes Synapse Maturation and Axonal Stabilization *In Vivo*. J. Neurosci. 37, 6277–6288. https://doi.org/10.1523/JNEUROSCI.3158-16.2017

Vanhoutte, P., Bading, H., 2003. Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. Current Opinion in Neurobiology 13, 366–371. https://doi.org/10.1016/S0959-4388(03)00073-4

Vazdarjanova, A., 2004. Differences in Hippocampal Neuronal Population Responses to Modifications of an Environmental Context: Evidence for Distinct, Yet Complementary, Functions of CA3 and CA1 Ensembles. Journal of Neuroscience 24, 6489–6496. https://doi.org/10.1523/JNEUROSCI.0350-04.2004

Vicini, S., Wang, J.F., Li, J.H., Zhu, W.J., Wang, Y.H., Luo, J.H., Wolfe, B.B., Grayson, D.R., 1998. Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. Journal of neurophysiology 79, 555–66. https://doi.org/10.1016/0014-5793(92)80648-Z

Vissel, B., Krupp, J.J., Heinemann, S.F., Westbrook, G.L., 2001. A use-dependent tyrosine dephosphorylation of NMDA receptors is independent of ion flux. Nature Neuroscience 4, 587–596. https://doi.org/10.1038/88404

Volianskis, A., France, G., Jensen, M.S., Bortolotto, Z.A., Jane, D.E., Collingridge, G.L., 2015. Long-term potentiation and the role of N-methyl-D-aspartate receptors. Brain Research 1621, 5–16. https://doi.org/10.1016/j.brainres.2015.01.016

Wenthold, R.J., Petralia, R.S., Blahos II, J., Niedzielski, A.S., 1996. Evidence for Multiple AMPA Receptor Complexes in Hippocampal CAl/CA2 Neurons 16, 1982–9.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., Bear, M.F., 2006. Learning Induces Long-Term Potentiation in the Hippocampus. Science 313, 1093–1097. https://doi.org/10.1126/science.1128134

Williams, J.M., Guévremont, D., Kennard, J.T.T., Mason-Parker, S.E., Tate, W.P., Abraham, W.C., 2003. Long-term regulation of N-methyl-D-aspartate receptor subunits and associated synaptic proteins following hippocampal synaptic plasticity. Neuroscience 118, 1003–1013. https://doi.org/10.1016/S0306-4522(03)00028-9

Williams, J.M., Mason-Parker, S.E., Abraham, W.C., Tate, W.P., 1998. Biphasic changes in the levels of N-methyl-D-aspartate receptor-2 subunits correlate with the induction and persistence of long-term potentiation. Molecular Brain Research 60, 21–27. https://doi.org/10.1016/S0169-328X(98)00154-5

Wong, J.M., Folorunso, O.O., Barragan, E.V., Berciu, C., Harvey, T.L., Coyle, J.T., Balu, D.T., Gray, J.A., 2020. Postsynaptic Serine Racemase Regulates NMDA Receptor Function. J. Neurosci. 40, 9564–9575. https://doi.org/10.1523/JNEUROSCI.1525-20.2020

Wong, J.M., Gray, J.A., 2018. Long-term depression is independent of GluN2 subunit composition. The Journal of Neuroscience 38, 0394–18. https://doi.org/10.1523/JNEUROSCI.0394-18.2018

Yang, S., Tang, Y., Zucker, R., 1999. Selective induction of LTP and LTD by postsynaptic i elevation. Journal of neurophysiology 81, 781–787. https://doi.org/10.1152/jn.1999.81.2.781

Yashiro, K., Philpot, B.D., 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55, 1081–1094. https://doi.org/10.1016/j.neuropharm.2008.07.046

Yassa, M.A., Stark, C.E.L., 2011. Pattern separation in the hippocampus. Trends in Neurosciences 34, 515–525. https://doi.org/10.1016/j.tins.2011.06.006

Yiu, A.P., Mercaldo, V., Yan, C., Richards, B., Rashid, A.J., Hsiang, H.-L.L., Pressey, J., Mahadevan, V., Tran, M.M., Kushner, S.A., Woodin, M.A., Frankland, P.W., Josselyn, S.A., 2014. Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training. Neuron 83, 722–735. https://doi.org/10.1016/j.neuron.2014.07.017

Zhang, W., Linden, D.J., 2003. The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci 4, 885–900. https://doi.org/10.1038/nrn1248

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., Silva, A.J., 2009. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12, 1438–1443. https://doi.org/10.1038/nn.2405

Zhu, Y., Pak, D., Qin, Y., Mccormack, S.G., Kim, M.J., Baumgart, J.P., Velamoor, V., Auberson, Y.P., Osten, P., Van Aelst, L., Sheng, M., Zhu, J.J., 2005. Rap2-JNK removes synaptic AMPA receptors during depotentiation. Neuron 46, 905–916. https://doi.org/10.1016/j.neuron.2005.04.037

Zukin, R.S., Bennett, M.V.L., 1995. Alternatively spliced isoforms of the NMDARI receptor subunit. Trends in Neurosciences 18, 306–313. https://doi.org/10.1016/0166-2236(95)93920-S

Chapter 2

D-SERINE IS A DETERMINANT OF NON-IONOTROPIC LTD

Preface

The following finding represent a preliminary story regarding D-serine-specific effects on non-ionotropic LTD induction. This chapter is the result of a collaborative work with Margarita Anisimova, Karen Zito, and John A. Gray. I wrote the following chapter and contributed Figures 1-8, 10-11. Margarita Anisimova contributed Figure 9. JAG and KZ were involved in experimental design, interpretation and editing. The authors report no conflicts of interest. This work was supported by R21MH116315 and R01MH117130 (JAG) and T32GM099608 (EVB).

Abstract

NMDARs are one of the most complex subfamilies of ionotropic glutamate receptors and master regulators of synaptic plasticity. NMDARs are unique among most receptors in that, in addition to glutamate binding, they also require simultaneous binding of a co-agonist – either glycine or D-serine – to the co-agonist site located on the GluN1 subunit. Without co-agonist binding, the receptor cannot fully activate, and the channel will remain closed. Yet, the fundamental role of the co-agonist requirement remains poorly understood. Recent evidence has shown that NMDAR-mediated LTD occurs in the absence of ion flux, a phenomenon that has been termed non-ionotropic LTD (niLTD). Interestingly, blocking the co-agonist site during an LTP-inducing stimulus also results in LTD. Based on these results, we hypothesized that the occupancy of the NMDAR co-agonist site may regulate niLTD. To investigate this hypothesis, we manipulated co-agonist availability both directly and indirectly during the induction of synaptic plasticity using pharmacological approaches and enzymatic scavenging. We showed that in the third postnatal week, blockade of the co-agonist site facilitates niLTD induction independent of induction frequency. We also demonstrated that increasing co-agonist saturation, specifically D-serine concentration, blocks niLTD. These results largely support our hypothesis that the NMDAR co-agonist modulates niLTD and provide a novel role for the co-agonist site and the co-agonist, D-serine. Additionally, this study highlights the emerging role of NMDAR non-ionotropic signaling. Understanding the full signaling and regulatory capabilities of the NMDAR will greatly expand our knowledge of basic neurobiological processes as well as how they may go awry in neurological disease states.

Introduction

The NMDA receptor is an incredibly sensitive postsynaptic device shown to be critical for proper synaptic development, functioning, and plasticity (Bliss and Collingridge, 1993; Lau and Zukin, 2007). NMDARs are ubiquitously expressed throughout the brain and operate as heterotetramers composed of two obligatory GluN1 subunits and two glutamate binding GluN2 subunits. Unlike other receptors in the brain, NMDARs have a requirement for co-agonist binding (either glycine or D-serine) to the GluN1 subunit for receptor activation and channel opening. Like other ionotropic glutamate receptors, canonical NMDAR signaling is mediated via ion conductance through the channel pore (Hansen et al., 2018; Traynelis et al., 2010). However, there is an increasing amount of data describing non-ionotropic NMDAR functioning in the absence of ion conductance through the channel.

Non-ionotropic actions of the NMDAR are caused by binding of glutamate and/or co-agonist to the receptor (Abrahamsson et al., 2017; Barria and Malinow, 2005; Coultrap et al., 2014; Malenka et al., 1989; Malinow et al., 1989; Nong et al., 2004, 2003; Vissel et al., 2001), which may trigger a conformational change of the receptor. Considering NMDARs are well positioned within the postsynaptic compartment to interact with other important synaptic molecules (Ehlers et al., 1996; McBain and Mayer, 1994; Paoletti, 2011), it is thought that conformational movement of the receptor's C-terminal domains (CTDs), in the absence of full receptor activation, may affect the distribution and activity of neighboring postsynaptic proteins. A growing body of evidence has demonstrated multiple types of NMDAR signaling without channel opening and ion flux. Non-ionotropic NMDAR signaling has been shown to prime receptors for endocytosis (Nong et al., 2004, 2003), govern receptor trafficking (Coultrap et al., 2014; Malenka et al., 1989; Malinow et al., 1989), regulate posttranslational modifications of GluN2A (Vissel et al., 2001), regulate spontaneous neurotransmitter release (Abrahamsson et

al., 2017), and give rise to functional and structural forms of synaptic depression (Nabavi et al., 2013; Stein et al., 2020, 2015; Wong and Gray, 2018).

Unlike ionotropic LTD, non-ionotropic NMDAR-mediated long-term depression (niLTD) requires only glutamate binding and basal Ca^{2+} levels for induction, not complete receptor activation. niLTD occurs independent of ion flux through the NMDAR (Nabavi et al., 2013), but the determinants and conditions in which this cellular phenomenon occurs are not fully known. GluN2 subunit identity does not affect niLTD induction, despite the absolute requirement for glutamate binding (Wong and Gray, 2018). However, blockade of ion flux during high-frequency stimulation (HFS) causes a conversion of the expected potentiation into synaptic depression (Nabavi et al., 2013). This finding has been supported by similar studies using co-agonist site antagonists during glutamate uncaging, which resulted in robust and reproducible spine shrinkage (Stein et al., 2015). Together, these results indicate that blocking the co-agonist site may result in niLTD induction, suggesting that the GluN1 subunit may play a role in regulating this form of non-ionotropic synaptic plasticity. Most work studying the differential roles of NMDAR subunits in synaptic plasticity has focused on the GluN2 subtypes and not the requisite GluN1 subunit. Indeed, our understanding of the role of the co-agonist and its binding site in regulating NMDAR signaling is rather limited.

Recent work has shed light on the molecular processes underlying niLTD, putting forth a putative mechanism of action that niLTD is mediated by glutamate binding to the GluN2 subunit. Yet, we still do not know whether glutamate binding induces niLTD directly through conformational movement of the GluN2 intracellular domains or via allosteric interactions with the GluN1 subunit that may then induce a conformational change of the GluN1 CTD. What has been shown is that application of NMDA (which binds to the GluN2 subunit) in the presence of NMDAR antagonists, 7CK and MK801, initiates a change in the GluN1 subunit CTD (Dore et al., 2015); but it is unknown

what the contribution of this conformational change is to the induction of synaptic depression. Additionally, the movement of the GluN1 subunit is accompanied by a temporary movement in PP1 relative to the CTD. It is thought that this might expose the catalytic site of the protein phosphatase PP1 to CaMKII, a postsynaptic target that is normally unavailable during baseline conditions (Aow et al., 2015). The dephosphorylated CaMKII could potentially initiate AMPAR endocytosis via phosphorylation of Ser-567 on the GluA1 subunit, which has previously been implicated in synaptic depression (Coultrap et al., 2014; Kim et al., 2001; Lin et al., 2000; Lüscher et al., 1999; Shi et al., 2001). It is unknown whether extracellular conditions also influence niLTD induction.

Given that niLTD is induced in the presence of a competitive antagonist of the co-agonist site, perhaps extracellular glycine and D-serine availability play a role in regulating this form of plasticity. Indeed, changes in co-agonist levels and regulation in the brain over development influence saturation of the co-agonist binding site. Adding another layer of complexity, the identity of the endogenous ligand of the co-agonist site varies across developmental stages, brain regions and cellular localization of the receptors (Balu and Coyle, 2015; Le Bail et al., 2015; Mothet et al., 2015; Papouin et al., 2012). Interestingly, most of the contradictory results regarding the existence of niLTD have been recorded in the presence of the NMDAR open channel blocker, MK-801. Unlike using co-agonist site antagonists, MK-801 blocks ion flow through the receptor without significantly altering the affinity and occupancy of the co-agonist binding site (MacDonald et al., 1991). Based on the basal extracellular glycine and D-serine (2-10 μM) concentrations (Hashimoto et al., 1995; Le Douce et al., 2020; Matsui et al., 2002; Pernot et al., 2012; Westergren et al., 2008; Wood et al., 1996), one would predict that the NMDAR co-agonist site (EC_{50} ~0.1-1 μM) is saturated under normal physiological conditions (Danysz and Parsons, 1998; Priestley and Kemp, 1994). However, both co-agonists' synaptic concentrations are tightly regulated by GlyT1 and Asc-1 transporters (Adam-Vizi, 1992; Attwell et al., 1993; Fukasawa et al., 2000; Rosenberg

et al., 2013). Numerous studies have shown that the co-agonist site of synaptic NMDARs is not saturated (Fossat et al., 2012; Henneberger et al., 2010; Mothet et al., 2000; Panatier et al., 2006; Papouin et al., 2012; Schell et al., 1997; Yang et al., 2003) and can be regulated by activity (Li et al., 2013; Mothet et al., 2015; Panatier et al., 2006; Rosenberg et al., 2013). Therefore, while the exact synaptic concentration of co-agonist is still unknown, these findings suggest the concentration falls on the hyperbolic part of the binding isotherm, rendering small changes in ligand concentration capable of inducing large changes in the NMDAR co-agonist site. We hypothesize that changes in co-agonist concentration may influence non-ionotropic LTD induction. To study the role of changes in extracellular co-agonist availability on non-ionotropic LTD, we varied co-agonist concentration while administering plasticity-inducing stimuli. Surprisingly, we found that saturating levels of D-serine completely blocked niLTD. This effect was specific to D-serine and was not observed in the presence of added glycine. These results suggest that D-serine alone regulates niLTD, which could influence synaptic plasticity as the brain shifts from predominantly glycine co-agonism to D-serine co-agonism during development.

Materials and Methods

Animals

For electrophysiology experiments we used male and female wildtype mice were group housed in polycarbonate cages and maintained on a 12h light/dark cycle at a constant temperature of 24 ± 1ºC. For two-photon imaging and uncaging experiments we used GFP-M mice (Feng et al., 2000) in a C57BL/6J background to obtain sparsely GFP-labelled pyramidal neurons in CA1 area of hippocampus. Animals were given access to food and water *ad libitum*. All experiments were carried out in accordance with the National Institutes of Health guidelines and were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC).

Electrophysiology

For extracellular field EPSP (fEPSP) recordings, P13-P21 mice were anesthetized with isoflurane and decapitated. Brains were rapidly removed and placed in ice-cold sucrose cutting buffer, containing the following (in mM): 210 sucrose, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 7 glucose, 7 $MgCl_2$, and 0.5 $CaCl_2$. Acute transverse 400 µM slices were made by dissecting the hippocampus out of each hemisphere and mounting on agar. Slices were cut on a Leica VT1200 vibratome (Buffalo Grove, IL) in ice-cold sucrose cutting buffer, then recovered for at least 45 min in 34ºC artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 2.5 $CaCl_2$, and 1.3 $MgSO_4$. Slices were stored submerged in room temperature ACSF for up to 5h, before they were transferred to a submersion chamber on an upright Olympus microscope, perfused with room temperature ACSF containing picrotoxin (0.1mM), and saturated with 95% O_2/5% CO_2. All solutions were vigorously perfused with 95% O_2/5% CO_2. All recordings were done with 3-5MΩ borosilicate pipettes filled with ACSF and were collected with a Multiclamp 700B amplifier (Molecular Devices). Analysis was performed with the Clampex software suite (Molecular Devices).

Experimental design and statistical analysis

All data represent the mean ± SEM of n = number of slices (1 recording per slice). Experiments were performed with interleaved controls and include both male and female mice. Data were analyzed using Clampfit 11.0 (Axon Instruments) and Prism 8 software (GraphPad). Plasticity experiments were analyzed by averaging the final 10 min of the recording and normalizing as a percentage of the baseline fEPSP slope.

Two-photon imaging and glutamate uncaging

Acute hippocampal slices were prepared from P16-P20 GFP-M mice of both sexes as previously described (Stein et al., 2021). GFP-expressing CA1 pyramidal neurons at average depth of 37 ± 2 µM were imaged using a custom two-photon microscope (Woods et al., 2011). For each neuron, image stacks (512 × 512 pixels; 0.02 µM per pixel; 1µm z-steps) were collected from one segment of secondary or tertiary basal dendrite at 5 min intervals at 27-30°C in recirculating ACSF 2 Ca/0 Mg, containing the following (in mM): 127 NaCl, 25 NaHCO3, 1.2 NaH2PO4, 2.5 KCl, 25 D-glucose, 0 Mg, and 2 Ca, aerated with 95% O_2/5% CO_2, ~310 mOsm, pH 7.2) with 5mM MNI-glutamate and 1µM TTX. Slices were preincubated for 1h with 100 µM MK801 and for 10 min with either vehicle, 10 µM D-serine or enzymes (DsdA + GO) cocktail prior taking first image. Vehicle and D-serine were washed out with bubbled ACSF 2 Ca/0 Mg containing 100 µM MK-801 and 1µM TTX after LFU. Enzymes were not washed out.

Low-frequency uncaging (LFU) consisted of 90 pulses (720 nm; 0.2 ms duration, 10.6±0.4 mW at the sample) at 0.1 Hz. The beam was parked at a point ~0.5-1µm from the spine at the position farthest from the dendrite. Estimated spine volume was measured from background-subtracted green fluorescence using the integrated pixel intensity of a boxed region surrounding the spine head, as described (Woods et al., 2011). Representative images are maximum projections of three-dimensional image stacks after applying a median filter (3 × 3) to raw image

data. Statistical comparisons were made with ordinary one-way ANOVA or unpaired t-test (GraphPad Prism 9.2.0).

Drugs and enzymes

All drugs used in this study (10 µM L689, 50 µM APV, 100 µM MK-801) were bath applied throughout the entirety of the recording, except for the co-agonists (10 µM or 100 µM D-serine, 100 µM glycine), which were only washed on during the baseline and induction periods of each recording. Purified enzymes (D-serine deaminase and glycine oxidase) were obtained from our collaborator Herman Wolosker.

Results

The complex pharmacology of the NMDAR provides a variety of ways in which the receptor's function can be modulated. Co-agonist site competitive antagonists prevent channel opening and therefore ion flux, which makes them useful tools for studying niLTD. The competitive co-agonist site antagonist 7-chlorokynurenic acid (7CK) has predominantly been used to investigate niLTD (Carter and Jahr, 2016; Dore et al., 2015; Nabavi et al., 2013; Stein et al., 2015). However, at concentrations needed for complete NMDAR-EPSC block, 7CK also inhibits a large portion of AMPAR-EPSCs (Wong and Gray, 2018). It is unknown whether the concentration required for complete inhibition of NMDAR currents affects fEPSP responses, or if other more potent and selective antagonists like 5,7-dichlorokynurenic acid (5,7DCK) or L689,560 (L689) should be used (Grimwood et al., 1995; Leeson et al., 1991). Using concentrations known to fully block NMDAR-EPSCs (McNamara et al., 1990; Wong and Gray, 2018), we found that compared to 100 µM 7CK and 50 µM 5,7DCK, bath application of 10 µM L689 does exhibit any block of fEPSP responses in comparison to baseline (100 µM 7CK: 47.09 ± 3.826%, n=5; 50 µM 5,7DCK: 44.66 ± 3.598%, n=5; 10 µM L689: 101.8 ± 5.648%, n=7) (Figure 1).

Co-agonist binding and ion flux are not requirements for niLTD

Whole-cell experiments can be altered by both the composition of the internal pipette solution and dilution of important intracellular molecules (Kato et al., 1993; Malinow and Tsien, 1990). Extracellular field recordings allow us to bypass these caveats, as they are not affected by intracellular solutions. Consistent with previous work (Wong and Gray, 2018), we show in interleaved experiments, using low-frequency stimulation (LFS; 1Hz, 900 pulses), LTD is induced in the presence of the co-agonist site antagonist 10 µM L689 (vehicle: 77.94 ± 3.836% of baseline, n=27; 10 µM L689: 77.82 ± 3.230% of baseline, n= 12; *, $p < 0.05$) (Figure 2). Application of the glutamate binding site competitive antagonist, D-AP5 (50 µM) blocked any observed depression of fEPSP responses (50 µM AP5: 101.3 ± 4.183% of baseline, n=11). This confirms that glutamate

binding is required for niLTD induction, but co-agonist binding, and therefore ion conductance through the NMDAR is not.

Blockade of the co-agonist site permits niLTD independent of stimulation frequency

It has been shown that varying the induction frequency while keeping the number of induction stimuli constant can yield alter the directionality of plasticity (Bienenstock et al., 1982; Dudek and Bear, 1992). To determine whether blockade of the co-agonist site can still drive niLTD during non-LTD inducing stimuli, we bath applied 10 µM L689 during neutral (10Hz, 900 pulses) and LTP (50Hz, 300 pulses) induction protocols (Figure 3). During neutral induction, which does not induce any change in fEPSP response (102.2. ± 2.353% of baseline, n=11), 10 µM L689 induced a significant depression (83.06 ± 2.685% of baseline, n= 10; *, p=0.0192). This effect was dependent on glutamate binding as well, as application of APV did not permit LTD expression (96.30 ± 3.572% of baseline, n=5) (Figure 3A-C). Blockade of the co-agonist site with L689 also reversed the potentiation induced by 50Hz, 300 pulse stimulation (L689: 80.06 ± 5.543% of baseline, n=10; vehicle: 130.0 ± 11.02% of baseline, n=12; ****, $p < 0.0001$) (Figure 3D-F). This demonstrates that blocking the co-agonist site, which is a proxy for low co-agonist availability, shifts the plasticity curve towards depression and facilitates niLTD regardless of induction frequency (Figure 3G). This indicates a relationship between co-agonist site saturation and non-ionotropic synaptic depression.

Decreased co-agonist availability facilitates niLTD

Previous studies examining niLTD have used L689 as a proxy of low co-agonist availability but lack direct manipulations of exogenous co-agonist concentrations (Stein et al., 2021; Wong and Gray, 2018). Thus, concern has remained that competitive antagonists of the co-agonist site may alter the conformational dynamics of the receptor in non-physiological ways. To isolate the contribution of co-agonist site occupancy while still inhibiting ion flow through the receptor, we used the open channel blocker, MK-801 during LFS. MK-801 exhibits “trapping block”, in which

the molecule enters the open pore, but becomes trapped inside as the channel returns to its closed state. Once the pore is closed, the receptor deactivates, and the LBDs can release their respective agonists (MacDonald et al., 1991). Thus, MK-801 can block ion flow through the NMDAR channel without significantly affecting the affinity and occupancy of glutamate and co-agonist for the LBDs. Slices were pre-incubated for at least 1h with 100 µM MK-801 and subsequently perfused with ACSF containing MK-801 throughout the entirety of the recording. We found that LTD was not blocked in the presence of 100 µM MK-801 (68.60 ± 7.939% of baseline, n=9; **, p=0.0012), and addition of L689 also permitted LTD induction (62.05 ± 4.290% of baseline, n=8; ***, p=0.0003). Only in the presence of AP5 was depression blocked (105.3 ± 5.820% of baseline, n=9) (Figure 4). niLTD is not always observed in the presence of MK-801 (Babiec et al., 2014), which could be due to differences in experimental conditions that may affect endogenous co-agonist concentrations. Our data suggest that niLTD observed in the presence of MK-801+L689 has reduced variance compared to MK-801 because the co-agonist binding site is blocked, mimicking low co-agonist availability.

To directly determine whether low co-agonist availability, consistent with L689 results, facilitates niLTD, we co-administered MK801 with the co-agonist scavenging enzymes, D-serine deaminase (DsdA) and glycine oxidase (GO) during LFS. DsdA is a bacterial enzyme that is at least three orders of magnitude more efficient than the more commonly used D-amino acid oxidase (DAAO) at degrading D-serine. DsdA also has a higher affinity (Km = 0.1mM) for D-serine compared to DAAO (Km = 50mM) (Shleper et al., 2005). DsdA was used in conjunction with GO to drastically reduce the endogenous concentrations of co-agonist in the following experiment. Slices were preincubated for at least 1h with 0.2U/mL and 0.12U/mL DsdA and GO, respectively to optimally degrade endogenous co-agonist. Slices were then continuously perfused throughout the entirety of the recording with ACSF containing the same concentrations of enzymes. To conserve enzymes, these experiments were also performed in a recirculating bath.

Interestingly, recirculating the bath solution significantly affected LTD induction in the vehicle condition (Vehicle(normal): 77.94 ± 3.836% of baseline, n=27 and Vehicle(recirculating): 113.1 ± 5.839% of baseline, n=14; ****, $p < 0.0001$). Recirculating conditions also caused a slight decrease in the magnitude of LTD in the presence of 100 µM MK-801 (MK-801(nl): 68.60 ± 7.9393% of baseline, n=9 and MK-801(rec): 87.61 ± 2.868% of baseline, n=11; ns, p=0.0640), while the depression observed with the application of 10 µM L689 stayed maximally low (L689(nl): 77.82 ± 3.230% of baseline, n=12 and L689(rec): 79.70 ± 4.479, n=9; ns, p=0.9935). Because the recirculating system affected ionotropic LTD but not LTD during blockade of the co-agonist site, we hypothesize that there may be an increase in ambient co-agonist concentration as solution is recycled. Further experiments are required to confirm this idea.

Assuming that the block of LTD in the recirculating solution was due to potential accumulation of co-agonist throughout the experiment, we proceeded with the enzymatic depletion of endogenous co-agonist with the enzymes, DsdA and GO. Significant depression was observed in the enzyme condition (89.00 ± 4.510% of baseline, n=10; *, p=0.0171) compared to vehicle (113.1 ± 5.839% of baseline, n=14). Notably, this depression was smaller in magnitude than that observed in the presence of L689 (79.70 ± 4.479% of baseline, n=9; ***, p=0.0010), but was AP5-sensitive (105.1 ± 6.560% of baseline, n=9) (Figure 6A-C). This could be because the enzymes do not completely eliminate endogenous co-agonist, they merely reduce the overall levels. In contrast, L689 is a competitive antagonist and is therefore only mimicking lack of co-agonist availability. These results show that decreasing extracellular co-agonist concentrations facilitates synaptic depression.

To determine whether low co-agonist availability also supports non-ionotropic synaptic depression, we then tested the effects of the enzymes in the presence of MK-801, which allows us to investigate the effects of decreased co-agonist concentration in the absence of ion flux. In the recirculating bath, LTD induction in the presence of 100 µM MK-801 evoked a slight

depression (87.61 ± 2.868% of baseline, n=11) although not significantly less than the control condition of MK-801 and APV (100.9 ± 12.44% of baseline, n=5). However, addition of both L689 and DsdA/GO enhanced the observed depression (MK-801+L689: 69.78 ± 4.470% of baseline, n=10; *, p=0.0444; MK-801+DsdA/GO: 74.53 ± 4.986% of baseline, n=8) (Figure 6D-F). These results suggest that low co-agonist availability supports niLTD induction.

Increased D-serine availability blocks niLTD

Given that low co-agonist site occupancy facilitates niLTD, we investigated whether the inverse is true. If increased co-agonist availability also affects niLTD, then that would provide a novel role for the NMDAR co-agonism. To investigate whether increased co-agonist site occupancy also influences niLTD induction, we simultaneously applied saturating concentrations – 100 µM glycine and 100 µM D-serine – of individual co-agonists with 100 µM MK-801 and administered LFS. MK-801 was included to isolate the contribution of increased co-agonist site occupancy without the increased Ca^{2+} influx that may be a consequence of activating otherwise subsaturated NMDARs. As previously shown in Figure 4, niLTD is reliably induced in the presence of MK-801 (68.60 ± 7.939% of baseline, n=9), an effect that is blocked by the addition AP5 (105.3 ± 5.820% of baseline, n=9; **, p=0.0024). Surprisingly, we found that application of 100 µM D-serine significantly inhibited the observed depression (102.8 ± 7.388% of baseline, n=9; **, p=0.0051). Interestingly, addition of 100 µM glycine did not affect the magnitude of niLTD (70.79 ± 14.46% of baseline, n=4) (Figure 8).

Spine shrinkage can occur independent of ion flux through the NMDAR. Indeed, shrinkage can be induced by low frequency glutamatergic stimulation and blockade of the co-agonist site (Stein et al., 2021, 2020, 2015). In collaboration with the Zito lab, we investigated whether D-serine availability can also modulate non-ionotropic spine shrinkage, we bath applied 10 µM D-serine or the scavenging enzymes (DsdA and GO) in combination with 100 µM MK-801. We found that similarly to our results from the electrophysiology experiments, Administration of 10 µM D-

serine and MK-801 blocked non-ionotropic spine shrinkage (109.9 ± 9.144% of baseline, n=7; ***, p=0.009). Additionally, enzymatic reduction of endogenous co-agonist levels in the presence of MK-801 also induced spine shrinkage of a similar magnitude to MK-801 alone (63.41 ± 4.524% of baseline, n=6 and 69.35 ± 2.906% of baseline, n=6, respectively), The D-serine specific block of synaptic depression was not a consequence of D-serine application (Figure 10). Together, these results demonstrate that the NMDAR co-agonist D-serine, and not glycine, gates niLTD induction.

Lastly, we also tested the effects of saturating the co-agonist binding site in the absence of MK-801. To determine the effects of increased co-agonist availability on canonical LTD induction, we bath applied D-serine and glycine during LFS. We found that compared to the sizeable depression observed in vehicle conditions (77.94 ± 3.836% of baseline, n=27), 100 µM D-serine cause a slight potentiation in lieu of LTD (119.8 ± 11.57, n=9; ****, $p < 0.0001$). Additionally, 100 µM glycine and a lower D-serine concentration (10 µM) induced a less robust blockade of synaptic depression (95.23 ± 9.455, n=6; ns, p=0.3590 and 96.63 ± 8.900, n=7; ns, p=0.2295, respectively) (Figure 11). This suggests that saturating the co-agonist binding site blocks ion flux-dependent LTD, which suggests a unique effect of D-serine on niLTD.

Discussion

D-serine availability gates non-ionotropic LTD induction

NMDARs are unique among all other ionotropic glutamate receptors in their requirement for co-agonist binding. Indeed, despite the extensive amount of research performed on the structure and function of the NMDAR, the fundamental role of the co-agonist remains unknown. NMDARs are also unique among ionotropic glutamate receptors in their numerous metabotropic, or non-ionotropic, signaling capabilities. Traditionally, blockade of the co-agonist binding site (using 7CK or L689) during low-frequency stimulation (LFS) or low-frequency uncaging (LFU) has been used as a reliable method for niLTD induction (Nabavi et al., 2013; Stein et al., 2020, 2015; Wong and Gray, 2018). This was our first indication that co-agonist binding is not a requirement of niLTD, but instead may serve a modulatory role in this process. The idea of the co-agonist as a regulator of non-ionotropic NMDAR-mediated LTD has been supported by evidence that blockade of the co-agonist site during high-frequency uncaging (HFU) converts structural LTP to LTD (Stein et al., 2015). Given that different patterns and frequencies of presynaptic stimulation may increase release of glycine and D-serine (Li et al., 2013; Panatier et al., 2006; Rosenberg et al., 2013), niLTD in the presence of HFU indicates that co-agonist availability may influence the direction of synaptic plasticity. Additionally, D-serine binding has been shown to specifically trigger a conformational movement of the GluN1 CTD, while glycine does not (Ferreira et al., 2017). We provide evidence that application of exogenous D-serine, but not glycine, also blocks niLTD. Thus, demonstrating a novel role for the NMDAR co-agonist D-serine as a regulator of niLTD.

In the electrophysiology experiments in this study, we increase D-serine availability by bath applying a saturating concentration (100 µM) of D-serine, which is much more than basal endogenous D-serine levels (~2-10 µM) (Sherwood et al., 2021). We do not know if addition of a high concentration of D-serine is causing off-target effects like the electrogenic reversal of cellular transporters or binding to other glycinergic ionotropic glutamate receptors – GluN3 and GluD

subunits are known to bind both glycine and D-serine (Stroebel et al., 2021). However, in the spine shrinkage experiments, a lower D-serine concentration was used (10 μM) and non-ionotropic NMDAR-mediated shrinkage was still significantly blocked. An additional concern is that we do not know if D-serine application enhances the removal of MK-801, such that ion flux through the receptor is still occurring even in the presence of the pore blocker. However, it is thought that both glutamate and D-serine enhance MK-801 binding, since MK-801 can only access its binding site within the pore when the receptor is activated by concurrent binding of both ligands (Reynolds, 2000). Future experiments are required to determine the allosteric interactions, if any, between co-agonist binding and the channel pore.

Decreased co-agonist availability creates a bias for synaptic depression

In this study we also show that niLTD occurs independent of stimulation frequency, resulting in synaptic depression when the co-agonist site is blocked. Additionally, we show that decreasing endogenous co-agonist concentration via glycine and D-serine scavenging enzymes, enhances niLTD. In contrast to our findings, at least three studies have reported the opposite – that increasing D-serine increases the amount of synaptic depression (Duffy et al., 2008; Pinto-Duarte et al., 2019; Zhang et al., 2008). Two of these studies (Duffy et al., 2008; Pinto-Duarte et al., 2019), however, used older animals for their electrophysiological experiments. Co-agonism within in the brain is highly dynamic on a developmental, as well as spatiotemporal, level, so it is hard to interpret whether the difference in these results is merely a consequence of the different ages used. While the third study used similarly aged animals as our study (Zhang et al., 2008), this study (along with the other two) examined the contribution of D-serine availability to canonical LTD, not niLTD. Future experiments are needed to determine whether these two forms of LTD are entirely separate processes, and if so, where their mechanisms diverge.

D-serine specific roles in synaptic regulation

While NMDAR overall affinity for both D-serine and glycine is similar (EC_{50} ~0.1-1 µM), D-serine is a more potent and efficient ligand for the GluN1 co-agonist site (Priestley and Kemp, 1994). There is a growing body of work highlighting endogenous D-serine as critical for proper network development, synaptic development, dendritic arborization, and spine density (Balu et al., 2014, 2013, 2012; Miya et al., 2008; Van Horn et al., 2017). During the second and third weeks of postnatal development, D-serine gradually replaces glycine as the main endogenous co-agonist of NMDARs at CA3-CA1 synapses. This co-agonist shift mirrors the developmental switch in the forebrain from mostly GluN2B-containing to GluN2A-containing NMDARs (Mothet et al., 2015). Changes in the GluN2A/GluN2B synaptic ratio have been shown to bidirectionally control LTP at CA3-CA1 synapses and influence essential neuronal properties like synaptic maturation (Kellermayer et al., 2018; Yashiro and Philpot, 2008). Other D-serine specific roles in proper network development, synaptic plasticity, and learning (Henneberger et al., 2010; Labrie et al., 2008; Rosenberg et al., 2013; Yang et al., 2003) have previously been established. Additionally, there is evidence that increased D-serine concentration may drive synapses to have a higher GluN2A/GluN2B ratio by limiting the surface dynamics of GluN2B-containing NMDARs (Ferreira et al., 2017). LTP-inducing high frequency priming stimulation also increases the GluN2A/GluN2B ratio, resulting in a sliding of the synaptic plasticity threshold. This suggests that synaptic NMDAR composition can regulate long-term synaptic plasticity (Ladépêche et al., 2014; Xu et al., 2009). Indeed, SRKO mice, which lack serine racemase and exhibit D-serine levels, display a shift in their structural plasticity capability toward spine shrinkage (Park et al., BioRxiv); perhaps a mechanism by which D-serine blocks niLTD is through shifting the plasticity curve toward LTP through an increase in the synaptic GluN2A/GluN2B ratio and limiting diffusion of GluN2B-containing NMDARs. Hippocampal synapses, in SRKO mice and individual neurons lacking SR, display increased levels of GluN2B (Basu et al., 2009; Wong et al., 2020). It is unclear whether

these D-serine-specific observed effects still operate in the same manner in the absence of traditional ionotropic NMDAR signaling.

Physiological roles for niLTD

Since D-serine is not the predominant co-agonist during early CNS development, it is tempting to think of niLTD as an exclusively developmental phenomenon. In the second and third postnatal week, niLTD may facilitate the recruitment of signaling proteins that foster spine shrinkage, AMPAR trafficking, and synaptic plasticity (Aow et al., 2015; Nabavi et al., 2013; Stein et al., 2020). The synaptic bias toward AMPAR internalization and spine shrinkage that accompany niLTD induction at this young age may serve to prevent the induction of long-lasting LTP, such that memory storage is impaired (Keith et al., 2021). Additionally, niLTD could play a potential early developmental role in the maintenance of silent synapses. At silent synapses, NMDARs act as detectors of non-coincident synaptic activity since presynaptic glutamate occurs but sufficient postsynaptic depolarization – to eject the NMDAR Mg^{2+} block – may be absent due to lack of AMPARs. After the third postnatal week niLTD is speculated to affect long-term memory by repressing contextual and spatial memory retrieval, however the exact mechanisms by which this may occur are still unclear (Keith et al., 2021). Further studies are needed to determine whether niLTD exists only during certain developmental stages and, if not, what roles this synaptic process serves in the juvenile and mature brain.

Figures and Figure Legends

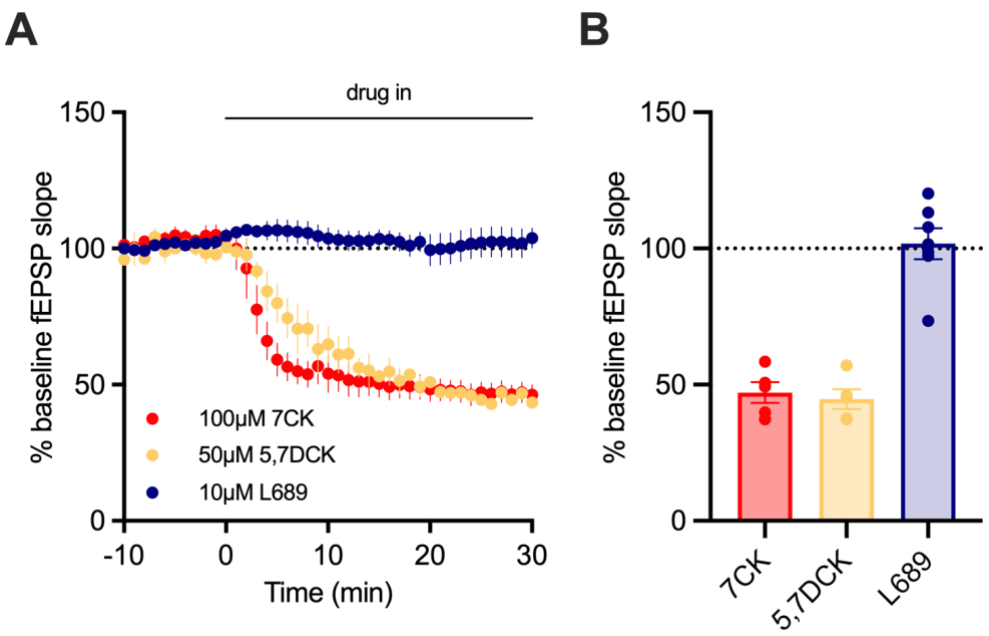

Figure 1. The NMDAR co-agonist site antagonist L689 does not affect AMPAR fEPSPs. A. Inhibition of AMPAR fEPSPs by NMDAR co-agonist site antagonists. **B.** Percentage block of AMPAR fEPSPs by 7CK, 5,7DCK, and L689 averaging from 20-30 min after drug application. 100 μM 7CK and 50 μM 5,7DCK reduced AMPAR fEPSPs to 47.09 ± 3.826% and 44.66 ± 3.598% of baseline, respectively (n=5 for both conditions).10 μM L689 did not inhibit responses (101.8 ± 5.648% of baseline, n=7).

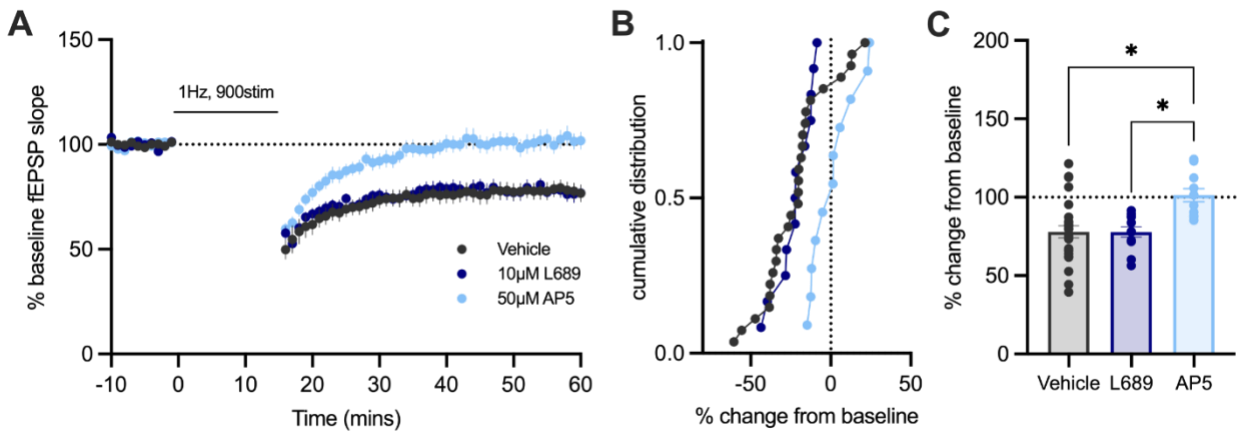

Figure 2. Co-agonist binding is not required for non-ionotropic LTD (niLTD). A-C. NMDAR-mediated non-ionotropic LTD occurs in the presence of 10 μM L689 and is blocked by 50 μM D-AP5. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in A. **C.** L689 administration resulted in depression (77.82 ± 3.230% of baseline, n= 12) of a similar magnitude to vehicle conditions (77.94 ± 3.836% of baseline, n=27). This depression is significantly inhibited in the presence of AP5 (101.3 ± 4.183% of baseline, n=11; *, $p < 0.05$). All data represent mean ± SEM.

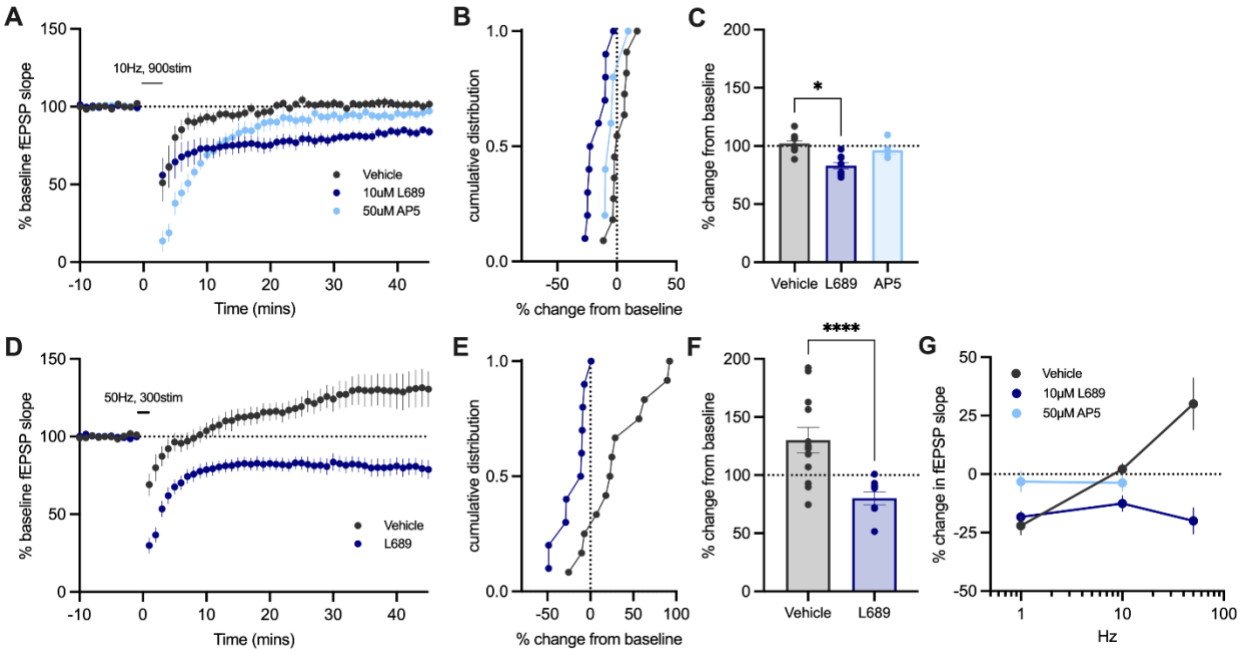

Figure 3. Blockade of the co-agonist site permits niLTD independent of stimulation frequency. A-C. NMDAR-mediated niLTD occurs in the presence of a neutral induction protocol (10Hz, 900 pulses). **A.** Averaged fEPSP experiments. **B.** Cumulative distribution of experiments in A. **C.** 10 μM L689 administration significantly depressed fEPSP responses (83.06 ± 2.685% of baseline, n= 10; *, p=0.0192) in comparison to vehicle conditions (102.2. ± 2.353% of baseline, n=11). Depression was not permitted in the presence of AP5 (96.30 ± 3.572% of baseline, n=5). **D-F.** NMDAR-mediated LTD occurs in the presence of an LTP induction protocol (50Hz, 300 pulses). **D.** Averaged fEPSP experiments. **E.** Cumulative distribution of experiments in D. **F.** 10 μM L689 reverses the direction of synaptic plasticity induced by 50Hz stimulation (L689: 80.06 ± 5.543% of baseline, n=10; vehicle: 130.0 ± 11.02% of baseline, n=12; ****, $p < 0.0001$). **G.** Plasticity curve of averaged percent change in fEPSP response. Blockade of co-agonist site with 10 μM L689 facilitates depression in response at all stimulation frequencies. All data represent mean ± SEM.

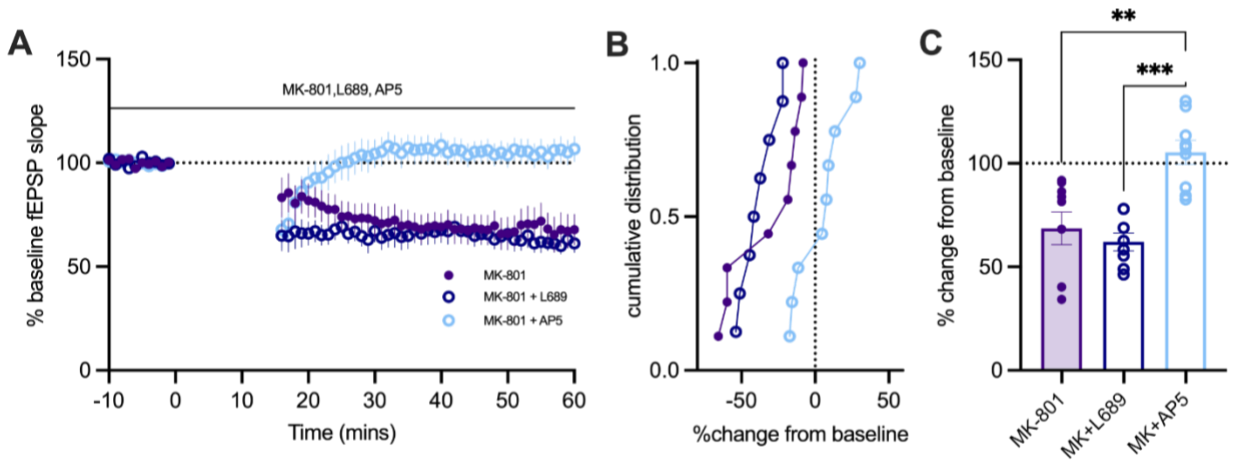

Figure 4. Ion flux is not required for non-ionotropic LTD. A-C. 100 µM MK801 permits niLTD induction and is blocked by 50 µM D-AP5. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in E. **C.** MK-801 application resulted in niLTD (68.60 ± 7.939% of baseline, n=9) and addition of L689 did not affect this depression (62.05 ± 4.290% of baseline, n=8). MK-801 niLTD was significantly blocked by administration of AP5 (105.3 ± 5.820% of baseline, n=9; **, p=0.0012; ***, p=0.0003). All data represent mean ± SEM.

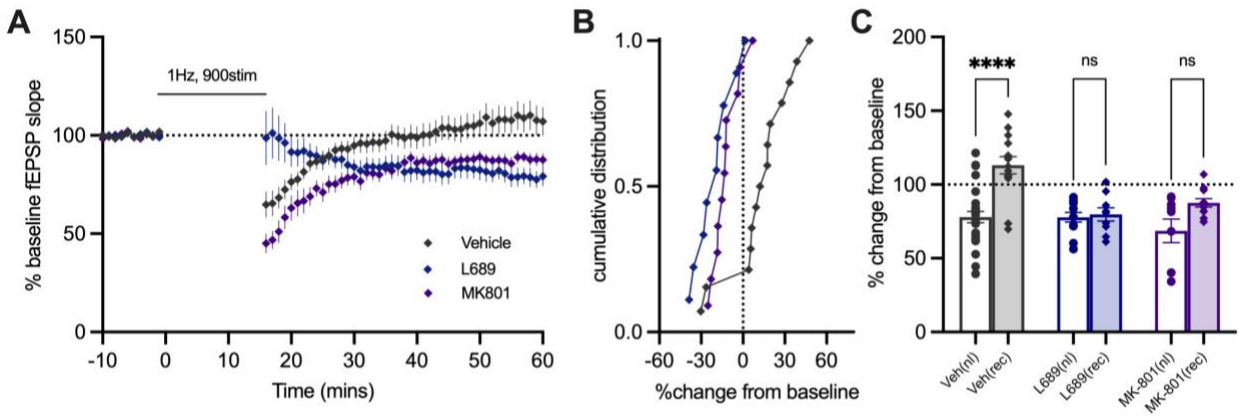

Figure 5. Recirculating solution affects LTD but not niLTD induction. A-C. LTD induction (1Hz, 900 pulses) is affected by recirculation of bath solution. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in A. **C.** Recirculating solution significantly affected LTD induction in vehicle conditions (Vehicle(normal): 77.94 ± 3.836% of baseline, n=27 and Vehicle(recirculating): 113.1 ± 5.839% of baseline, n=14; ****, $p < 0.0001$). Recirculation did not affect LTD in the L689 (L689(nl): 77.82 ± 3.230% of baseline, n=12 and L689(rec): 79.70 ± 4.479, n=9; ns, p=0.9935) or MK-801 conditions (MK-801(nl): 68.60 ± 7.9393% of baseline, n=9 and MK-801(rec): 87.61 ± 2.868% of baseline, n=11; ns, p=0.0640). All data represent mean ± SEM.

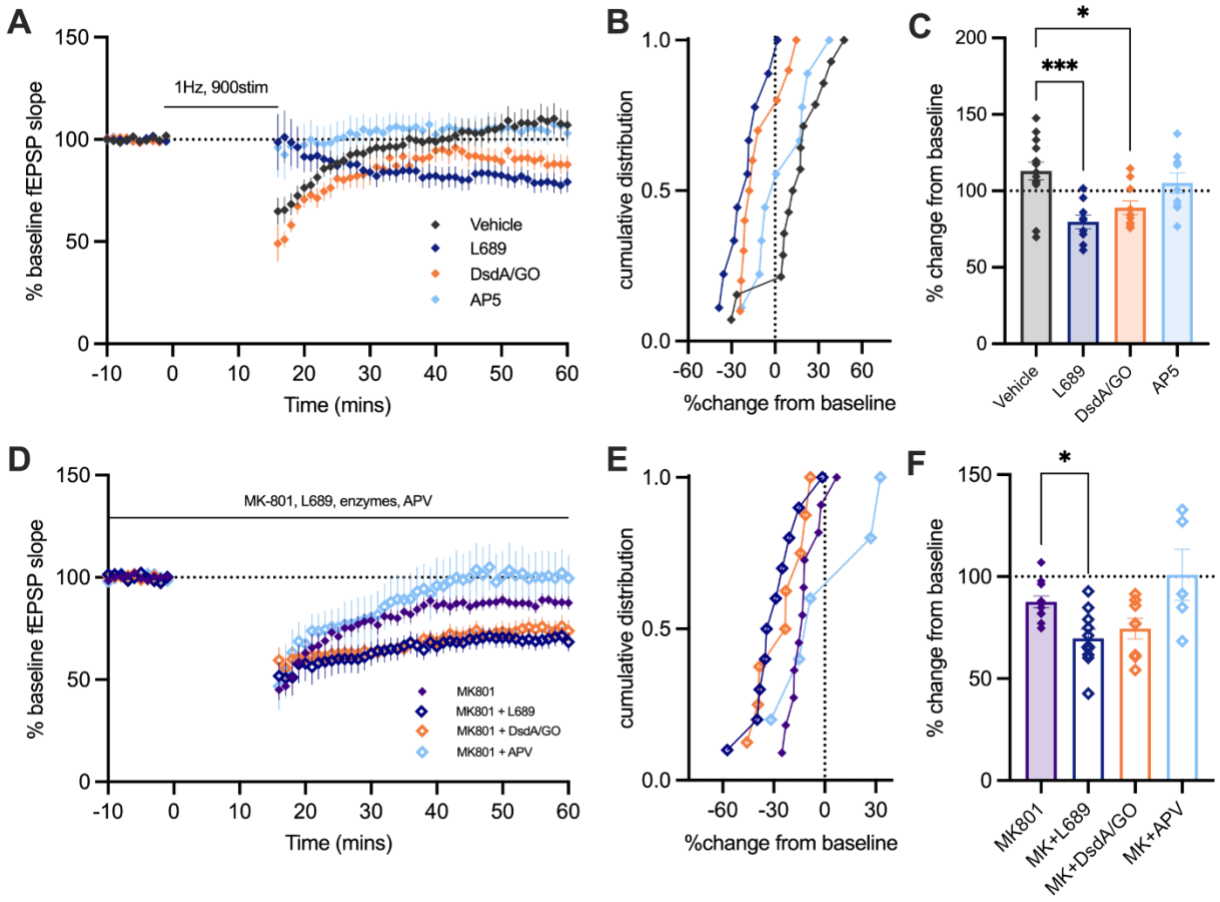

Figure 6. Decreased co-agonist availability promotes synaptic depression. A-C. Low co-agonist availability facilitates LTD induction. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in A. **C.** Recirculation of solution blocks LFS-LTD (113.1 ± 5.839% of baseline, n=14), but robust LTD can be reliably induced using co-agonist scavenging enzymes, D-serine deaminase (DsdA) and glycine oxidase (GO) (89.00 ± 4.510% of baseline, n=10; *, p=0.0171). This depression was smaller in magnitude than that observed in the presence of L689 (79.70 ± 4.479% of baseline, n=9; ***, p=0.0010), but was blocked by AP5 (105.1 ± 6.560% of baseline, n=9). **D-F.** Low co-agonist availability in the absence of ion flux permits non-ionotropic LTD induction. **D.** Averaged fEPSP LTD experiments. **E.** Cumulative distribution of experiments in D. **F.** niLTD can be induced in the recirculating bath in the presence of 100 µM MK-801 (87.61 ± 2.868% of baseline, n=11). Addition of L689 and DsdA/GO augmented this observed depression (MK-801+L689: 69.78 ± 4.470% of baseline, n=10; *, p=0.0444; MK-801+DsdA/GO: 74.53 ±

4.986% of baseline, n=8; ns, p= 0.2398). niLTD was not induced in the presence of MK-801 and APV (100.9 ± 12.44% of baseline, n=5). All data represent mean ± SEM.

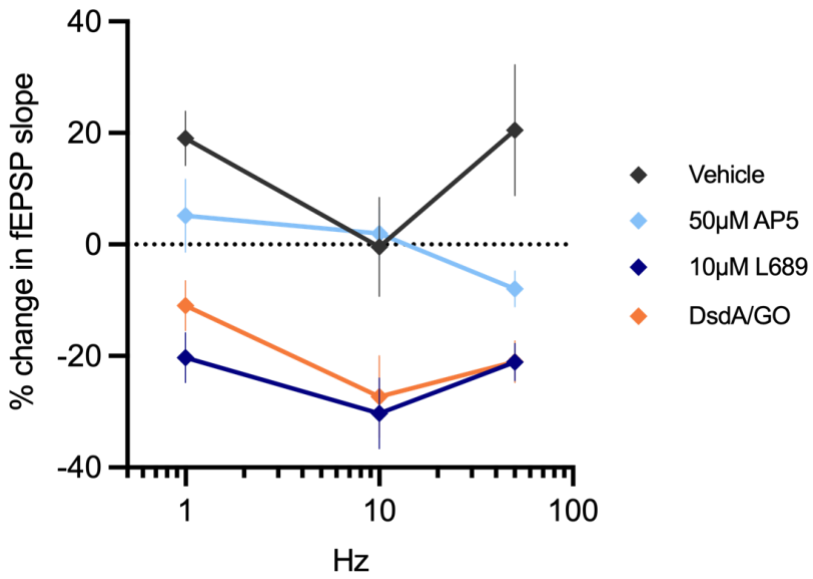

Figure 7. Decreased co-agonist availability biases toward synaptic depression. In recirculating solution, similar to blocking the co-agonist site, decreasing extracellular co-agonist concentration with the enzymes DsdA and GO, shifts the plasticity curve toward depression independent of induction frequency.

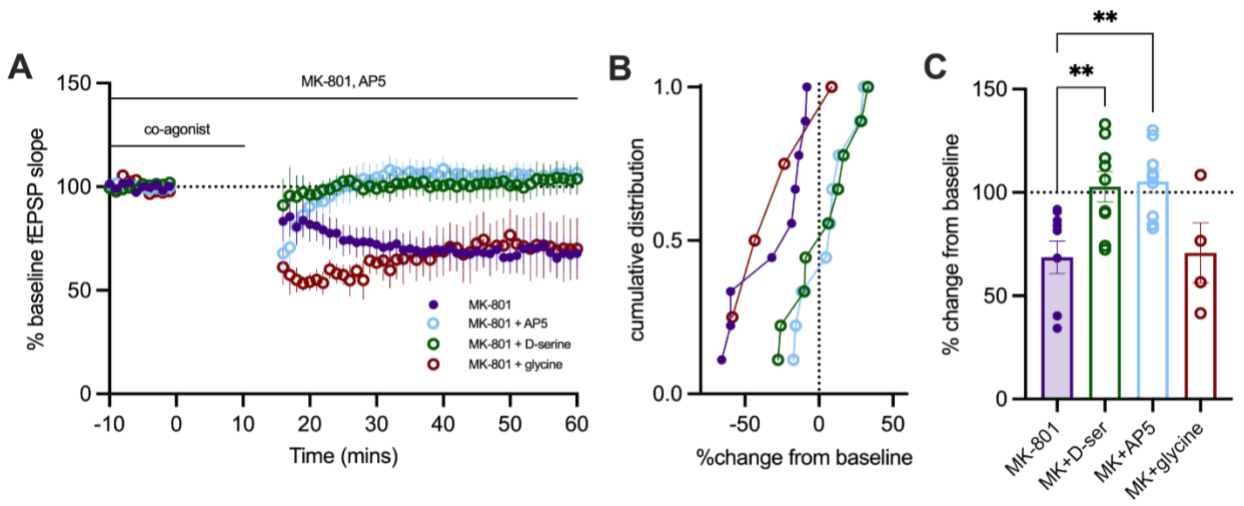

Figure 8. Increasing D-serine concentration blocks niLTD. A-C. Increasing glycine and D-serine concentration differentially affects non-ionotropic LTD. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in A. **C.** niLTD occurs in the presence of 100 µM MK-801 (68.60 ± 7.939% of baseline, n=9), an effect that is blocked with the addition of 50 µM AP5 (105.3 ± 5.820% of baseline, n=9; **, p=0.0024). Addition of 10 µM glycine did not affect niLTD induction (70.79 ± 14.46% of baseline, n=4; ns, p=0.9996), while application of 100 µM D-serine significantly inhibited the observed depression (102.8 ± 7.388% of baseline, n=9; **, p=0.0051). All data represent mean ± SEM.

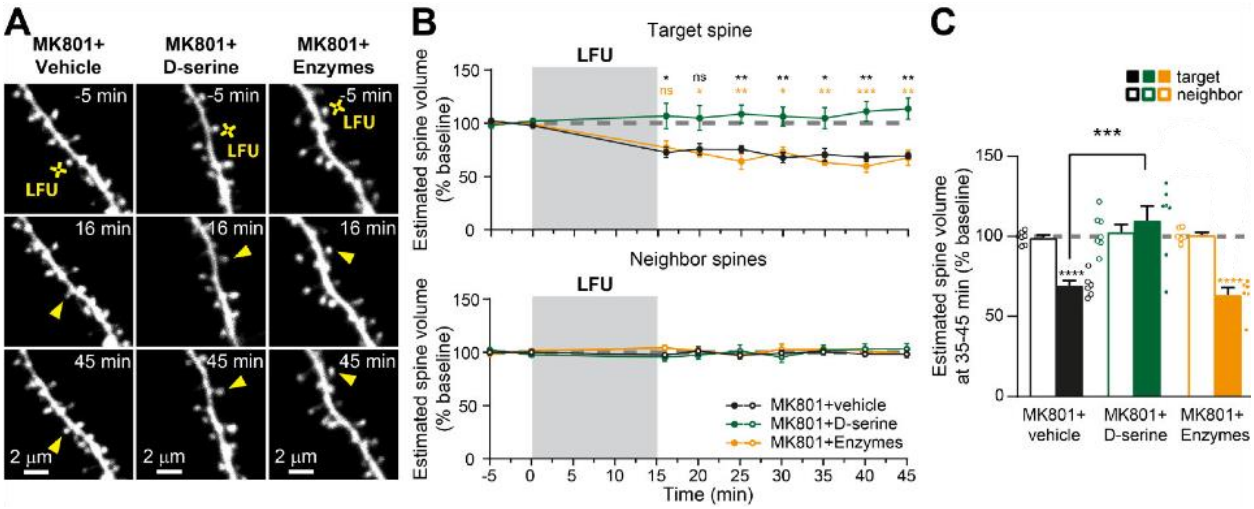

Figure 9. Increasing D-serine availability blocks non-ionotropic spine shrinkage. A. Representative images of CA1 pyramidal neuron basal dendrites in acute hippocampal slices. Yellow cross and arrows indicate stimulated spine (LFU of MNI-glutamate). **B.** Averaged spine shrinkage experiments. **C.** Low frequency uncaging (LFU) in the presence of MK-801 induced robust spine shrinkage which was also permitted with the addition of DsdA and GO. Presence of 10 µM D-serine blocks non-ionotropic spine shrinkage (109.9 ± 9.144% of baseline, n=7; ***, p=0.009) compared to vehicle and enzyme conditions (69.35 ± 2.906% of baseline, n=6 and 63.41 ± 4.524% of baseline, n=6, respectively), which were not significantly different. Volume of unstimulated neighbouring spines was also not affected by LFU (open circles, bottom). Estimated spine volume at 35-45 min. Data are represented as mean ± SEM. These experiments were performed in by Margarita Anisimova in the Zito lab.

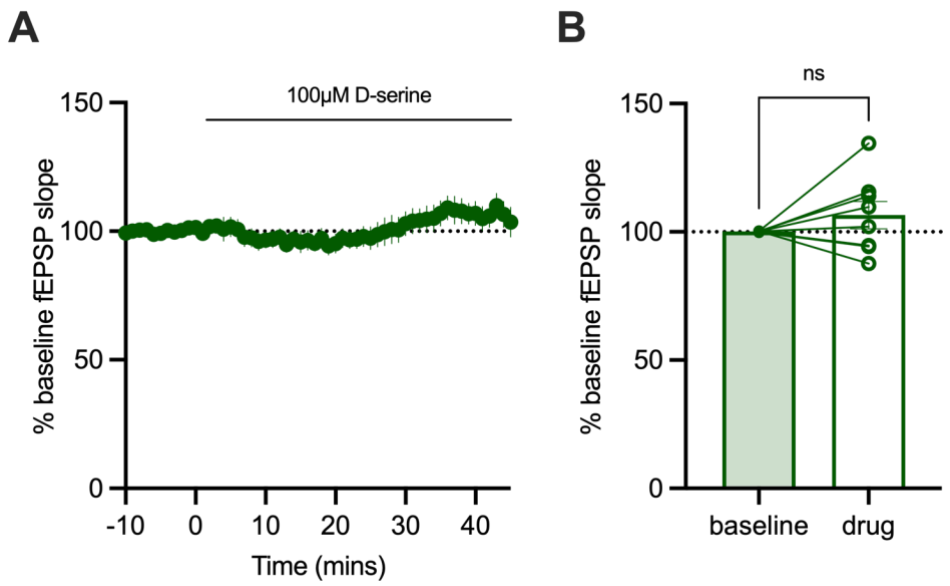

Figure 10. Application of D-serine does not affect AMPAR-mediated fEPSP responses. A. Time course of D-serine wash. **B.** 100 μM D-serine wash does not significantly affect AMPAR fEPSP slope averaging from 20-30 min post drug application (106.6 ± 5.336% of baseline, n=8). All data represent mean ± SEM.

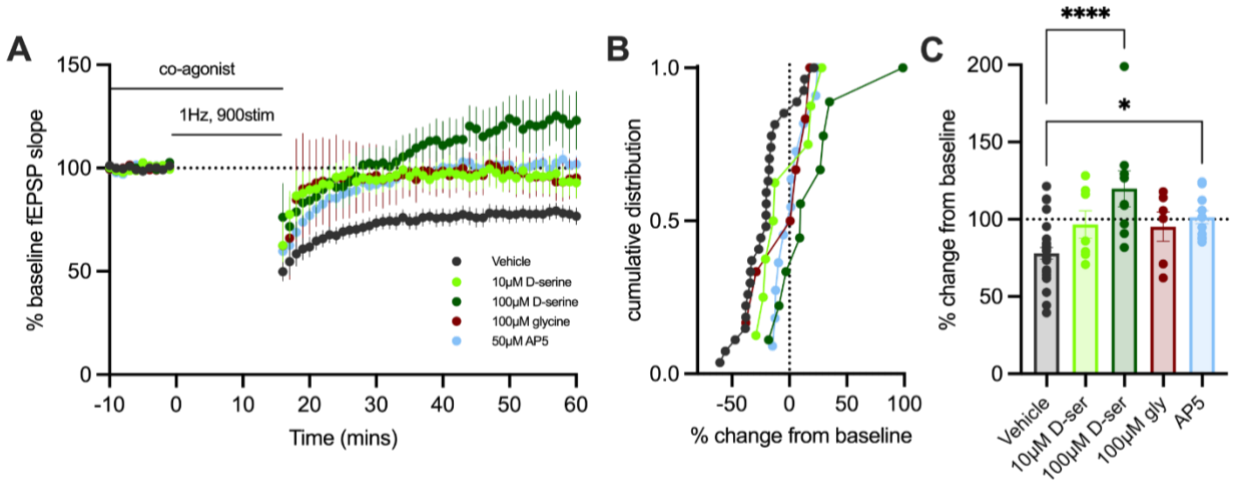

Figure 11. Increased co-agonist concentration blocks synaptic depression. A-C. Application of saturating co-agonist concentrations in non-recirculating solution inhibits LTD induction. **A.** Averaged fEPSP LTD experiments. **B.** Cumulative distribution of experiments in A. **C.** Using an LFS induction protocol, we observed a robust depression in the vehicle condition (77.94 ± 3.836% of baseline, n=27) that was blocked in the presence of AP5 (101.3 ± 4.183% of baseline, n=4.183; *, p=0.0193). 100 μM D-serine significantly blocked LFS-induced LTD (119.8 ± 11.57, n=9; ****, p < 0.0001). Interestingly high (100 μM) glycine and low (10 μM) D-serine concentrations also exhibited a weak block of synaptic depression (glycine: 95.23 ± 9.455, n=6; ns, p=0.3590; D-serine: 96.63 ± 8.900, n=7; ns, p=0.2295). All data represent mean ± SEM.

References

Abrahamsson, T., Chou, C.Y.C., Li, S.Y., Mancino, A., Costa, R.P., Brock, J.A., Nuro, E., Buchanan, K.A., Elgar, D., Blackman, A.V., Tudor-Jones, A., Oyrer, J., Farmer, W.T., Murai, K.K., Sjöström, P.J., 2017. Differential Regulation of Evoked and Spontaneous Release by Presynaptic NMDA Receptors. Neuron 96, 839-855.e5. https://doi.org/10.1016/j.neuron.2017.09.030

Adam-Vizi, V., 1992. External Ca $^{2+}$ -Independent Release of Neurotransmitters. J Neurochem 58, 395–405. https://doi.org/10.1111/j.1471-4159.1992.tb09736.x

Aow, J., Dore, K., Malinow, R., 2015. Conformational signaling required for synaptic plasticity by the NMDA receptor complex. Proceedings of the National Academy of Sciences 112, 14711–14716. https://doi.org/10.1073/pnas.1520029112

Attwell, D., Barbour, B., Szatkowski, M., 1993. Nonvesicular release of neurotransmitter. Neuron 11, 401–407. https://doi.org/10.1016/0896-6273(93)90145-H

Babiec, W.E., Guglietta, R., Jami, S.A., Morishita, W., Malenka, R.C., Dell, T.J.O., 2014. Ionotropic NMDA Receptor Signaling Is Required for the Induction of Long-Term Depression in the Mouse Hippocampal CA1 Region 34, 5285–5290. https://doi.org/10.1523/JNEUROSCI.5419-13.2014

Balu, D.T., Basu, A.C., Corradi, J.P., Cacace, A.M., Coyle, J.T., 2012. The NMDA receptor co-agonists, d-serine and glycine, regulate neuronal dendritic architecture in the somatosensory cortex. Neurobiology of Disease 45, 671–682. https://doi.org/10.1016/j.nbd.2011.10.006

Balu, D.T., Coyle, J.T., 2015. The NMDA receptor ‘ glycine modulatory site ’ in schizophrenia : D-serine, glycine , and beyond. Current Opinion in Pharmacology 20, 109–115. https://doi.org/10.1016/j.coph.2014.12.004

Balu, D.T., Li, Y., Puhl, M.D., Benneyworth, M.A., Basu, A.C., Takagi, S., Bolshakov, V.Y., Coyle, J.T., 2013. Multiple risk pathways for schizophrenia converge in serine racemase knockout mice, a mouse model of NMDA receptor hypofunction. Proceedings of the National Academy of Sciences of the United States of America 110, E2400-9. https://doi.org/10.1073/pnas.1304308110

Balu, D.T., Takagi, S., Puhl, M.D., Benneyworth, M.A., Coyle, J.T., 2014. d-Serine and Serine Racemase are Localized to Neurons in the Adult Mouse and Human Forebrain. Cell Mol Neurobiol 34, 419–435. https://doi.org/10.1007/s10571-014-0027-z

Barria, A., Malinow, R., 2005. NMDA receptor subunit composition controls synaptic plasticity by regulating binding to CaMKII. Neuron 48, 289–301. https://doi.org/10.1016/j.neuron.2005.08.034

Basu, A.C., Tsai, G.E., Ma, C.-L., Ehmsen, J.T., Mustafa, A.K., Han, L., Jiang, Z.I., Benneyworth, M.A., Froimowitz, M.P., Lange, N., Snyder, S.H., Bergeron, R., Coyle, J.T., 2009. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Molecular psychiatry 14, 719–27. https://doi.org/10.1038/mp.2008.130

Bienenstock, L., Cooper, N., Munro, W., 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex 17.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39. https://doi.org/10.1038/361031a0

Carter, B.C., Jahr, C.E., 2016. Postsynaptic, not presynaptic NMDA receptors are required for spike-timing-dependent LTD induction. Nature Neuroscience 19, 1218–1224. https://doi.org/10.1038/nn.4343

Coultrap, S.J., Freund, R.K., O'Leary, H., Sanderson, J.L., Roche, K.W., Dell'Acqua, M.L., Bayer, K.U., 2014. Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection. Cell Reports 6, 431–437. https://doi.org/10.1016/j.celrep.2014.01.005

Danysz, W., Parsons, C.G., 1998. Glycine and N-Methyl-D-Aspartate Receptors: Physiological Significance and Possible Therapeutic Applications. Pharmalogical Reviews 50, 598–650.

Dore, K., Aow, J., Malinow, R., 2015. Agonist binding to the NMDA receptor drives movement of its cytoplasmic domain without ion flow. Proceedings of the National Academy of Sciences 112, 14705–14710. https://doi.org/10.1073/pnas.1520023112

Dudek, S.M., Bear, M.F., 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences of the United States of America 89, 4363–7. https://doi.org/10.1073/pnas.89.10.4363

Duffy, S., Labrie, V., Roder, J.C., 2008. D-Serine Augments NMDA-NR2B Receptor-Dependent Hippocampal Long-Term Depression and Spatial Reversal Learning. Neuropsychopharmacol 33, 1004–1018. https://doi.org/10.1038/sj.npp.1301486

Ehlers, M.D., Zhang, S., Bernhardt, J.P., Huganir, R.L., 1996. Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. Cell 84, 745–755. https://doi.org/10.1016/S0092-8674(00)81052-1

Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Nerbonne, J.M., Lichtman, J.W., Sanes, J.R., 2000. Imaging Neuronal Subsets in Transgenic Mice Expressing Multiple Spectral Variants of GFP. Neuron 28, 41–51. https://doi.org/10.1016/S0896-6273(00)00084-2

Ferreira, J.S., Papouin, T., Ladépêche, L., Yao, A., Langlais, V.C., Bouchet, D., Dulong, J., Mothet, J.P., Sacchi, S., Pollegioni, L., Paoletti, P., Richard Oliet, S.H., Groc, L., 2017. Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses. eLife 6, 1–22. https://doi.org/10.7554/eLife.25492

Fossat, P., Turpin, F.R., Sacchi, S., Dulong, J., Shi, T., Rivet, J.M., Sweedler, J.V., Pollegioni, L., Millan, M.J., Oliet, S.H.R., Mothet, J.P., 2012. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cerebral Cortex 22, 595–606. https://doi.org/10.1093/cercor/bhr130

Fukasawa, Y., Segawa, H., Kim, J.Y., Chairoungdua, A., Kim, D.K., Matsuo, H., Cha, S.H., Endou, H., Kanai, Y., 2000. Identification and Characterization of a Na+-independent Neutral Amino Acid Transporter That Associates with the 4F2 Heavy Chain and Exhibits Substrate Selectivity for Small Neutral d- and l-Amino Acids. Journal of Biological Chemistry 275, 9690–9698. https://doi.org/10.1074/jbc.275.13.9690

Grimwood, S., Le Bourdellès, B., Whiting, P.J., 1995. Recombinant Human NMDA Homomeric NR1 Receptors Expressed in Mammalian Cells Form a High-Affinity Glycine Antagonist Binding Site. Journal of Neurochemistry 64, 525–530. https://doi.org/10.1046/j.1471-4159.1995.64020525.x

Hansen, K.B., Yi, F., Perszyk, R.E., Furukawa, H., Wollmuth, L.P., Gibb, A.J., Traynelis, S.F., 2018. Structure, function, and allosteric modulation of NMDA receptors. J. Gen. 150. https://doi.org/10.1085/jgp.201812032

Hashimoto, A., Oka, T., Nishikawa, T., 1995. Extracellular Concentration of Endogenous Free D-serine int he Rat Brain as Revealed by In Vivo Microdialysis. Neuroscience 66, 635–43.

Henneberger, C., Papouin, T., Oliet, S.H.R., Rusakov, D. a, 2010. Long-term potentiation depends on release of D-serine from astrocytes. Nature 463, 232–236. https://doi.org/10.1038/nature08673

Kato, K., Clifford, D.B., Zorumski, C.F., 1993. Long-term potentiation during whole-cell recording in rat hippocampal slices. Neuroscience 53, 39–47. https://doi.org/10.1016/0306-4522(93)90282-K

Keith, R.E., Ogoe, R.H., Dumas, T.C., 2021. Behind the scenes: Are latent memories supported by calcium independent plasticity? Hippocampus hipo.23332. https://doi.org/10.1002/hipo.23332

Kellermayer, B., Ferreira, J.S., Dupuis, J., Levet, F., Grillo-Bosch, D., Bard, L., Linarès-Loyez, J., Bouchet, D., Choquet, D., Rusakov, D.A., Bon, P., Sibarita, J.B., Cognet, L., Sainlos, M., Carvalho, A.L., Groc, L., 2018. Differential Nanoscale Topography and Functional Role of GluN2-NMDA Receptor Subtypes at Glutamatergic Synapses. Neuron 100, 106-119.e7. https://doi.org/10.1016/j.neuron.2018.09.012

Kim, C.H., Chung, H.J., Lee, H.K., Huganir, R.L., 2001. Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. Proceedings of the National Academy of Sciences of the United States of America 98, 11725–30. https://doi.org/10.1073/pnas.211132798

Labrie, V., Duffy, S., Wang, W., Barger, S.W., Baker, G.B., Roder, J.C., 2008. Genetic inactivation of D-amino acid oxidase enhances extinction and reversal learning in mice. Learning & Memory 16, 28–37. https://doi.org/10.1101/lm.1112209

Ladépêche, L., Dupuis, J.P., Groc, L., 2014. Surface trafficking of NMDA receptors: Gathering from a partner to another. Seminars in Cell & Developmental Biology 27, 3–13. https://doi.org/10.1016/j.semcdb.2013.10.005

Lau, C.G., Zukin, R.S., 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nature Reviews Neuroscience 8, 413–426. https://doi.org/10.1038/nrn2153

Le Bail, M., Martineau, M., Sacchi, S., Yatsenko, N., Radzishevsky, I., Conrod, S., Ait Ouares, K., Wolosker, H., Pollegioni, L., Billard, J.-M., Mothet, J.-P., 2015. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 112, E204-13. https://doi.org/10.1073/pnas.1416668112

Le Douce, J., Maugard, M., Veran, J., Matos, M., Jégo, P., Vigneron, P.-A., Faivre, E., Toussay, X., Vandenberghe, M., Balbastre, Y., Piquet, J., Guiot, E., Tran, N.T., Taverna, M., Marinesco, S., Koyanagi, A., Furuya, S., Gaudin-Guérif, M., Goutal, S., Ghettas, A., Pruvost, A., Bemelmans, A.-P., Gaillard, M.-C., Cambon, K., Stimmer, L., Sazdovitch, V., Duyckaerts, C., Knott, G., Hérard, A.-S., Delzescaux, T., Hantraye, P., Brouillet, E., Cauli, B., Oliet, S.H.R., Panatier, A., Bonvento, G., 2020. Impairment of Glycolysis-Derived l-

Serine Production in Astrocytes Contributes to Cognitive Deficits in Alzheimer's Disease. Cell Metabolism 31, 503-517.e8. https://doi.org/10.1016/j.cmet.2020.02.004
Leeson, P.D., Baker, R., Carling, R.W., Curtis, N.R., Moore, K.W., Williams, B.J., Foster, A.C., Donald, A.E., Kemp, J.A., Marshall, G.R., 1991. Kynurenic acid derivatives. Structure-activity relationships for excitatory amino acid antagonism and identification of potent and selective antagonists at the glycine site on the N-methyl-D-aspartate receptor. J. Med. Chem. 34, 1243–1252. https://doi.org/10.1021/jm00108a002
Li, Y., Sacchi, S., Pollegioni, L., Basu, A.C., Coyle, J.T., Bolshakov, V.Y., 2013. Identity of endogenous NMDAR glycine site agonist in amygdala is determined by synaptic activity level. Nature Communications 4, 1760–1760. https://doi.org/10.1038/ncomms2779
Lin, J.W., Ju, W., Foster, K., Lee, S.H., Ahmadian, G., Wyszynski, M., Wang, Y.T., Sheng, M., 2000. Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nature Neuroscience 3, 1282–1290. https://doi.org/10.1038/81814
Lüscher, C., Xia, H., Beattie, E.C., Carroll, R.C., Von Zastrow, M., Malenka, R.C., Nicoll, R.A., 1999. Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron 24, 649–658. https://doi.org/10.1016/S0896-6273(00)81119-8
MacDonald, J.F., Bartlett, M.C., Mody, I., Pahapill, P., Reynolds, J.N., Salter, M.W., Schneiderman, J.H., Pennefather, P.S., 1991. Actions of ketamine, phencyclidine and MK-801 on NMDA receptor currents in cultured mouse hippocampal neurones. The Journal of Physiology 432, 483–508. https://doi.org/10.1113/jphysiol.1991.sp018396
Malenka, R.C., Kauer, J.A., Perkel, D.J., Mauk, M.D., Kelly, P.T., Nicoll, R.A., Waxham, M.N., 1989. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340, 554–557. https://doi.org/10.1038/340554a0
Malinow, R., Schulman, H., Tsien, R.W., 1989. Inhibition of Postsynaptic PKC or CaMKII Blocks Induction But Not Expression of LTP. Science 245, 862–866. https://doi.org/10.1126/science.2549638
Malinow, R., Tsien, R.W., 1990. Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. Nature 346, 177–180. https://doi.org/10.1038/346177a0
Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., Wada, K., 2002. Functional Comparison of d-Serine and Glycine in Rodents: The Effect on Cloned NMDA Receptors and the Extracellular Concentration. Journal of Neurochemistry 65, 454–458. https://doi.org/10.1046/j.1471-4159.1995.65010454.x
McBain, C.J., Mayer, M.L., 1994. N-Methyl-D-Aspartic Acid Receptor Structure. Physiological Reviews 74, 723–753.
McNamara, D., Smith, E.C.R., Calligaro, D.O., O'Malley, P.J., McQuaid, L.A., Dingledine, R., 1990. 5,7-Dichlorokynurenic acid, a potent and selective competitive antagonist of the glycine site on NMDA receptors. Neuroscience Letters 120, 17–20. https://doi.org/10.1016/0304-3940(90)90157-5
Miya, K., Inoue, R., Takata, Y., Abe, M., Natsume, R., Sakimura, K., Hongou, K., Miyawaki, T., Mori, H., 2008. Serine racemase is predominantly localized in neurons in mouse brain. Journal of Comparative Neurology 510, 641–654. https://doi.org/10.1002/cne.21822

Mothet, J., Parent, T., Wolosker, H., Brady, R.O., Linden, D.J., Ferris, C.D., Rogawski, M.A., Snyder, S.H., 2000. D -Serine is an endogenous ligand for the glycine site of the N-methyl-D -aspartate receptor. Proceedings of the National Academy of Sciences 97, 4926–4931.

Mothet, J.P., Le Bail, M., Billard, J.M., 2015. Time and space profiling of NMDA receptor co-agonist functions. Journal of Neurochemistry 135, 210–225. https://doi.org/10.1111/jnc.13204

Nabavi, S., Kessels, H.W., Alfonso, S., Aow, J., Fox, R., Malinow, R., 2013. Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression 110. https://doi.org/10.1073/pnas.1219454110

Nong, Y., Huang, Y.-Q., Ju, W., Kalia, L.V., Ahmadian, G., Wang, Y.T., Salter, M.W., 2003. Glycine binding primes NMDA receptor internalization. Nature 422, 302–307. https://doi.org/10.1038/nature01497

Nong, Y., Huang, Y.-Q., Salter, M.W., 2004. NMDA receptors are movin' in. Current Opinion in Neurobiology 14, 353–361. https://doi.org/10.1016/j.conb.2004.05.001

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H.R., 2006. Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. Cell 125, 775–784. https://doi.org/10.1016/j.cell.2006.02.051

Paoletti, P., 2011. Molecular basis of NMDA receptor functional diversity. European Journal of Neuroscience 33, 1351–1365. https://doi.org/10.1111/j.1460-9568.2011.07628.x

Papouin, T., Lad??p??che, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., Groc, L., Pollegioni, L., Mothet, J.P., Oliet, S.H.R., 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150, 633–646. https://doi.org/10.1016/j.cell.2012.06.029

Pernot, P., Maucler, C., Tholance, Y., Vasylieva, N., Debilly, G., Pollegioni, L., Cespuglio, R., Marinesco, S., 2012. d-Serine diffusion through the blood–brain barrier: Effect on d-serine compartmentalization and storage. Neurochemistry International 60, 837–845. https://doi.org/10.1016/j.neuint.2012.03.008

Pinto-Duarte, A., Roberts, A.J., Ouyang, K., Sejnowski, T.J., 2019. Impairments in remote memory caused by the lack of Type 2 IP_3 receptors. Glia glia.23679. https://doi.org/10.1002/glia.23679

Priestley, T., Kemp, J.A., 1994. Kinetic Study of the Interactions Between the Glutamate and Glycine Recognition Sites on the N-Methyl-D-aspartic Acid Receptor Complex. Molecular Pharmacology 46, 1191–1196.

Reynolds, I.J., 2000. [^{3}H](+)MK801 Radioligand Binding Assay at the *N* -Methyl- D -Aspartate Receptor. Current Protocols in Pharmacology 11. https://doi.org/10.1002/0471141755.ph0120s11

Rosenberg, D., Artoul, S., Segal, A.C., Kolodney, G., Radzishevsky, I., Dikopoltsev, E., Foltyn, V.N., Inoue, R., Mori, H., Billard, J.-M., Wolosker, H., 2013. Neuronal D-Serine and Glycine Release Via the Asc-1 Transporter Regulates NMDA Receptor-Dependent Synaptic Activity. Journal of Neuroscience. https://doi.org/10.1523/JNEUROSCI.3836-12.2013

Schell, M.J., Brady, R.O., Molliver, M.E., Snyder, S.H., 1997. D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 17, 1604–15.

Sherwood, M.W., Oliet, S.H.R., Panatier, A., 2021. NMDARs, Coincidence Detectors of Astrocytic and Neuronal Activities. IJMS 22, 7258. https://doi.org/10.3390/ijms22147258
Shi, S.-H., Hayashi, Y., Esteban, J.A., Malinow, R., 2001. Subunit-Specific Rules Governing AMPA Receptor Trafficking to Synapses in Hippocampal Pyramidal Neurons. Cell 105, 331–343. https://doi.org/10.1016/S0092-8674(01)00321-X
Shleper, M., Kartvelishvily, E., Wolosker, H., 2005. D-Serine Is the Dominant Endogenous Coagonist for NMDA Receptor Neurotoxicity in Organotypic Hippocampal Slices. Journal of Neuroscience 25, 9413–9417. https://doi.org/10.1523/JNEUROSCI.3190-05.2005
Stein, I.S., Gray, J.A., Zito, K., 2015. Non-Ionotropic NMDA Receptor Signaling Drives Activity-Induced Dendritic Spine Shrinkage 35, 12303–12308. https://doi.org/10.1523/JNEUROSCI.4289-14.2015
Stein, I.S., Park, D.K., Claiborne, N., Zito, K., 2021. Non-ionotropic NMDA receptor signaling gates bidirectional structural plasticity of dendritic spines. Cell Reports 34, 108664. https://doi.org/10.1016/j.celrep.2020.108664
Stein, I.S., Park, D.K., Flores, J.C., Jahncke, J.N., Zito, K., 2020. Molecular Mechanisms of Non-ionotropic NMDA Receptor Signaling in Dendritic Spine Shrinkage. J. Neurosci. 40, 3741–3750. https://doi.org/10.1523/JNEUROSCI.0046-20.2020
Stroebel, D., Mony, L., Paoletti, P., 2021. Glycine agonism in ionotropic glutamate receptors. Neuropharmacology 193, 108631. https://doi.org/10.1016/j.neuropharm.2021.108631
Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate Receptor Ion Channels: Structure, Regulation, and Function. Catheterization and cardiovascular diagnosis 62, 405–496. https://doi.org/10.1124/pr.109.002451.405
Van Horn, M.R., Strasser, A., Miraucourt, L.S., Pollegioni, L., Ruthazer, E.S., 2017. The Gliotransmitter d-Serine Promotes Synapse Maturation and Axonal Stabilization *In Vivo*. J. Neurosci. 37, 6277–6288. https://doi.org/10.1523/JNEUROSCI.3158-16.2017
Vissel, B., Krupp, J.J., Heinemann, S.F., Westbrook, G.L., 2001. A use-dependent tyrosine dephosphorylation of NMDA receptors is independent of ion flux. Nature Neuroscience 4, 587–596. https://doi.org/10.1038/88404
Westergren, I., Nystrom, B., Hamberger, A., Nordborg, C., Johansson, B.B., 2008. Concentrations of Amino Acids in Extracellular Fluid After Opening of the Blood-Brain Barrier by Intracarotid Infusion of Protamine Sulfate. Journal of Neurochemistry 62, 159–165. https://doi.org/10.1046/j.1471-4159.1994.62010159.x
Wong, J.M., Folorunso, O.O., Barragan, E.V., Berciu, C., Harvey, T.L., Coyle, J.T., Balu, D.T., Gray, J.A., 2020. Postsynaptic Serine Racemase Regulates NMDA Receptor Function. J. Neurosci. 40, 9564–9575. https://doi.org/10.1523/JNEUROSCI.1525-20.2020
Wong, J.M., Gray, J.A., 2018. Long-term depression is independent of GluN2 subunit composition. The Journal of Neuroscience 38, 0394–18. https://doi.org/10.1523/JNEUROSCI.0394-18.2018
Wood, P.L., Hawkinson, J.E., Goodnough, D.B., 1996. Formation of d-Serine from l-Phosphoserine in Brain Synaptosomes. Journal of Neurochemistry 67, 1485–1490. https://doi.org/10.1046/j.1471-4159.1996.67041485.x

Woods, G.F., Oh, W.C., Boudewyn, L.C., Mikula, S.K., Zito, K., 2011. Loss of PSD-95 Enrichment Is Not a Prerequisite for Spine Retraction. Journal of Neuroscience 31, 12129–12138. https://doi.org/10.1523/JNEUROSCI.6662-10.2011

Xu, Z., Chen, R.-Q., Gu, Q.-H., Yan, J.-Z., Wang, S.-H., Liu, S.-Y., Lu, W., 2009. Metaplastic Regulation of Long-Term Potentiation/Long-Term Depression Threshold by Activity-Dependent Changes of NR2A/NR2B Ratio. Journal of Neuroscience 29, 8764–8773. https://doi.org/10.1523/JNEUROSCI.1014-09.2009

Yang, Y., Ge, W., Chen, Y., Zhang, Z., Shen, W., Wu, C., Poo, M., Duan, S., 2003. Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proceedings of the National Academy of Sciences of the United States of America 100, 15194–9. https://doi.org/10.1073/pnas.2431073100

Yashiro, K., Philpot, B.D., 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55, 1081–1094. https://doi.org/10.1016/j.neuropharm.2008.07.046

Zhang, Z., Gong, N., Wang, W., Xu, L., Xu, T.L., 2008. Bell-shaped d-serine actions on hippocampal long-term depression and spatial memory retrieval. Cerebral Cortex 18, 2391–2401. https://doi.org/10.1093/cercor/bhn008

Chapter 3

Contextual Fear Conditioning Increases Intrinsic Excitability in CA1 Pyramidal Cells

Preface

The following chapter contains a figure from published data titled “Metaplasticity contributes to memory formation in the hippocampus” that was accepted in Neuropsychopharm on May 11th, 2018. It also contains unpublished data further characterizing a learning-induced change in intrinsic excitability in CA1 pyramidal neurons which is the result of a collaborative work with Jacob H. Wilmot, Brian J. Wiltgen, and John A. Gray. All text and figures in the following chapter are my own. BJW provided the mice for these experiments and JHW performed all the contextual fear training. JAG was involved in experimental design, interpretation, and editing. The authors report no conflicts of interest. This work was supported by R21MH116315 and R01MH117130 (JAG) and T32GM099608 (EVB).

Abstract

Memory is central to who we are and how we behave – learning from the past to interact with the present. Within the brain, memories are generated during experience when learning activates a subpopulation of neurons – a neural ensemble – that then encodes that memory in the hippocampus. Synaptic plasticity is a key cellular substrate underlying learning and memory, but learning also produces intrinsic changes in neuronal excitability, whose contribution to memory is not completely understood. It has been suggested that changes in intrinsic excitability enhance responsiveness and thus, chances of integration into the neural ensemble. While previous studies have only compared CA1 neurons between trained and untrained animals, recent technological advances allow us to examine, in a single animal, the learning-induced increase in excitability specifically in engram cells – neurons activated by the contextual fear conditioning (CFC). To investigate whether intrinsic excitability changes occur specifically in cells activated during CFC, TetTag mice were trained, and 48 hours later acute hippocampal slices were prepared. Consistent with previous studies, we found that CA1 neurons that were activated (GFP^+) during learning display increased firing rate compared to neighboring neurons that were not activated (GFP^-). This increased excitability in GFP^+ neurons persisted for at least two days following contextual fear conditioning. Additionally, high excitability state was highly correlated to high percent freezing time. Interestingly, in contrast to prior studies examining trace eyeblink conditioning in rabbits, changes in the medium afterhyperpolarization (mAHP) do not account for the differential excitability between GFP^+ and GFP^- neurons. These results suggest a unique mechanism exists for learning-induced enhancement of intrinsic excitability in CA1 pyramidal neurons activated during learning.

Introduction

Memories are formed via activation of neural ensembles in the hippocampus. The idea of memory being stored as a neural ensemble predates modern neuroscience, and it wasn't until the 20th century that Richard Semon would coin the term 'engram' to encompass the entirety of that concept. Semon posited that a population of neurons would become an engram – a tangible memory within the brain – after a series of experience-induced physical and chemical changes. He also thought these memories could be retrieved by reactivating the engram with cues from the original experience – suggesting the engram is both necessary and sufficient for memory formation, storage, and retrieval. The hunt for the engram was largely unfruitful, and for some time a discernable underlying cellular mechanism of learning and memory remained elusive. A promising candidate emerged, first theoretically, in the form of Donald Hebb's postulate that neuronal connectivity is dependent on changes in synaptic strength, then experimentally, showing strong correlations between potentiated synaptic strength and memory (Morris et al., 1986; Sakimura et al., 1995). Synaptic changes that occur during learning are rapidly induced, long-lasting, and input specific, rendering them ideal for information storage. Yet without more sophisticated and advanced techniques, it was difficult to establish a causal relationship between long-lasting changes in synaptic connectivity with a distinct, behavioral memory at the level of the neural ensemble.

Learning also produces changes in intrinsic neuronal properties, whose contribution to memory are not well understood. Unlike synaptic plasticity, intrinsic plasticity is transient and therefore not ideal for long-term memory storage. However, in CA1, these short-lived changes in neuronal properties could serve to enhance the retrieval of recent memories via facilitating the consolidation of new memories. Previous studies have compared differences in activity following learning in trained and untrained animals. In these studies, it has overwhelmingly been shown that behavioral stimulates robust changes in neuronal excitability (Disterhoft et al., 1986;

Kaczorowski et al., 2007; McKay et al., 2013; Moyer et al., 2000; Oh and Disterhoft, 2015; Rabinak et al., 2008). But these experiments did not address whether the changes were specific to the engram encoding that experience. A more recent study showed that contextual fear conditioning enhances excitability in neurons in the dentate gyrus (DG) in a way that is correlated to memory retrieval. The learning-induced enhancement of DG engram cell excitability is very transient, lasting for less than 2 hours, and is causally linked with a short-term increase in strength of cue recognition. It is unknown whether the change in neuronal excitability in CA1 engram cells can also affect behavioral performance.

Investigation of this phenomenon requires a system in which we have temporal specificity that can be adapted to behavioral tasks, by allowing control over windows of activity-dependent labeling of neurons. Following contextual fear conditioning, roughly 40% of CA1 pyramidal neurons express c-Fos (Reijmers et al., 2007; Tayler et al., 2013), an immediate early gene typically used as an indicator of recent neuronal activity. Reactivation of neurons that express elevated c-Fos levels after training increases freezing, which suggests that these cells have been incorporated into the neural ensemble corresponding to that memory (Liu et al., 2012; Reijmers and Mayford, 2009). Transgenic fos-tTa (TetTag) mice utilize the tetracycline transactivator (tTA) system to regulate activity-induced stimulation of the c-Fos promoter to tag recently activated neurons – tTA binds to tetO and then drives expression of the "tag" (Gossen et al., 1995; Mayford et al., 1996; Smeyne et al., 1992). Administration of doxycycline (dox) inhibits tTA binding, while removal of dox allows neural activity to drive expression of the tag in activated cells (Nakazawa et al., 2016; Tanaka et al., 2014). When using the long-lasting fluorophore H2B-GFP as the tag, there is now an elegant system capable of fluorescently labeling neurons that were incorporated into a neural ensemble in a dox-dependent manner. We utilized this system so that learning-induced intrinsic changes can be directly compared within a single animal between cells that were activated during the contextual fear conditioning experience and those that were not.

While increased firing capability after learning has been extensively documented, the role of this enhanced excitability in CA1 remains poorly understood. Additionally, disruption of intrinsic plasticity may contribute to disease states as well as dysfunction in long-term memory formation. In epilepsy, networks of neurons become synchronously active, which in part may be mediated by pathologies of intrinsic plasticity. In chronic pain, changes in intrinsic plasticity can lead to an amplified sensitivity to previously neutral stimuli (hyperalgesia) and to perception of pain after the noxious stimuli is removed (paresthesia). Changes in intrinsic excitability also naturally arise with aging, such that normal aging subjects, including humans, have difficulty learning hippocampus-dependent tasks. An important cause is the reduced intrinsic excitability observed in hippocampal pyramidal neurons from normal aging subjects, as reflected by an enlarged after-hyperpolarization (AHP) and an increased spike-frequency adaptation (accommodation). Therefore, a more detailed, fundamental understanding of the role changes in intrinsic plasticity play in memory formation and learning is necessary. The important question of whether changes in intrinsic plasticity are necessary for learning remains to be answered. In addition, the identity of the mechanism underlying these changes in neuronal excitability in CA1 remain to be elucidated.

Materials and Methods

Animals

Male and female C57/BL6 and female B6/129 F1 hybrid TetTag transgenic mice were used. These mice express H2B-GFP under the c-Fos promoter. In these mice, activation of the c-Fos promoter during learning leads to the expression of H2B-GFP in active neurons (Reijmers and Mayford, 2009). Mice were removed from doxycycline (40mg/kg) for 2 days prior to fear conditioning. Immediately following training, animals were put on high doxycycline (1g/kg) for 1 day, then returned to low doxycycline for the remainder of the experiment. Mice were single housed in plastic cages under a 12h light/dark cycle at a constant temperature of 24 ± 1ºC, with water and food ad libitum. Behavioral tests and recordings were performed during the light phase of the cycle. All experiments were carried out in accordance with the National Institutes of Health guidelines and were approved by the UC Davis Institutional Animal Care and Use Committee (IACUC).

Behavioral Procedure

Prior to contextual fear conditioning, mice were handled 2 min per day for 5-6 days. The chamber used for fear conditioning in these experiments was described previously (Nakazawa et al., 2016; Tanaka et al., 2014). The chamber consisted of a stainless-steel grid floor and side walls, as well as a Plexiglass door. Overhead LED lighting provided broad spectrum or near infrared light, and a high-speed monochrome video camera (Med Associates) captured animals/ movement during the training sessions. Mice underwent one training session in which they were placed into the chamber and a single 0.4mA/2s foot shock was delivered before the mouse was returned to its cage. Homecage control animals did not undergo any contextual fear training.

Electrophysiology

For whole-cell recordings, 6- to 12-week old male TetTag mice were anesthetized with isoflurane and transcardially perfused with ice-cold carbogen (95% O_2/5% CO_2)-bubbled NMDG-

based cutting solution (Ting et al., 2018) containing (in mM): 93 NMDG, 2.5 KCl, 1.25 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 10 $MgSO_4$, 0.5 $CaCl_2$ (titrated to pH 7.3-7.4 with concentrated HCl; osmolality of 200-310 mOsm/kg). Acute transverse slices (300 µM) of dorsal hippocampus were cut on a Leica VT1200 vibratome (Buffalo Grove, IL) in the ice-cold and oxygenated NMDG cutting solution described above, then transferred to an incubation chamber containing the same NMDG cutting solution for 15 min at 34ºC. Before recording, slices were recovered for at least 45 min then stored submerged for up to 5 h in room temperature oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 2.5 $CaCl_2$, and 1.3 $MgSO_4$. Whole-cell current clamp recordings were obtained from CA1 pyramidal neurons under visual guidance (DIC/infrared optics). GFP^+ neurons were identified by epifluorescence microscopy. Whenever possibly, neighboring GFP^+ and GFP^- neurons were recorded simultaneously. For all recordings, borosilicate glass pipettes were fabricated with resistances of 4-6MΩ. Pipettes were filled with the following intracellular solution (in mM): 135 K^+ gluconate, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.6 EGTA, 4 NaATP, 0.4 NaGTP, pH 7.3, 290 mOsm. Recordings were collected with a Multiclamp 700B amplifier (Molecular Devices), filtered at 2 kHz, digitized at 10kHz, and data analyzed using pClamp 10 software (Molecular Devices). Series resistance was monitored and cells in which series resistance varied by more than 20% during a recording were discarded. Frequency-current relationships for evoked firing were determined by injecting 500ms current steps with amplitudes increasing by 25pA, from 0 to 500 pA from the resting potential. Liquid junction potentials were not corrected.

Results

Hippocampal CA1 pyramidal neurons become more excitable after context fear learning

To determine if contextual fear conditioning (CFC) enhances excitability in the hippocampus, we patched and recorded from CA1 neurons following CFC (Figure 1A). The long-lasting fluorescent protein H2B-GFP was used to identify cells that were active during training. Expression of this protein was controlled by the c-Fos promoter and suppressed by doxycycline as previously described. Doxycycline chow was removed two days prior to fear conditioning, to allow for tagging, and reintroduced immediately after learning. Two days later, GFP^+ and GFP^- cells were patched in CA1, and excitability was examined (Figure 1B). We found a significant increase in intrinsic excitability (slope of the spike frequency vs injected current curve) in GFP^+ cells compared with neighboring GFP^- neurons (Figures 1C-E) (two-way repeated measures ANOVA with injected current · cell: $F_{6,90} = 4.55$, $p < 0.05$). The average firing rate of GFP^- neurons with current injections between 100 and 150pA demonstrates the distribution (10.6 ± 2.0Hz for GFP^- and 20.2 ± 1.4Hz for GFP^+; $t_{(15)} = 4.07$, $p = 0.001$). This increase was not accompanied by a change in the resting membrane potential (Figure 1f) (-63.8 ± 1.2mV for GFP^- and -64.1 ± 1.0mV for GFP^+). Therefore, as predicted from previous work, this learning-dependent enhancement in excitability in CA1 pyramidal neurons persisted for at least two days after CFC.

These findings were then repeated using a wider range of current injections to further compare firing capabilities of GFP^+ and GFP^- neurons and uncover the mechanism behind the enhancement in intrinsic excitability. Surprisingly, there was a difference in observed firing rates between the control (GFP^-) neurons in the original experiments (Crestani et al., 2018) and the control neurons of the repeated (unpublished) experiments in response to the same injected current step. This difference in firing rate was also noticeable in the GFP^+ neurons (Figure 2A). Despite this, a significant increase in the number of spikes per injected current was still found in GFP^+ neurons compared with neighboring GFP^- cells at injections of 300pA (35.19 ± 3.99Hz for

GFP$^-$ and 47.20 ± 2.12Hz for GFP$^+$, p = 0.0119) or more (Figure 2B). This increase in intrinsic plasticity was not due to any substantial differences in resting membrane potential (-66.76 ± 1.40mV for GFP$^-$ and -66.29 ± 1.08mV for GFP$^+$) or input resistance (194.8 ±11.06MΩ for GFP$^-$ and 188.2 ± 11.47MΩ for GFP$^+$) between the two groups of cells (Figure 2C-D). These findings are consistent with our previous work showing a learning-induced increase in intrinsic excitability in CA1 pyramidal neurons.

Increased excitability is not due to changes in neuronal properties

Earlier studies examining the excitability changes in hippocampal CA1 neurons following trace eyeblink conditioning in rabbits identified reductions in the medium (peak) post-burst afterhyperpolarization as the mechanism underlying the learning-induced increase in firing rate (Disterhoft et al., 2012; Matthews et al., 2008). The afterhyperpolarization (AHP) plays a large role in determining how a cell responds to stimulation, which in turn can affect the network's ability to form memories. To test this in our system, we compared the amplitudes of the medium and slow AHPs following current injection in activated (GFP$^+$) and control (GFP$^-$) cells. Despite observing a higher firing rate in response to various current injections in GFP$^+$ cells compared to GFP$^-$ cells, no significant difference in medium (-1.85mV ± 0.29 in GFP$^-$ and -1.56mV ± 0.32 in GFP$^+$) or slow (-0.52mV ± 0.15 in GFP$^-$ and -0.63mV ± 0.14) AHPs was observed following contextual fear conditioning (Figure 3). The average medium and slow AHPs were similar in amplitude to those collected from homecage control animals (-1.62mV ± 0.31 for mAHP and -0.496mV ± 0.17 for sAHP), which did not undergo CFC training and would reveal if any global changes occur when an animal undergoes CFC training that are independent of GFP expression. The average mAHP amplitude across all three groups is surprisingly similar to the mAHP amplitude seen in trained animals in prior studies but is inconsistent with the values seen in untrained animals (Moyer et al., 2000). These earlier studies compared CA1 neurons between trained and untrained animals. In contrast, our experiments compare activated and control CA1

neurons from within the same animal following CFC training. Other neuronal and action potential specific properties were compared between GFP^+ and GFP^- neurons, but no significant differences were found (Table 1). Therefore, the process underlying the change in enhancement of neuronal excitability in CA1 neurons following CFC remains unknown.

Variability in excitability is due to differences in percent freezing

Our previous study showed a consistent and significant learning-induced increase in average firing rate; the animals used for these experiments spent on average around 50% of the time freezing during CFC (Crestani et al., 2018). Further examination of the unpublished data (that was later collected across a wider range of current injections) revealed a high amount of variability in both GFP^+ and GFP^- average firing rates following training from day to day. Observation of the freezing data collected by the Wiltgen lab also showed a corresponding broad spread in percent freezing time during CFC training. Data collected from these animals was then sorted into groups based on the amount of time spent freezing during training. Animals that froze for less than 10% of the training (n=2 animals) showed no difference in GFP^+ (20.67Hz ± 2.12) and GFP^- (24.61Hz ± 2.86) neurons' average firing rates in response to a 300pA current injection (Figure 4A-B). However, animals that froze more than 55% of the CFC training time (n=2 animals) showed a significant difference in average firing rate during current injection (24.92Hz ± 5.63 in GFP^- neurons and 44.67Hz ± 2.85 in GFP^+, p=0.0055) (Figure 4C-D). This suggests there is a strong relationship between the variability in percent freezing time during CFC training and the variability in excitability state.

Increased intrinsic excitability is highly correlated with increased percent freezing

It has been shown that constitutively reduced neuronal excitability (via Kir2.1 overexpression) in activated hippocampal DG granule cells affects learning and memory retrieval, demonstrating a causal relationship between enhanced context recognition during CFC and high engram cell excitability (Pignatelli et al., 2019). In our own data, we see a similar trend in increased freezing

time during CFC in animals that exhibit a substantially higher average firing rate in GFP^+ versus GFP^- CA1 neurons. In GFP^+ cells, enhanced average firing rate is strongly correlated to percent freezing time during CFC training (n=9 animals, 20 cells; R^2=0.8592). Variability in GFP^- neurons does not show a correlative relationship to changes in percent freezing (n=9 animals, 19 cells; R^2=0.0611) (Figure 5). The percentage of GFP^+ cells was not noticeably different between low and high freezing animals (observation), meaning GFP expression occurs independent of learning-induced changes in excitability. Together, this data suggests that augmented intrinsic excitability in activated (engram) cells is highly correlated to the percent time spent freezing during CFC, which in this study is used as a proxy for learning.

Discussion

The biological basis of memory in the hippocampus has been extensively studied in relation to synaptic plasticity (Kandel, 2009; Langille and Brown, 2018; Morris et al., 1990). It has long been posited that memories are formed via activation of postsynaptic NMDARs, with the resultant calcium influx initiating downstream molecular pathways that trigger gene expression and ultimately long-lasting synaptic changes. It is undeniable that NMDAR-mediated plasticity plays a critical role in learning and memory. For example, NMDAR blockade has been shown to severely impair performance in spatial tasks (Morris water maze), as well as olfactory learning and contextual fear conditioning (Bannerman et al., 1995; Quinlan et al., 2004; Sanders and Fanselow, 2003; Saucier and Cain, 1995; Tayler et al., 2011; Wiltgen et al., 2011, 2010). However, even within these studies, as well as in our own collaboration with the Wiltgen lab (Crestani et al., 2018), a distinct NMDAR-independent learning component exists. Additionally, there is the growing body of evidence demonstrating the role of intrinsic neuronal changes in memory (Disterhoft et al., 1986; Matthews et al., 2008; McKay et al., 2009; Moyer et al., 2000; Moyer Jr. et al., 1996; Oh et al., 2009), especially as a candidate for facilitating integration of populations of neurons into an engram (Cai et al., 2016; Chandra and Barkai, 2018; Pignatelli et al., 2019; Titley et al., 2017). Indeed, the dogma that synaptic plasticity is the sole cellular mechanism underlying learning and memory, two incredibly complicated cognitive processes, is beginning to change to also encompass more nuanced and temporary cellular changes that occur as a result of learning.

Increased freezing is correlated to high excitability state

Increasing the excitability of neurons in the lateral amygdala via chronic (CREB overexpression) and acute (DREADDs and opsins) manipulations has demonstrated a relationship of high neuronal excitability and its assignment into an engram (Yiu et al., 2014; Zhou et al., 2009). Our published data shows a dramatic increase in average firing rate in CA1 neurons

specifically activated by CFC in comparison to neighboring control cells and this enhanced excitability persisted for at least two days following the learning experience. This is consistent with earlier work in which behavioral training stimulated robust changes in neuronal excitability that can last for up to a week (Disterhoft et al., 1986; Kaczorowski et al., 2007; McKay et al., 2013; Moyer et al., 2000; Oh et al., 2009; Rabinak et al., 2008). Surprisingly, the duration of increased excitability is starkly different from the brief excitability change observed in neurons in the dentate gyrus after learning, which returns to baseline within two hours (Pignatelli et al., 2019). This may indicate a differential role for increased excitability in CA1 pyramidal neurons, which, unlike the neurons in the dentate, undergo repetitive hippocampal replay during sleep and wakefulness following learning that may play a role in memory consolidation. Persistent enhanced excitability may facilitate neuronal firing during hippocampal replay and thus play a role in memory consolidation.

It is also worth noting that our published data was generated using fos-tTA mice bred directly from the Mayford lab, while the second round of unpublished experiments used fos-tTA/fos-shEGFP mice from Jackson Labs, which have been shown to overexpress c-Fos in the hippocampus in comparison to their wildtype littermates. The strain from Jackson Labs contains an additional transgene, fos-shEGFP, which contains a half-life variant of GFP whose expression is driven by the c-Fos promotor. The mice obtained from the Mayford lab do not contain a copy this transgene. Preliminary data indicates that fos-tTA mice, which do not encode shEGFP, show no difference in c-Fos expression between transgenic mice and their wildtype littermates, suggesting the overexpression of c-Fos is due to the fos-shEGFP gene and not the fos-tTA gene (Wiltgen lab, unpublished). We think overexpression of c-Fos may be contributing to the differences in baseline intrinsic excitability between the two rounds of experiments described in this chapter.

In CA1, cells with higher baseline activity are more likely than neighboring neurons to be integrated into a neural ensemble at the time of learning and memory formation. Once a part of the engram, those cells maintain that enhanced excitability state for several days. However, it is unclear whether the excitability state of the neurons within the engram are correlated directly to behavioral performance, or if the neurons must simply meet a 'threshold' of excitability for learning and memory formation to take place. We show that engram cells in animals exhibiting high freezing have the highest average firing rate in comparison to neighboring control (non-engram) cells. This difference in firing rate between the engram cells and non-engram cells is absent if the animal does not freeze during CFC training. Overall, the average firing rate of engram cells two days after CFC training is highly correlated to the percent freezing time. It is unknown whether the amount of freezing is driven by the change in excitability state or vice versa, although previous studies would suggest the former. Additionally, we do not know the extent to which intrinsic firing capabilities are altered in engram cells in response to activation by the behavioral task. More rigorous testing involving specific manipulation of excitability state in CA1 engram cells is needed to answer these questions.

Mechanisms of increased intrinsic excitability following learning

Many studies have shown an alteration in AHP amplitude following learning-induced changes in excitability state; however, we were unable to replicate these findings in our system. Because the mAHP and sAHP average amplitudes were not significantly different between GFP^+ and GFP^- neurons, alternative underlying mechanisms must be considered since the learning-induced intrinsic changes are consistent and correlated to the behavioral outcome of the CFC training. There are three promising candidates: the H-, M-, and A-currents, whose conductances would potentially modulate neuronal excitability state. The H-current (I_h) which is mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels and is known to regulate resting membrane potential, neuronal response to hyperpolarization, and synchronous

activity among populations of neurons (Gasselin et al., 2015; He et al., 2014; Lüthi and McCormick, 1998). Previous groups have shown in CA1 pyramidal neurons that I_h changes in an activity-dependent manner and that these changes can alter input resistance, EPSP summation and firing rate (Gasselin et al., 2015). Based on these data, reduction in I_h could mediate the enhanced excitability observed following contextual fear conditioning. The potassium channels KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3) comprise the M-current (I_M), which is a potassium conductance with slow gating kinetics that plays a critical role in determining the responsiveness to synaptic inputs and subthreshold electrical excitability of neurons (Wang et al., 1998). I_M is typically activated at subthreshold potentials near resting membrane potential (around -60mV) and will persist as long as the membrane potential is depolarized because the channel does not inactivate, only slowly deactivates (Halliwell and Adams, 1982; Miceli et al., 2009; Robbins, 2001; Wang et al., 1998). It has been shown that suppression of I_M leads to transient increases in excitability (Brown and Adams, 1980; Delmas and Brown, 2005) and additionally may also contribute to the mAHP and sAHP (Soh et al., 2014; Stocker et al., 1999; Tzingounis and Nicoll, 2008). These data would indicate that the learning-induced increase in intrinsic excitability could be due to decreased I_M, as channel suppression has also been shown to allow for the accumulation of excitatory inputs that leading to repetitive firing or complex spikes (Hu et al., 2007; Yue and Yaari, 2004). Lastly, the A-current (I_A) in hippocampal pyramidal neurons is mediated by A-type voltage-gated potassium channels (Kv1, Kv3, Kv4 and Kv12), and is encoded by multiple Kv subunits (Angelova and Müller, 2009; Cai et al., 2004; Chen and Johnston, 2006; Cooper et al., 1998; Sheng et al., 1992; Zhang et al., 2010). These channels are rapidly activating and inactivate at subthreshold voltages. They also enable repetitive firings at low frequencies (Bean, 2007; Connor and Stevens, 1971a, 1971b). It has been shown that increased expression of A-type channels increases firing rate (Drion et al., 2015; Shibata et al., 2000) due to faster action potential repolarization (Simkin et al., 2015). In addition to the fast afterhyperpolarization (fAHP), A-type channel density can influence action potential (AP) duration (Kim et al., 2005;

Simkin et al., 2015; Storm, 1987; Zhang and McBain, 1995). These data suggest that increased intrinsic excitability could be due to increased I_A, which would facilitate repetitive firing. While some of these conductances were already indirectly tested (Table 1), future experiments involving animals with more robust freezing responses will be helpful to elucidate the underlying mechanism of change in intrinsic excitability.

In summary, our data suggest that CFC training induces changes in intrinsic excitability in hippocampal CA1 pyramidal neurons lasting at least two days after learning. This enhancement in firing rate is highly correlated to the amount of learning, although it is unknown on what temporal scale this change is required in relation to the behavioral training. Previous studies supporting a role for increased excitability in learning have only been demonstrated with chronic manipulations (i.e. overexpression of potassium channels). However, changes in intrinsic excitability are transient in comparison to synaptic changes. Therefore, further studies are needed to demonstrate whether altering the enhanced intrinsic excitability in CA1 neurons during CFC is sufficient to impair proper learning and contextual memory formation.

Figures and Figure Legends

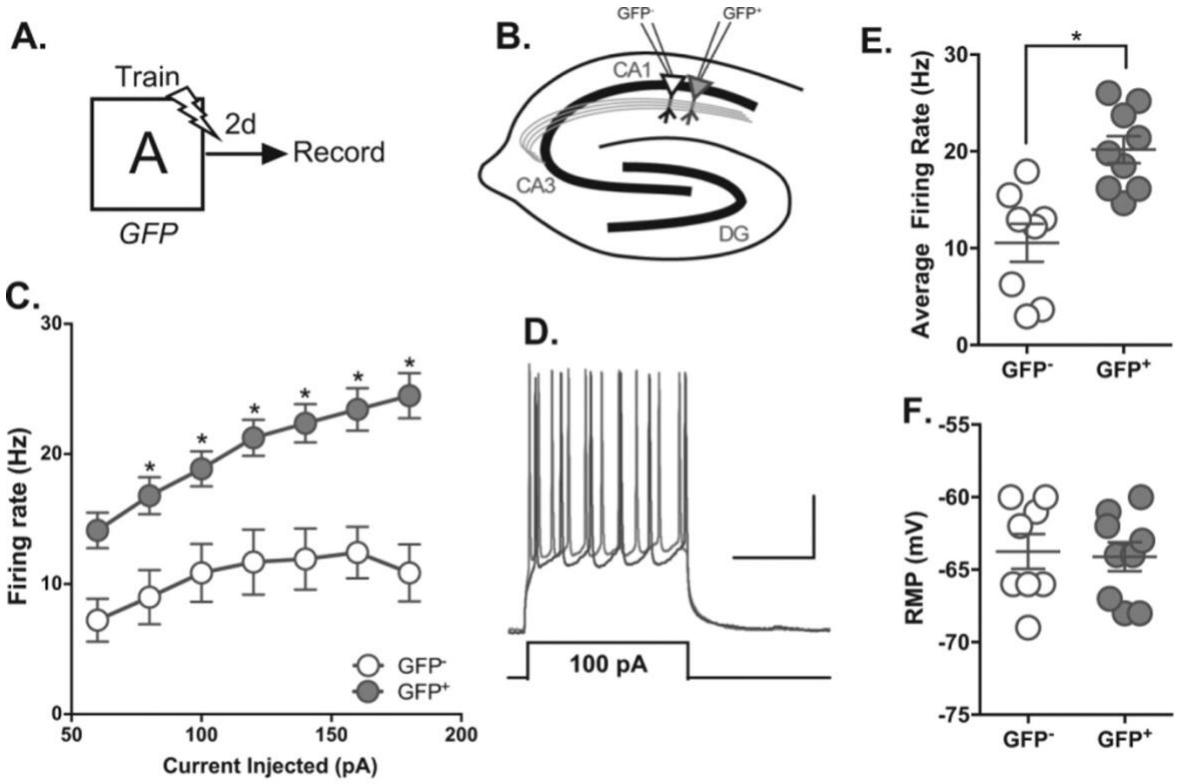

Figure 1. Hippocampal neurons become more excitable after context fear learning. A. Experimental design. Animals were trained and active neurons tagged with GFP. Hippocampal slices were made 2 days later. **B.** Whole-cell patch clamp recordings were obtained from GFP^+ and GFP^- CA1 pyramidal neurons. **C.** Action potential firing rates evoked by depolarizing current injection from GFP^+ and GFP^- CA1 pyramidal neurons recorded from TetTag mice after fear conditioning. Data represent mean ± SEM. A two-way repeated measures ANOVA (group X current step) had a significant effect of the group. The GFP^+ group (n=9 cells, 4 animals) had a higher firing rate per current injection than the GFP^- group (n=8 cells, 5 animals). **D.** Sample traces from GFP^+ (gray) and GFP^- (black) neurons with a 100pA current injection (scale bars: 250ms, 20mV). **E.** Average firing rates from each cell from 100-150pA of injected current. GFP^+ cells (20.2 ± 1.4Hz) had significantly higher firing rates than GFP^- neurons (10.6 ± 2.0Hz). **F.** Resting membrane potential is unchanged between GFP^+ (-64.1 ± 1.0mV) and GFP^- (-63.8 ± 1.2mV) neurons. Data represent mean ± SEM. Significance values were set at $p < 0.05$ (*). Adapted from Crestani et al., 2018.

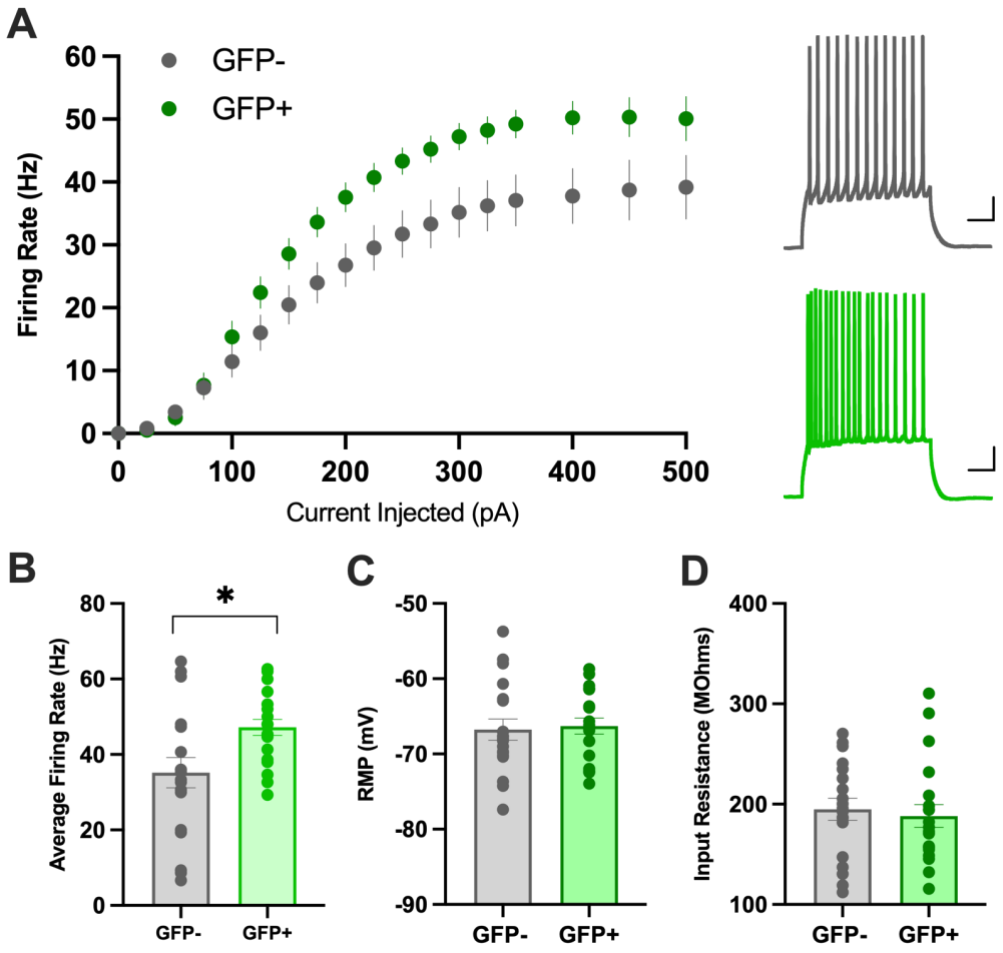

Figure 2. Firing rate is increased in GFP⁺ cells after contextual fear conditioning. A. GFP⁺ neurons (n=14 cells, 3 animals) had a higher firing rate per current injection than the GFP⁻ group (n=13 cells, 3 animals). Sample traces from GFP⁺ (green) and GFP⁻ (gray) neurons in response to a 150pA current injection (scale bars: 100ms, 10mV). **B.** Average firing rates from each cell (300pA of injected current). GFP⁺ cells (47.20Hz ± 2.12) had significantly higher firing rates than GFP⁻ neurons (35.19Hz ± 3.99). **C.** The resting membrane potential is unchanged between GFP⁺ (-66.29mV ± 1.08mV) and GFP⁻ (-66.76mV ± 1.40mV) neurons. **D.** Input resistance is also unchanged between GFP⁺ (188.2 ± 11.47MΩ) and GFP⁻ (194.8 ±11.06MΩ) neurons. Data represent mean ± SEM. (*, p=0.0119).

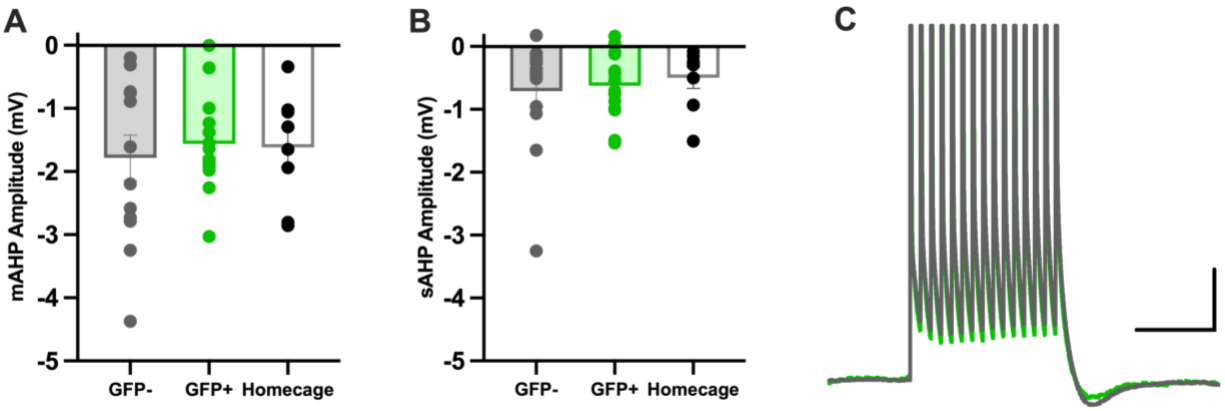

Figure 3. Increased excitability is not due to changed afterhyperpolarization. **A.** Medium afterhyperpolarization (AHP) is unchanged between GFP^- (-1.78mV ± 0.36), GFP^+ (-1.56mV ± 0.20) and homecage control (-1.62mV ± 0.31) neurons. **B.** Slow AHP is also similar in GFP^- (-0.708mV ± 0.25), GFP^+ (-0.621mV ± 0.14), and homecage (-0.496mV ± 0.17) control CA1 pyramidal neurons. Data represent mean ± SEM. **C.** Sample traces from GFP^-, and GFP^+ neurons in response to a 100pA current injection (scale bars: 200ms, 5mV).

Table 1: Intrinsic Excitability in Hippocampal CA1 Pyramidal Neurons				
Property	GFP- n=19	GFP+ n=20	Student's t-test (unpaired)	P value
RMP (mV)	-66.76 ± 1.402 [-77.4 to -53.7]	-66.29 ± 1.08 [-73.9 to -58.7]	t(37)=0.2672	0.7908
R_{input} (MΩ)	194.8 ± 11.06 [112 to 270]	188.2 ± 11.47 [115 to 310]	t(37)=0.4181	0.6783
Rheobase (pA)	75.25 ± 12.23 [12.0 to 192]	57.3 ± 7.05 [7.33 to 130]	t(37)=1.283	0.2076
Sag (mV)[A]	0.1403 ± 0.009 [0.050 to 0.198]	0.1414 ± 0.010 [0.053 to 0.240]	t(37)=0.0740	0.9414
Afterdepolarization (mV)	7.883 ± 0.7397 [0.676 to 14.89]	7.482 ± 0.7461 [0.052 to 11.8]	t(37)=0.3812	0.7052
AP Threshold (mV)[A]	-54.66 ± 1.774 [-65.1 to-34.1]	-53.51 ± 1.338 [-62.8 to -44.7]	t(37)=0.5235	0.6037
AP Peak (mV)	139.7 ± 3.859 [117 to 179]	139.3 ± 3.214 [116 to 177]	t(37)=0.0835	0.9339
AP Half-width (ms)	1.514 ± 0.06258 [1.11 to 2.00]	1.534 ± 0.05166 [1.24 to 1.97]	t(37)=0.2385	0.8128
Mean ± SEM [range] * Significant at p < 0.05 [A] junction potential not adjusted				

Table 1. Intrinsic excitability properties of hippocampal pyramidal neurons. No significant difference was found between GFP^- and GFP^+ cells in any of the listed neuronal properties. Data represent mean ± SEM, significance was defined as $p < 0.05$.

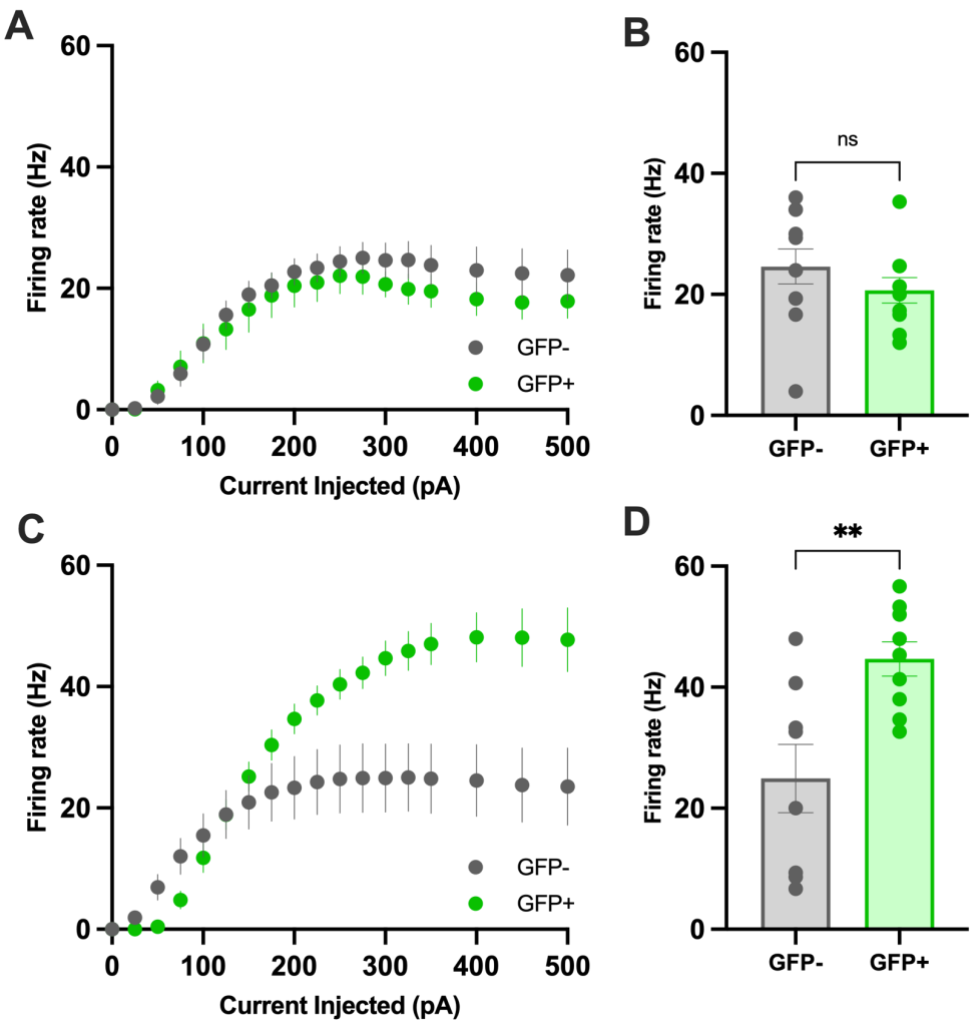

Figure 4. Variability due to difference in percent freezing. A. Animals exhibiting freezing less than 10% during CFC training show no significant excitability increase in GFP^+ neurons (n=10 cells, 2 animals) compared to GFP^- neurons (n=11 cells, 2 animals). **B.** Average firing rates from each cell in response to 300pA current injection. There was no observed difference in intrinsic firing capabilities between GFP^+ cells (20.67Hz ± 2.12) and GFP^- neurons (24.61Hz ± 2.86). **C.** Animals exhibiting freezing greater than 55% during CFC training show increased excitability in GFP^+ neurons (n=9 cells, 2 animals) compared to GFP^- neurons (n=8 cells, 2 animals). **D.** Average firing rates from each cell with a 300pA current injection. Under these conditions, GFP^+ neurons (44.67Hz ± 2.85) exhibited a significantly higher firing rate than GFP^- cells (24.92Hz ± 5.63). Data represent mean ± SEM. (**, p=0.0055).

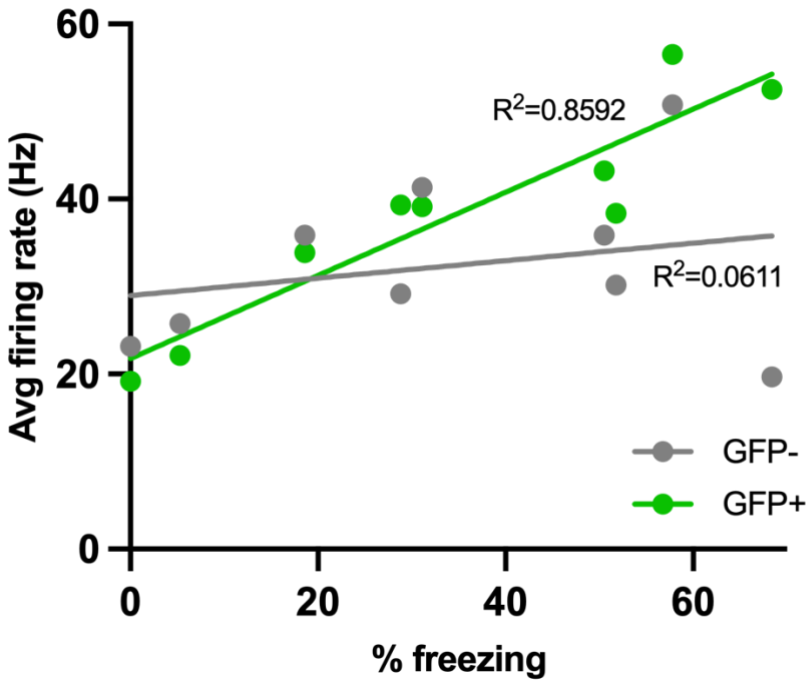

Figure 5. Increased intrinsic excitability is highly correlated to increased percent freezing. Mean firing rate of GFP^+ and GFP^- neurons for each animal (n=9 animals) in response to a 300pA current compared to the percent freezing exhibited during CFC training. Enhanced average firing rate seen in GFP^+ cells are strongly correlated to percent freezing time during CFC training (R^2=0.8592). Variability in GFP^- neurons is not correlated to changes in percent freezing (R^2=0.0611).

References

Angelova, P.R., Müller, W.S., 2009. Arachidonic acid potently inhibits both postsynaptic-type Kv4.2 and presynaptic-type Kv1.4 IA potassium channels. European Journal of Neuroscience 29, 1943–1950. https://doi.org/10.1111/j.1460-9568.2009.06737.x

Bannerman, D.M., Good, M.A., Butcher, S.P., Ramsay, M., Morris, R.G.M., 1995. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature 378, 182–186. https://doi.org/10.1038/378182a0

Bean, B.P., 2007. The action potential in mammalian central neurons. Nature Reviews Neuroscience 8, 451–465. https://doi.org/10.1038/nrn2148

Brown, D.A., Adams, P.R., 1980. Muscarinic suppression of a novel voltage-sensitvie K+ current in a vertebrate neuron. Nature 283, 673–676. https://doi.org/10.2307/814290

Cai, D.J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., Wei, B., Veshkini, M., La-Vu, M., Lou, J., Flores, S.E., Kim, I., Sano, Y., Zhou, M., Baumgaertel, K., Lavi, A., Kamata, M., Tuszynski, M., Mayford, M., Golshani, P., Silva, A.J., 2016. A shared neural ensemble links distinct contextual memories encoded close in time. Nature 534, 115–118. https://doi.org/10.1038/nature17955

Cai, X., Liang, C.W., Muralidharan, S., Kao, J.P.Y., Tang, C.-M., Thompson, S.M., 2004. Unique Roles of SK and Kv4.2 Potassium Channels in Dendritic Integration influence of intrinsic voltage-dependent channels comes from direct dendritic recordings from apical trunk dendrites within ف052 □m of the cell body. These studies. Neuron 44, 351–364.

Chandra, N., Barkai, E., 2018. A non-synaptic mechanism of complex learning: Modulation of intrinsic neuronal excitability. Neurobiology of Learning and Memory 154, 30–36. https://doi.org/10.1016/j.nlm.2017.11.015

Chen, X., Johnston, D., 2006. Voltage-gated ion channels in dendrites of hippocampal pyramidal neurons. Pflugers Archiv European Journal of Physiology 453, 397–401. https://doi.org/10.1007/s00424-006-0097-y

Connor, J.A., Stevens, C.F., 1971a. Inward and Delayed Outward Membrane Currents in Isolated Neuronal Somata under Voltage Clamp. Journal of Physiology 213, 1–19. https://doi.org/10.1152/jn.1986.56.6.1739

Connor, J.A., Stevens, C.F., 1971b. Voltage-clamp studies of transient outward membrane current in gastropos neural somata. The Journal of Physiology 213, 21–30. https://doi.org/10.1113/jphysiol.1982.sp014230

Cooper, E.C., Milroy, A., Jan, Y.N., Jan, L.Y., Lowenstein, D.H., 1998. Presynaptic Localization of Kv1.4-Containing A-Type Potassium Channels Near Excitatory Synapses in the Hippocampus. The Journal of Neuroscience 18, 965–974. https://doi.org/10.1523/jneurosci.18-03-00965.1998

Crestani, A.P., Krueger, J.N., Barragan, E.V., Nakazawa, Y., Nemes, S.E., Quillfeldt, J.A., Gray, J.A., Wiltgen, B.J., 2018. Metaplasticity contributes to memory formation in the hippocampus. Neuropsychopharmacology 44, 408–414. https://doi.org/10.1038/s41386-018-0096-7

Delmas, P., Brown, D.A., 2005. Pathways modulating neural KCNQ/M (Kv7) potassium channels. Nature Reviews Neuroscience 6, 850–862. https://doi.org/10.1038/nrn1785

Disterhoft, J.F., Coulter, D.A., Alkon, D.L., 1986. Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro. Proceedings of the National Academy of Sciences 83, 2733–2737. https://doi.org/10.1073/pnas.83.8.2733

Disterhoft, J.F., Weiss, C., Burgdorf, J., Oh, M.M., McKay, B.M., Kroes, R.A., Galvez, R., Moskal, J.R., Adelman, J.P., 2012. Increasing SK2 channel activity impairs associative learning. Journal of Neurophysiology 108, 863–870. https://doi.org/10.1152/jn.00025.2012

Drion, G., O'Leary, T., Marder, E., 2015. Ion channel degeneracy enables robust and tunable neuronal firing rates. Proceedings of the National Academy of Sciences 112, E5361–E5370. https://doi.org/10.1073/pnas.1516400112

Gasselin, C., Inglebert, Y., Debanne, D., 2015. Homeostatic regulation of h-conductance controls intrinsic excitability and stabilizes the threshold for synaptic modification in CA1 neurons. Journal of Physiology 593, 4855–4869. https://doi.org/10.1113/JP271369

Gossen, J.A., Martus, H.J., Wei, J.Y., Vijg, J., 1995. Spontaneous and X-ray-induced deletion mutations in a LacZ plasmid-based transgenic mouse model. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 331, 89–97. https://doi.org/10.1016/0027-5107(95)00055-N

Halliwell, J.V., Adams, P.R., 1982. Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. Brain Research 250, 71–92. https://doi.org/10.1016/0006-8993(82)90954-4

He, C., Chen, F., Li, B., Hu, Z., 2014. Neurophysiology of HCN channels: From cellular functions to multiple regulations. Progress in Neurobiology 112, 1–23. https://doi.org/10.1016/j.pneurobio.2013.10.001

Hu, H., Vervaeke, K., Storm, J.F., 2007. M-Channels (Kv7/KCNQ Channels) That Regulate Synaptic Integration, Excitability, and Spike Pattern of CA1 Pyramidal Cells Are Located in the Perisomatic Region. Journal of Neuroscience 27, 1853–1867. https://doi.org/10.1523/jneurosci.4463-06.2007

Kaczorowski, C.C., Disterhoft, J., Spruston, N., 2007. Stability and plasticity of intrinsic membrane properties in hippocampal CA1 pyramidal neurons: Effects of internal anions. Journal of Physiology 578, 799–818. https://doi.org/10.1113/jphysiol.2006.124586

Kandel, E.R., 2009. The Biology of Memory: A Forty-Year Perspective. Journal of Neuroscience 29, 12748–12756. https://doi.org/10.1523/JNEUROSCI.3958-09.2009

Kim, J., Wei, D.S., Hoffman, D.A., 2005. Kv4 potassium channel subunits control action potential repolarization and frequency-dependent broadening in rat hippocampal CA1 pyramidal neurones. Journal of Physiology 569, 41–57. https://doi.org/10.1113/jphysiol.2005.095042

Langille, J.J., Brown, R.E., 2018. The Synaptic Theory of Memory: A Historical Survey and Reconciliation of Recent Opposition. Front. Syst. Neurosci. 12, 52. https://doi.org/10.3389/fnsys.2018.00052

Liu, X., Ramirez, S., Pang, P.T., Puryear, C.B., Govindarajan, A., Deisseroth, K., Tonegawa, S., 2012. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484, 381–385. https://doi.org/10.1038/nature11028

Lüthi, A., McCormick, D.A., 1998. H-current: Properties of a neuronal and network pacemaker. Neuron 21, 9–12. https://doi.org/10.1016/S0896-6273(00)80509-7

Matthews, E.A., Weible, A.P., Shah, S., Disterhoft, J.F., 2008. The BK-mediated fAHP is modulated by learning a hippocampus-dependent task. Proceedings of the National Academy of Sciences 105, 15154–15159. https://doi.org/10.1073/pnas.0805855105

Mayford, M., Mayford, M., Bach, M.E., Bach, M.E., Huang, Y.Y., Huang, Y.Y., Wang, L., Wang, L., Hawkins, R.D., Hawkins, R.D., Kandel, E.R., Kandel, E.R., 1996. Control of memory formation through regulated expression of a CaMKII transgene. Science 274, 1678–1683.

McKay, B.M., Matthews, E.A., Oliveira, F.A., Disterhoft, J.F., 2009. Intrinsic Neuronal Excitability Is Reversibly Altered by a Single Experience in Fear Conditioning. Journal of Neurophysiology 102, 2763–2770. https://doi.org/10.1152/jn.00347.2009

McKay, B.M., Oh, M.M., Disterhoft, J.F., 2013. Learning Increases Intrinsic Excitability of Hippocampal Interneurons. Journal of Neuroscience 33, 5499–5506. https://doi.org/10.1523/jneurosci.4068-12.2013

Miceli, F., Cilio, M.R., Taglialatela, M., Bezanilla, F., 2009. Gating currents from neuronal KV7.4 channels: General features and correlation with the ionic conductance. Channels 3, 1–10. https://doi.org/10.4161/chan.3.4.9477

Morris, R.G.M., Anderson, E., Lynch, G.S., Baudry, M., 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319, 774–776. https://doi.org/10.1038/319774a0

Morris, R.G.M., Davis, S., Butcher, S.P., 1990. Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? Philosophical Transactions of the Royal Society B: Biological Sciences 329, 187–204.

Moyer Jr., J.R., Thompson, L.T., Disterhoft, J.F., 1996. Trace Eyeblink Conditioning Increases CA1 Excitability in a Transient and Learning-Specific Manner. The Journal of Neuroscience 16, 5536–5546. https://doi.org/10.1523/jneurosci.16-17-05536.1996

Moyer, J.R., Power, J.M., Thompson, L.T., Disterhoft, J.F., 2000. Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 5476–82.

Nakazawa, Y., Pevzner, A., Tanaka, K.Z., Wiltgen, B.J., 2016. Memory retrieval along the proximodistal axis of CA1 37, 784–790. https://doi.org/10.1183/09031936.00063810.The

Oh, M.M., Disterhoft, J.F., 2015. Increased excitability of both principle neurons and interneurons during associative learning. Neuroscientist 21, 372–384. https://doi.org/10.1586/14737175.2015.1028369.Focused

Oh, M.M., McKay, B.M., Power, J.M., Disterhoft, J.F., 2009. Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. Proceedings of the National Academy of Sciences 106, 1620–1625. https://doi.org/10.1073/pnas.0807708106

Pignatelli, M., Ryan, T.J., Roy, D.S., Lovett, C., Smith, L.M., Muralidhar, S., Tonegawa, S., 2019. Engram Cell Excitability State Determines the Efficacy of Memory Retrieval. Neuron 101, 1–11. https://doi.org/10.1016/j.neuron.2018.11.029

Quinlan, E.M., Lebel, D., Brosh, I., Barkai, E., 2004. A Molecular Mechanism for Stabilization of Learning-Induced Synaptic Modifications. Neuron 41, 185–192. https://doi.org/10.1016/S0896-6273(03)00874-2

Rabinak, C.A., Zimmerman, J.M., Chang, C., Orsini, C.A., 2008. Bidirectional Changes in the Intrinsic Excitabiity of Infralimbic Neuorns Reflect a Possible Regulatory Role in the

Acquisition and Extinction of Pavlovian Conditioned Fear. The Journal of Neuroscience 28, 7245–7247. https://doi.org/10.1523/JNEUROSCI.2130-08.2008
Reijmers, L.G., Mayford, M., 2009. Genetic control of active neural circuits. Frontiers in molecular neuroscience 2, 1–8. https://doi.org/10.3389/neuro.02.027
Reijmers, L.G., Perkins, B.L., Matsuo, N., Mayford, M., 2007. Localization of a stable neural correlate of associative memory. Science 317, 1230–1234. https://doi.org/10.1126/science.1143839
Robbins, J., 2001. KCNQ potassium channels: physiology, pathophysiology, and pharmacology. Pharmacology & Therapeutics 90, 1–19.
Sakimura, K., Kutsuwada, T., Manabet, T., Takayama, C., Kushiya, E., Inoue, Y., Suglyamat, H., Mishina, M., 1995. Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor t1 subunit 373, 5.
Sanders, M.J., Fanselow, M.S., 2003. Pre-training prevents context fear conditioning deficits produced by hippocampal NMDA receptor blockade. Neurobiology of Learning and Memory 80, 123–129. https://doi.org/10.1016/S1074-7427(03)00040-6
Saucier, D., Cain, D.P., 1995. Spatial learning without NMDA receptor-dependent long-term potentiation. Nature 378, 186–189. https://doi.org/10.1038/378186a0
Sheng, M., Tsaur, M.L., Nung Jan, Y., Yeh Jan, L., 1992. Subcellular segregation of two A-type K+ channel proteins in rat central neurons. Neuron 9, 271–284. https://doi.org/10.1016/0896-6273(92)90166-B
Shibata, R., Nakahira, K., Shibasaki, K., Wakazono, Y., Imoto, K., Ikenaka, K., 2000. A-type K+ current mediated by the Kv4 channel regulates the generation of action potential in developing cerebellar granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 4145–55.
Simkin, D., Hattori, S., Ybarra, N., Musial, T.F., Buss, E.W., Richter, H., Oh, M.M., Nicholson, D.A., Disterhoft, J.F., 2015. Aging-Related Hyperexcitability in CA3 Pyramidal Neurons Is Mediated by Enhanced A-Type K+ Channel Function and Expression. Journal of Neuroscience 35, 13206–13218. https://doi.org/10.1523/jneurosci.0193-15.2015
Smeyne, R.J., Curran, T., Morgan, J.I., 1992. Temporal and spatial expression of a fos-lacZ transgene in the developing nervous system. Molecular Brain Research 16, 158–162. https://doi.org/10.1016/0169-328X(92)90206-Q
Soh, H., Pant, R., LoTurco, J.J., Tzingounis, A.V., 2014. Conditional Deletions of Epilepsy-Associated KCNQ2 and KCNQ3 Channels from Cerebral Cortex Cause Differential Effects on Neuronal Excitability. Journal of Neuroscience 34, 5311–5321. https://doi.org/10.1523/jneurosci.3919-13.2014
Stocker, M., Krause, M., Edarzani, P.P., 1999. An apamin-sensitive Ca2+-activated K+ current in hippocampal. Proceedings of the National Academy of Sciences 96, 4662–4667.
Storm, J.F., 1987. Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. Journal of Physiology 385, 733–759. https://doi.org/10.1016/0032-0633(64)90077-7
Tanaka, K.Z., Pevzner, A., Hamidi, A.B., Nakazawa, Y., Graham, J., Wiltgen, B.J., 2014. Cortical Representations Are Reinstated by the Hippocampus during Memory Retrieval. Neuron 84, 347–354. https://doi.org/10.1016/j.neuron.2014.09.037

Tayler, K.K., Lowry, E., Tanaka, K., Levy, B., Reijmers, L., Mayford, M., Wiltgen, B.J., 2011. Characterization of NMDAR-Independent Learning in the Hippocampus. Frontiers in Behavioral Neuroscience 5, 1–12. https://doi.org/10.3389/fnbeh.2011.00028

Tayler, K.K., Tanaka, K.Z., Reijmers, L.G., Wiltgen, B.J., 2013. Reactivation of neural ensembles during the retrieval of recent and remote memory. Current Biology 23, 99–106. https://doi.org/10.1016/j.cub.2012.11.019

Ting, J.T., Lee, B.R., Chong, P., Soler-Llavina, G., Cobbs, C., Koch, C., Zeng, H., Lein, E., 2018. Preparation of Acute Brain Slices Using an Optimized N-Methyl-D-glucamine Protective Recovery Method. JoVE 53825. https://doi.org/10.3791/53825

Titley, H.K., Brunel, N., Hansel, C., 2017. Toward a Neurocentric View of Learning. Neuron 95, 19–32. https://doi.org/10.1016/j.neuron.2017.05.021

Tzingounis, A.V., Nicoll, R.A., 2008. Contribution of KCNQ2 and KCNQ3 to the medium and slow afterhyperpolarization currents. Proceedings of the National Academy of Sciences 105, 19974–19979. https://doi.org/10.1073/pnas.0810535105

Wang, H.S., Pan, Z.M., Shi, W.M., Brown, B.S., Wymore, R.S., Cohen, I.S., Dixon, J.E., McKinnon, D., 1998. KCNQ2 and KCNQ3 potassium channel subunits. Science 282, 1890–1893.

Wiltgen, B.J., Royle, G.A., Gray, E.E., Abdipranoto, A., Thangthaeng, N., Jacobs, N., Saab, F., Tonegawa, S., Heinemann, S.F., O'Dell, T.J., Fanselow, M.S., Vissel, B., 2010. A Role for Calcium-Permeable AMPA Receptors in Synaptic Plasticity and Learning. PLoS ONE 5, e12818. https://doi.org/10.1371/journal.pone.0012818

Wiltgen, B.J., Wood, A.N., Levy, B., 2011. The cellular mechanisms of memory are modified by experience. Learning & Memory 18, 747–750. https://doi.org/10.1101/lm.024026.111

Yiu, A.P., Mercaldo, V., Yan, C., Richards, B., Rashid, A.J., Hsiang, H.-L.L., Pressey, J., Mahadevan, V., Tran, M.M., Kushner, S.A., Woodin, M.A., Frankland, P.W., Josselyn, S.A., 2014. Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training. Neuron 83, 722–735. https://doi.org/10.1016/j.neuron.2014.07.017

Yue, C., Yaari, Y., 2004. KCNQ/M Channels Control Spike Afterdepolarization and Burst Generation in Hippocampal Neurons. Journal of Neuroscience 24, 4614–4624. https://doi.org/10.1523/jneurosci.0765-04.2004

Zhang, L., McBain, C.J., 1995. Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurones. The Journal of Physiology 488, 661–672. https://doi.org/10.1113/jphysiol.1995.sp020998

Zhang, X., Bertaso, F., Yoo, J.W., Baumgärtel, K., Clancy, S.M., Lee, V., Cienfuegos, C., Wilmot, C., Avis, J., Hunyh, T., Daguia, C., Schmedt, C., Noebels, J., Jegla, T., 2010. Deletion of the potassium channel Kv12.2 causes hippocampal hyperexcitability and epilepsy. Nature Neuroscience 13, 1056–1058. https://doi.org/10.1038/nn.2610

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., Silva, A.J., 2009. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12, 1438–1443. https://doi.org/10.1038/nn.2405

Chapter 4

General Discussion

At this point, it bears repeating that memory is central to who we are. It is a dynamic, not static, process that is employed daily and for the entirely of our lives. In the past century, advancement in techniques and equipment have led to a surplus of findings in the learning and memory field. Yet, many open questions still remain regarding the intricacies of synaptic and intrinsic plasticity and their relative contributions to learning and memory. As the fundamental of memory, it is imperative to thoroughly understand the not only the proper functioning of these processes, but also the consequences of their dysfunction. When conducting research at the cellular level, it is quite easy for one to become lost in the details and forget just how impactful, these (at least at the level of the organism) seemingly insignificant changes can be. To close out this dissertation, I will leave you with some of the larger implications of my work.

Implications of D-serine Regulation of Non-ionotropic LTD

Ionotropic glutamate receptors (iGluRs) are abundant throughout the CNS and are responsible for most of the excitatory synaptic transmission that underlies proper brain function. One member of this family of receptors stands out among the others for its involvement in synaptic development, function, and plasticity. Indeed, as a master regulator of synaptic plasticity, the NMDA receptor has a unique ability to control information storage within the brain. Adding to their intrigue, NMDARs are equipped with distinctive properties, that no other iGluRs possess high Ca^{2+} permeability, voltage-dependent Mg^{2+} block of their channel pore, slow gating kinetics, and lastly an obligatory requirement for concurrent binding of agonist (glutamate) and co-agonist (glycine and D-serine). Indeed, NMDARs are the only known CNS receptors involved in intracellular signaling that have exhibit this dual agonist dependence. Despite the vast amounts of work characterizing the structure and function of NMDARs, the fundamental role of co-agonism and why two endogenous ligands of the co-agonist site exist are still unknown.

NMDARs subunits' sequences and functions are highly homologous among organisms containing a nervous system. For example, residues forming the GluN1 ligand binding pocket are strongly conserved from mammalian to cnidarian (a phylum of aquatic animals that includes jellyfish and sea anemones) GluN1, suggesting glycine co-agonism has always been an important property of NMDARs (Stroebel and Paoletti, 2021). Glycine selectivity at the GluN1 co-agonist binding site is largely determined by molecular size and steric hindrance – put simply, glutamate just does not fit at that binding site (Mayer, 2006). Robust D-serine binding at this site is most likely to due to its small size and reoriented stereochemistry, as L-amino acids larger than glycine would generate a steric clash with a serine residue within the binding site (Furukawa et al., 2005). Both co-agonists are present in the extracellular space – albeit at different levels, total brain D-serine concentration is only around 40% of glycine levels. In spite or perhaps because of the discrepancy in endogenous concentrations, D-serine is thought to be a more potent and efficient agonist, overall affinities of the NMDAR for glycine and D-serine are similar (EC_{50}= ~0.1-1μM) (Priestley and Kemp, 1994). Therefore, in terms of receptor activation, the two co-agonists are seemingly equivalent.

So, what is the reason for having two biologically available, seemingly redundant co-agonists, each with their own modes of regulation? It has been suggested that in the hippocampus, glycine acts as the endogenous co-agonist for GluN2B-containing NMDARS and D-serine for those with GluN2A (Papouin et al., 2012). However this may be an oversimplification, as prior work has shown that synaptic and extrasynaptic NMDARs are not pure populations of either GluN2B- or GluN2A-containing receptors (Fossat et al., 2012; Panatier et al., 2006) and relative affinities for the co-agonists are similar for both GluN2B- or GluN2A-containing receptors. Additionally, this does not take into account how this co-agonist preference would affect triheteromeric NMDARs (GluN1/GluN2A/GluN2B) which are thought to account for over 50% of native NMDARs in the hippocampus and cortex (Al-Hallaq et al., 2007; Sheng et al., 1994; Tovar

et al., 2013). What we can glean from this evidence is that there are instances of differential action of the two co-agonists, despite their functional overlap. We do know that the identity of the predominant co-agonist of the co-agonist site varies by developmental age, brain region, and receptor localization (Balu and Coyle, 2015; Le Bail et al., 2015; Mothet et al., 2015; Papouin et al., 2012). This heterogeneity within the brain suggests that roles unique to each co-agonist do exist.

I have shown evidence for one such role, that of D-serine as a determinant of non-ionotropic LTD. While reduction of endogenous amounts of both co-agonists in the absence of ion flux is sufficient to cause LTD, I found that application of D-serine, but not glycine, blocked non-ionotropic synaptic depression. This may be important in relation to the changing identity of the endogenous co-agonist at Schaffer collateral–CA1 synapses throughout development (Le Bail et al., 2015; Mothet et al., 2015). In early development, when glycine is the chief ligand of the NMDAR co-agonist site (Ferreira et al., 2017), non-ionotropic signaling will be facilitated. Some receptors may fully activate with both ligands bound and others may receive insufficient depolarization even with both glutamate and glycine bound. We now know that this second condition still results in robust NMDAR signaling even though ion flux through the channel is absent, triggering both structural and functional niLTD and even receptor endocytosis. In contrast, in adulthood, when D-serine is the predominating co-agonist at synaptic NMDARs (Le Bail et al., 2015; Papouin et al., 2012), niLTD and non-ionotropic NMDAR signaling will be blocked. Whether ion flux through the NMDAR occurs or not, we have shown that in the presence of D-serine, niLTD will not occur (Figure 1).

The discovery of this novel regulatory role for D-serine has brought to light many new questions and therefore future directions for this research. Continuation of this project will include the investigation of the mechanism by which D-serine blocks niLTD. It has been shown that D-serine appears to cause a conformational movement in the GluN1 CTD that is distinct from the

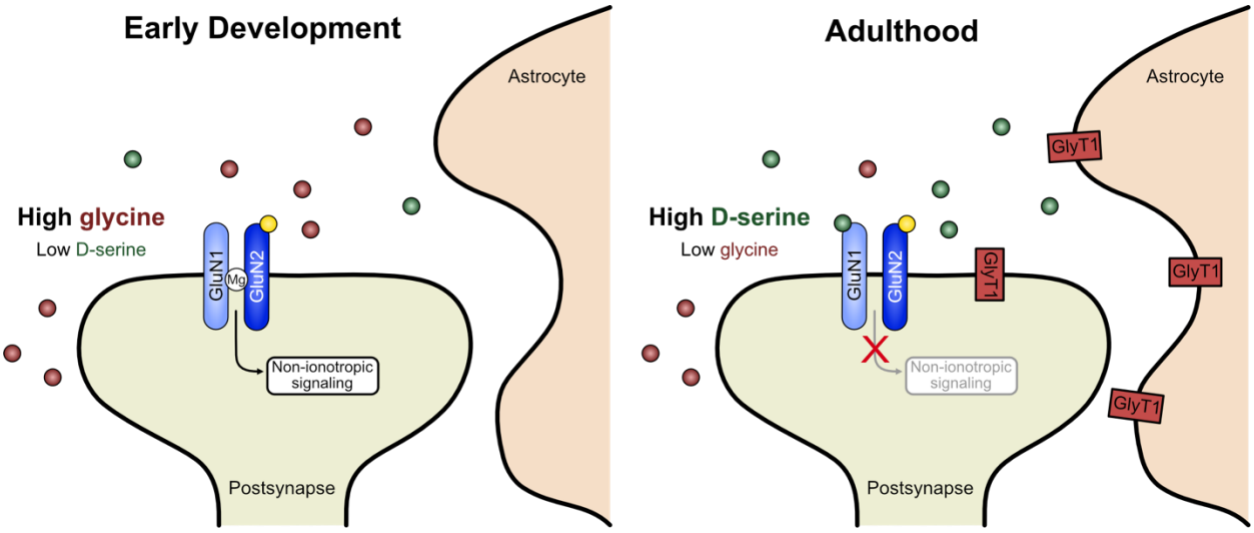

Figure 1. Model of D-serine effect on non-ionotropic signaling throughout development. At prototypical Schaffer collateral–CA1 synapses during early postnatal development, glycine is the principal ligand of the co-agonist site of synaptic NMDARs. Glutamate or glutamate and glycine binding to the receptor will result in non-ionotropic signaling and niLTD. During adulthood, synaptic glycine concentrations are limited by the expression of GlyT1 glycine transporters primarily on astrocytes surrounding the synaptic cleft (Danysz and Parsons, 1998), and D-serine becomes the primary endogenous co-agonist for NMDARs at forebrain glutamatergic synapses. This increased D-serine then limits niLTD and spine shrinkage.

conformational changes induced by NMDA during niLTD (Aow et al., 2015; Ferreira et al., 2017). Interestingly, glycine binding does not induce these conformation changes in the GluN1 CTD. Additionally, D-serine reduces the surface diffusion of GluN2B-containing NMDARs (Ferreira et al., 2017). From these observations, two potential mechanisms for the D-serine mediated block of niLTD arise: (1) the unique conformational change of the GluN1-CTD induced by D-serine binding may disrupt a key protein-protein interaction required for triggering niLTD, or (2) D-serine binding may prevent some crucial diffusion of GluN2B-containing NMDARs at synapses that is required for niLTD induction. (Yashiro and Philpot, 2008). Further experiments are required to

determine whether these mechanisms underlie the D-serine regulation of niLTD we observed, and it will be interesting to see if other forms of non-ionotropic signaling from NMDARs are also inhibited by D-serine.

Another avenue of study still open following this project is the elucidation of the intracellular molecules involved downstream of the NMDAR in functional niLTD. The molecular mechanisms of structural niLTD and how they underlie spine shrinkage have been very well characterized (Stein et al., 2020). However, only one such protein has been identified in functional niLTD: p38 MAPK whose phosphorylation is necessary for non-ionotropic NMDAR signaling (Nabavi et al., 2013). Indeed, the processes that underlie functional niLTD are still poorly understood and necessitate further exploration. These future questions are indeed critical and may take several years before they are fully answered. However, my current finding as it is already has many physiological implications considering that altered D-serine levels play a role in age-related decline in cognitive function, wakefulness/sleep states, and schizophrenia.

Age-related changes in D-serine levels affect cognitive function

Abnormally low levels of D-serine are associated with impairments in functional plasticity and deficits in memory (Billard, 2008). D-serine levels fluctuate with age, with an increase in D-serine early in development and a gradual reduction throughout adulthood (Billard, 2015; Potier et al., 2010). Aging is very normal part of life and with it comes steady decline in cognitive function. Age is one of the main risk factors for Alzheimer's disease (AD), a devastating and progressive neurological disorder, but AD is not part of the aging process. AD goes beyond normal age-related cognitive decline and is associated with advanced memory loss and cognitive impairment, as well as a host of other symptoms like aggression and anxiety (Huang et al., 2012). Aggregation of the ß-amyloid (Aß) peptide is a typical pathological feature of AD. Its precursor, amyloid precursor protein (APP), is involved in structural plasticity of dendritic spines and also plays a role in

maintaining extracellular D-serine levels (Orzylowski et al., 2021). APP knockout (APP-KO) mice display impaired synaptic plasticity and cognitive function; they also exhibit increased total D-serine but decreased extracellular D-serine. Application of exogenous D-serine restored extracellular D-serine levels and plasticity capability, as well as rescued cognitive deficits (Zou et al., 2016).

Increasing production of Aß causes synaptic depression, spine loss, and a reduced capability to undergo synaptic plasticity (Hsieh et al., 2006; Kamenetz et al., 2003; Lacor et al., 2007; Shankar et al., 2007; Texidó et al., 2011; Ting et al., 2007). Of particular interest to my work is the Aß-induced synaptic depression which occurs in an ion flux independent manner and has also been shown to selectively reduce currents from GluN2B-containing receptors (Kessels et al., 2013). Interestingly, D-serine binding has been shown to regulate NMDAR trafficking by limiting surface dynamics of GluN2B-containing NMDARs (Ferreira et al., 2017). Together, this data suggests Aß dysfunction underlies the reduced D-serine levels seen in AD models and patients and mechanistically may be depriving synapses of a means to stabilize GluN2B-receptors. This process could potentially occur in a non-ionotropic manner, since evidence of ion flux independent endocytosis of NMDARs has also been shown (Vissel et al., 2001)

D-serine availability fluctuates throughout wakefulness and sleep

Aside from development variations in D-serine levels, changes in D-serine availability also occur over a 24 hour period. Perhaps surprisingly, these daily oscillations are not driven by circadian rhythms. Rather, they are mediated by wakefulness state such that concentrations are low (~0.5µM) during rest and high (3µM) during wakefulness – all concentrations within the range of NMDAR co-agonist sensitivity (Paoletti et al., 2013; Papouin et al., 2017). The degree of co-agonist binding site occupancy directly determines the ability of glutamate to activate the NMDAR during synaptic transmission (Clements, 1996; Kleckner and Dingledine, 1988). Therefore, D-

serine availability is the limiting factor for NMDAR activation. During wakefulness, high D-serine levels may serve to promote the widespread LTP that occurs with the sensory overload of being awake and freely moving through an environment while suppressing non-ionotropic LTD. In contrast, during rest, low D-serine levels may promote synaptic downscaling, or other LTD-like processes. Homeostatic downscaling of synapses and elimination of weak connections are some of the central mechanisms of memory processing during sleep (Gais and Schönauer, 2017; Tononi and Cirelli, 2014). Additionally, there evidence that downregulation of D-serine release is suggested to weaken the effect of glutamatergic stimulation of the NMDAR such that only LTD can be induced, not LTP (Bains and Oliet, 2007). Therefore, during sleep when lower levels of D-serine are present, non-ionotropic LTD is permitted to scale down less critical synapses and acts as a mechanism of maintaining information storage.

Reduced D-serine levels as an underlying cause of schizophrenia

A number of human mutations commonly associated with schizophrenia point to a comprised function of the NMDAR co-agonist site (Balu et al., 2018; Balu and Coyle, 2015; Javitt, 2015; Moghaddam and Javitt, 2012). Impaired synthesis, availability, and binding of D-serine are all hallmarks of schizophrenia. Decreased D-serine and elevated levels of kynurenic acid – an endogenous NMDAR antagonist – may work in conjunction to bias the overall direction of synaptic plasticity toward synapse weakening, resulting in overpruning of synapses. This is consistent with the global reduction of dendritic spines that is exhibited in schizophrenia. Many of these schizophrenia-like symptoms are recapitulated in the SR-knockout (SRKO) mouse. SRKO mice are characterized by reduced LTP (Basu et al., 2009) and LTP-associated spine growth, increased number of synaptic NMDARs, and enhanced non-ionotropic NMDAR signaling. Together, these factors shift the synaptic modification threshold toward spine shrinkage (Park et al., Biorxiv). Additionally, the reduced D-serine levels in schizophrenia give rise to NMDAR

hypofunction which may lead to disinhibition of excitatory neurons; GABAergic disfunction underlies the altered E/I balance seen in these mice (Jami et al., 2021; Ploux et al., 2020; Steullet et al., 2017). Increased activity of excitatory neurons may lead to glutamate spillover at dendritic spines (Gallinat et al., 2016; Kraguljac et al., 2013). Augmented glutamate release coupled with reduced D-serine levels would drive non-ionotropic NMDAR activation (presumably functional or structural niLTD) and promote spine shrinkage (Stein et al., 2015), which ultimately may underlie the reduced spine density seen in schizophrenia patients and mouse models. Increased excitatory neuron activity may also represent a homeostatic means of compensating for the network's bias toward synaptic depression and spine shrinkage/elimination – highlighting another example of synaptic and cellular mechanisms working in conjunction to give rise to cognitive functions.

Contributions of Enhanced Intrinsic Excitability to Memory

Memory is one such cognitive process that is continuously regulated by both synaptic and cellular changes. Original insights into the role of the latter were derived from manipulations of the cAMP responsive element-binding protein (CREB) and the observation that CREB gene expression was directly related to excitability state (Dong et al., 2006; Viosca et al., 2009; Zhou et al., 2009). Excitability was then linked to memory allocation, such that neurons with higher intrinsic firing abilities were more likely to be allocated into the engram than neurons with lower intrinsic excitability. Direct manipulations of excitability are sufficient to drive engram formation. Additionally, changes in intrinsic excitability may promote memory consolidation. Learning-induced hyperexcitability and consolidation occur in the same temporal window following learning, and indeed, excitability may play a role during the hours and days post-learning to stabilize memory. However, enhanced excitability is not required to retrieve the memory (Moyer Jr. et al., 1996; Thompson et al., 1996), although retrieval can induce another wave of enhanced excitability upon activation (Chen et al., 2020).In summary, within the hippocampus, a transient increase in

intrinsic excitability is correlated with effective learning and may serve as a cellular mechanism to promote memory consolidation. Consolidation can be broken into two discrete components: synaptic consolidation which is thought to last up to hours after encoding within the local network as well as synapses, and systems consolidation which can take months or longer and involves other brain regions (Dudai, 2012). Following learning, a transient increase in intrinsic excitability is observed in CA1 that can last up to 7 days (Moyer et al., 2000; Moyer Jr. et al., 1996; Thompson et al., 1996). The temporal window in which this occurs coincides with systems consolidation, suggesting hyperexcitability may play a role in stabilizing the memory in the first few days after learning.

Memories need to maintain stability and fidelity, yet remain dynamic so that new information can be integrated into past memories to inform future decision making (Kroes and Fernández, 2012; Nadel et al., 2012; Routtenberg and Rekart, 2005; Rule et al., 2019) Here, I report an increase in intrinsic excitability in CA1 pyramidal neurons two days following contextual fear conditioning (CFC) (Crestani et al., 2018). The amount of change in intrinsic excitability was shown to be highly correlated to the amount of freezing during CFC, such that cells exhibited the highest firing capabilities also froze the most. This is an example of a direct relationship between cellular excitability state and behavior. Enhanced intrinsic excitability is also involved in the formation of place cells and the interaction of engrams encoding different experiences, thus making it imperative to understand the mechanisms underlying these changes.

Place cell formation

The hippocampus provides a spatial framework that allows both related and discrete memories to exist. Place cells are neurons in the hippocampus that are tuned to distinct locations within a context (Goode et al., 2020). Within dorsal CA1, place cells and engram cells are different, although engrams formed during novel context exploration involve recruitment of a subset of

hippocampal place cells (Tanaka et al., 2018). Formation of place cells has been shown to occur in response to specific spatial input and in an excitability-dependent manner, like the competition-based allocation seen in engram cells. Overall, CA1 cells are “silent” and exhibit relatively low firing, and therefore only a small subset of these neurons are place cells. Neurons with higher basal excitability are more likely to become place cells upon placement in a novel environment. It is also experimentally possible to create a place cell by enhancing the excitability of a silent cell (Cohen et al., 2017; Epsztein et al., 2011; Lee et al., 2012; Rich et al., 2014; Rickgauer et al., 2014). Together, these data demonstrate that modulation of intrinsic plasticity is a critical mechanism for not only engram assignment, but the formation of the spatial framework that can be used as a reference point for contextual memory representations, thus supporting memory function.

Interaction of engrams encoding different experiences

To keep up with the dynamic nature of our daily life, memory should be able to be adjusted over time, linked to other memories, and ultimately incorporated into a larger framework of general knowledge. Experimentally it is rather easy to boil memory down to single experiences. In the real world, memories are more likely to exist as an amalgamation of several related episodes than distinct, isolated events. While intrinsic changes are transient, there is a temporal window in which they exist (changes depending on brain region), and relatively more excitable cells are more likely to be allocated to an engram. It is advantageous for memories related in time to be stored in overlapping engrams, such that presentation of cues to activate one memory also activates linked memories. Competition-based mechanisms governing engram allocation (e.g. cells with higher excitability states are more likely to be allocated into the engram) following a single experience are also employed to link multiple experiences into overlapping engrams. Conversely, these mechanisms can also be engaged to separate unrelated experiences (Cai et al., 2016; Rashid et

al., 2016; Sehgal et al., 2018). Thus, bidirectional regulation of intrinsic excitability is critical for arranging different memory representations across the brain. Retrieval also temporarily reactivates engram cells and increases intrinsic firing capabilities. In doing so, a new temporal window for engram allocation is opened (Gouty-Colomer et al., 2016; Pignatelli et al., 2019; Rashid et al., 2016). This could explain how new knowledge is incorporated into prior knowledge and additionally, how memories and information separated by hours and even days can be linked (Sehgal et al., 2018).

Interaction of Synaptic and Intrinsic Plasticity in Learning and Memory

Learning has been shown to trigger changes in both synaptic and intrinsic plasticity. Additionally, there is a fair amount of commonality between the intracellular signaling pathways that regulate these two forms of plasticity. Activation of NMDARs, PKA, PKC and CaMKII has been shown to mediate synaptic and intrinsic plasticity (Daoudal and Debanne, 2003). However, it is still unclear which type of plasticity facilitates the other, as the two processes are so intimately linked. One example of their interconnectedness is during engram formation, when it is possible that synaptic stimulation during learning yield changes in intrinsic plasticity; the magnitude of this plasticity, in turn, may influence future experience-dependent synaptic plasticity (Sehgal et al., 2013). Memory formation and retrieval relies on a neuronal network's ability to manage long-term plasticity changes in synaptic transmission. For example, co-allocated memories may sustain their unique identities by engaging specific, enhanced synapses within shared engrams. Synaptic plasticity is responsible for constructing these unique identities by establishing precise functional connectivity within each engram. Maintenance of engram identity through preservation of specifically potentiated synapses is a mechanism by which the brain can maintain the uniqueness of a massive number of memory representations, while storing them in shared cell ensembles (Abdou et al., 2018). The demonstrated interplay between these two plasticity processes only

strengthens the case that learning models should be expanded to encompass this extra dimension of neuronal plasticity and complexity.

Concluding Remarks

As I scientist, I have nothing but the greatest appreciation for the ability to learn. At the organism level, learning is discovering a new fact, understanding how to perform a new technique, or reading a scientific article. At the cellular level, learning is changes in excitability and synapse strength. The brain's ability to regulate these changes in response to environmental stimuli is perhaps one of its most impressive capabilities and the overarching goal of this dissertation was to gain a deeper understanding of these two distinct types of cellular plasticity. This was accomplished by 1) discovering a novel role for the NMDAR co-agonist D-serine as a determinant of non-ionotropic synaptic depression and 2) demonstration of a correlative relationship between neuronal excitability state and behavior. These results in no way solve the immense puzzle that is memory, but they do serve to strengthen and expand the foundational knowledge we have about this phenomenon.

References

Abdou, K., Shehata, M., Choko, K., Nishizono, H., Matsuo, M., Muramatsu, S., Inokuchi, K., 2018. Synapse-specific representation of the identity of overlapping memory engrams. Science 360, 1227–1231. https://doi.org/10.1126/science.aat3810

Al-Hallaq, R.A., Conrads, T.P., Veenstra, T.D., Wenthold, R.J., 2007. NMDA Di-Heteromeric Receptor Populations and Associated Proteins in Rat Hippocampus. Journal of Neuroscience 27, 8334–8343. https://doi.org/10.1523/JNEUROSCI.2155-07.2007

Aow, J., Dore, K., Malinow, R., 2015. Conformational signaling required for synaptic plasticity by the NMDA receptor complex. Proceedings of the National Academy of Sciences 112, 14711–14716. https://doi.org/10.1073/pnas.1520029112

Bains, J.S., Oliet, S.H.R., 2007. Glia: they make your memories stick! Trends in Neurosciences 30, 417–424. https://doi.org/10.1016/j.tins.2007.06.007

Balu, D.T., Coyle, J.T., 2015. The NMDA receptor ‘ glycine modulatory site ’ in schizophrenia : D-serine, glycine , and beyond. Current Opinion in Pharmacology 20, 109–115. https://doi.org/10.1016/j.coph.2014.12.004

Balu, D.T., Presti, K.T., Huang, C.C.Y., Muszynski, K., Radzishevsky, I., Wolosker, H., Guffanti, G., Ressler, K.J., Coyle, J.T., 2018. Serine Racemase and D-serine in the Amygdala Are Dynamically Involved in Fear Learning. Biological Psychiatry 83, 273–283. https://doi.org/10.1016/j.biopsych.2017.08.012

Basu, A.C., Tsai, G.E., Ma, C.-L., Ehmsen, J.T., Mustafa, A.K., Han, L., Jiang, Z.I., Benneyworth, M.A., Froimowitz, M.P., Lange, N., Snyder, S.H., Bergeron, R., Coyle, J.T., 2009. Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Molecular psychiatry 14, 719–27. https://doi.org/10.1038/mp.2008.130

Billard, J.-M., 2015. d-Serine in the aging hippocampus. Journal of Pharmaceutical and Biomedical Analysis 116, 18–24. https://doi.org/10.1016/j.jpba.2015.02.013

Billard, J.-M., 2008. D-serine signalling as a prominent determinant of neuronal-glial dialogue in the healthy and diseased brain. Journal of Cellular and Molecular Medicine 12, 1872–1884. https://doi.org/10.1111/j.1582-4934.2008.00315.x

Cai, D.J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., Wei, B., Veshkini, M., La-Vu, M., Lou, J., Flores, S.E., Kim, I., Sano, Y., Zhou, M., Baumgaertel, K., Lavi, A., Kamata, M., Tuszynski, M., Mayford, M., Golshani, P., Silva, A.J., 2016. A shared neural ensemble links distinct contextual memories encoded close in time. Nature 534, 115–118. https://doi.org/10.1038/nature17955

Chen, L., Cummings, K.A., Mau, W., Zaki, Y., Dong, Z., Rabinowitz, S., Clem, R.L., Shuman, T., Cai, D.J., 2020. The role of intrinsic excitability in the evolution of memory: Significance in memory allocation, consolidation, and updating. Neurobiology of Learning and Memory 173, 107266. https://doi.org/10.1016/j.nlm.2020.107266

Clements, J.D., 1996. Transmitter timecourse in the synaptic cleft: its role in central synaptic function. Trends Neurosci 19, 163–71. https://doi.org/10.1016/s0166-2236(96)10024-2

Cohen, J.D., Bolstad, M., Lee, A.K., 2017. Experience-dependent shaping of hippocampal CA1 intracellular activity in novel and familiar environments. eLife 6, e23040. https://doi.org/10.7554/eLife.23040

Crestani, A.P., Krueger, J.N., Barragan, E.V., Nakazawa, Y., Nemes, S.E., Quillfeldt, J.A., Gray, J.A., Wiltgen, B.J., 2018. Metaplasticity contributes to memory formation in the hippocampus. Neuropsychopharmacology 44, 408–414. https://doi.org/10.1038/s41386-018-0096-7

Danysz, W., Parsons, C.G., 1998. Glycine and N-Methyl-D-Aspartate Receptors: Physiological Significance and Possible Therapeutic Applications. Pharmalogical Reviews 50, 598–650.

Daoudal, G., Debanne, D., 2003. Long-Term Plasticity of Intrinsic Excitability: Learning Rules and Mechanisms. Learning & Memory 10, 456–465. https://doi.org/10.1101/lm.64103

Dong, Y., Green, T., Saal, D., Marie, H., Neve, R., Nestler, E.J., Malenka, R.C., 2006. CREB modulates excitability of nucleus accumbens neurons. Nat Neurosci 9, 475–477. https://doi.org/10.1038/nn1661

Dudai, Y., 2012. The Restless Engram: Consolidations Never End. Annu. Rev. Neurosci. 35, 227–247. https://doi.org/10.1146/annurev-neuro-062111-150500

Epsztein, J., Brecht, M., Lee, A.K., 2011. Intracellular Determinants of Hippocampal CA1 Place and Silent Cell Activity in a Novel Environment. Neuron 70, 109–120. https://doi.org/10.1016/j.neuron.2011.03.006

Ferreira, J.S., Papouin, T., Ladépêche, L., Yao, A., Langlais, V.C., Bouchet, D., Dulong, J., Mothet, J.P., Sacchi, S., Pollegioni, L., Paoletti, P., Richard Oliet, S.H., Groc, L., 2017. Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses. eLife 6, 1–22. https://doi.org/10.7554/eLife.25492

Fossat, P., Turpin, F.R., Sacchi, S., Dulong, J., Shi, T., Rivet, J.M., Sweedler, J.V., Pollegioni, L., Millan, M.J., Oliet, S.H.R., Mothet, J.P., 2012. Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex. Cerebral Cortex 22, 595–606. https://doi.org/10.1093/cercor/bhr130

Furukawa, H., Singh, S.K., Mancusso, R., Gouaux, E., 2005. Subunit arrangement and function in NMDA receptors. Nature 438, 185–192. https://doi.org/10.1038/nature04089

Gais, S., Schönauer, M., 2017. Untangling a Cholinergic Pathway from Wakefulness to Memory. Neuron 94, 696–698. https://doi.org/10.1016/j.neuron.2017.05.010

Gallinat, J., McMahon, K., Kühn, S., Schubert, F., Schaefer, M., 2016. Cross-sectional Study of Glutamate in the Anterior Cingulate and Hippocampus in Schizophrenia. SCHBUL 42, 425–433. https://doi.org/10.1093/schbul/sbv124

Goode, T.D., Tanaka, K.Z., Sahay, A., McHugh, T.J., 2020. An Integrated Index: Engrams, Place Cells, and Hippocampal Memory. Neuron 107, 805–820. https://doi.org/10.1016/j.neuron.2020.07.011

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., Malinow, R., 2006. AMPAR Removal Underlies Aβ-Induced Synaptic Depression and Dendritic Spine Loss. Neuron 52, 831–843. https://doi.org/10.1016/j.neuron.2006.10.035

Huang, Y.-J., Lin, C.-H., Lane, H.-Y., E. Tsaid, G., 2012. NMDA Neurotransmission Dysfunction in Behavioral and Psychological Symptoms of Alzheimer's Disease. CN 10, 272–285. https://doi.org/10.2174/157015912803217288

Jami, S.A., Cameron, S., Wong, J.M., Daly, E.R., McAllister, A.K., Gray, J.A., 2021. Increased excitation-inhibition balance and loss of GABAergic synapses in the serine racemase knockout model of NMDA receptor hypofunction. Journal of Neurophysiology 126, 11–27. https://doi.org/10.1152/jn.00661.2020

Javitt, D.C., 2015. Current and Emergent Treatments for Symptoms and Neurocognitive Impairment in Schizophrenia. Curr Treat Options Psych 1, 107–120. https://doi.org/10.1007/s40501-014-0010-9

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., Sisodia, S., Malinow, R., 2003. APP Processing and Synaptic Function. Neuron 37, 925–937. https://doi.org/10.1016/S0896-6273(03)00124-7

Kessels, H.W., Nabavi, S., Malinow, R., 2013. Metabotropic NMDA receptor function is required for -amyloid-induced synaptic depression. Proceedings of the National Academy of Sciences 110, 4033–4038. https://doi.org/10.1073/pnas.1219605110

Kleckner, N.W., Dingledine, R., 1988. Requirement for Glycine in Activation of NMDA-Receptors Expressed in Xenopus Oocytes. Science 241, 835–837.

Kraguljac, N.V., White, D.M., Reid, M.A., Lahti, A.C., 2013. Increased Hippocampal Glutamate and Volumetric Deficits in Unmedicated Patients With Schizophrenia. JAMA Psychiatry 70, 1294. https://doi.org/10.1001/jamapsychiatry.2013.2437

Kroes, M.C.W., Fernández, G., 2012. Dynamic neural systems enable adaptive, flexible memories. Neuroscience & Biobehavioral Reviews 36, 1646–1666. https://doi.org/10.1016/j.neubiorev.2012.02.014

Lacor, P.N., Buniel, M.C., Furlow, P.W., Sanz Clemente, A., Velasco, P.T., Wood, M., Viola, K.L., Klein, W.L., 2007. A Oligomer-Induced Aberrations in Synapse Composition, Shape, and Density Provide a Molecular Basis for Loss of Connectivity in Alzheimer's Disease. Journal of Neuroscience 27, 796–807. https://doi.org/10.1523/JNEUROSCI.3501-06.2007

Le Bail, M., Martineau, M., Sacchi, S., Yatsenko, N., Radzishevsky, I., Conrod, S., Ait Ouares, K., Wolosker, H., Pollegioni, L., Billard, J.-M., Mothet, J.-P., 2015. Identity of the NMDA receptor coagonist is synapse specific and developmentally regulated in the hippocampus. Proceedings of the National Academy of Sciences of the United States of America 112, E204-13. https://doi.org/10.1073/pnas.1416668112

Lee, D., Lin, B.-J., Lee, A.K., 2012. Hippocampal Place Fields Emerge upon Single-Cell Manipulation of Excitability During Behavior. Science 337, 849–853. https://doi.org/10.1126/science.1221489

Mayer, M.L., 2006. Glutamate receptors at atomic resolution. Nature 440, 456–462. https://doi.org/10.1038/nature04709

Moghaddam, B., Javitt, D., 2012. From Revolution to Evolution: The Glutamate Hypothesis of Schizophrenia and its Implication for Treatment. Neuropsychopharmacol 37, 4–15. https://doi.org/10.1038/npp.2011.181

Mothet, J.P., Le Bail, M., Billard, J.M., 2015. Time and space profiling of NMDA receptor co-agonist functions. Journal of Neurochemistry 135, 210–225. https://doi.org/10.1111/jnc.13204

Moyer Jr., J.R., Thompson, L.T., Disterhoft, J.F., 1996. Trace Eyeblink Conditioning Increases CA1 Excitability in a Transient and Learning-Specific Manner. The Journal of Neuroscience 16, 5536–5546. https://doi.org/10.1523/jneurosci.16-17-05536.1996

Moyer, J.R., Power, J.M., Thompson, L.T., Disterhoft, J.F., 2000. Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 5476–82.

Nabavi, S., Kessels, H.W., Alfonso, S., Aow, J., Fox, R., Malinow, R., 2013. Metabotropic NMDA receptor function is required for NMDA receptor-dependent long-term depression 110. https://doi.org/10.1073/pnas.1219454110

Nadel, L., Hupbach, A., Gomez, R., Newman-Smith, K., 2012. Memory formation, consolidation and transformation. Neuroscience & Biobehavioral Reviews 36, 1640–1645. https://doi.org/10.1016/j.neubiorev.2012.03.001

Orzylowski, M., Fujiwara, E., Mousseau, D.D., Baker, G.B., 2021. An Overview of the Involvement of D-Serine in Cognitive Impairment in Normal Aging and Dementia. Front. Psychiatry 12, 754032. https://doi.org/10.3389/fpsyt.2021.754032

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H.R., 2006. Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. Cell 125, 775–784. https://doi.org/10.1016/j.cell.2006.02.051

Paoletti, P., Bellone, C., Zhou, Q., 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nature reviews. Neuroscience 14, 383–400. https://doi.org/10.1038/nrn3504

Papouin, T., Dunphy, J.M., Tolman, M., Dineley, K.T., Haydon, P.G., 2017. Septal Cholinergic Neuromodulation Tunes the Astrocyte-Dependent Gating of Hippocampal NMDA Receptors to Wakefulness. Neuron 94, 840-854.e7. https://doi.org/10.1016/j.neuron.2017.04.021

Papouin, T., Lad??p??che, L., Ruel, J., Sacchi, S., Labasque, M., Hanini, M., Groc, L., Pollegioni, L., Mothet, J.P., Oliet, S.H.R., 2012. Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. Cell 150, 633–646. https://doi.org/10.1016/j.cell.2012.06.029

Ploux, E., Bouet, V., Radzishevsky, I., Wolosker, H., Freret, T., Billard, J.-M., 2020. Serine Racemase Deletion Affects the Excitatory/Inhibitory Balance of the Hippocampal CA1 Network. IJMS 21, 9447. https://doi.org/10.3390/ijms21249447

Potier, B., Turpin, F.R., Sinet, P.M., Rouaud, E., Mothet, J.-P., Epelbaum, J., Dutar, P., Billard, J.-M., 2010. Contribution of the D-Serine-dependent pathway to the cellular mechanisms underlying cognitive aging. Front. Ag. Neurosci. 2. https://doi.org/10.3389/neuro.24.001.2010

Priestley, T., Kemp, J.A., 1994. Kinetic Study of the Interactions Between the Glutamate and Glycine Recognition Sites on the N-Methyl-D-aspartic Acid Receptor Complex. Molecular Pharmacology 46, 1191–1196.

Rashid, A.J., Yan, C., Mercaldo, V., Hsiang, H.-L. (Liz), Park, S., Cole, C.J., De Cristofaro, A., Yu, J., Ramakrishnan, C., Lee, S.Y., Deisseroth, K., Frankland, P.W., Josselyn, S.A., 2016. Competition between engrams influences fear memory formation and recall. Science 353, 383–387. https://doi.org/10.1126/science.aaf0594

Rich, P.D., Liaw, H.-P., Lee, A.K., 2014. Large environments reveal the statistical structure governing hippocampal representations. Science 345, 814–817. https://doi.org/10.1126/science.1255635

Rickgauer, J.P., Deisseroth, K., Tank, D.W., 2014. Simultaneous cellular-resolution optical perturbation and imaging of place cell firing fields. Nat Neurosci 17, 1816–1824. https://doi.org/10.1038/nn.3866

Routtenberg, A., Rekart, J.L., 2005. Post-translational protein modification as the substrate for long-lasting memory. Trends in Neurosciences 28, 12–19. https://doi.org/10.1016/j.tins.2004.11.006

Rule, M.E., O'Leary, T., Harvey, C.D., 2019. Causes and consequences of representational drift. Current Opinion in Neurobiology 58, 141–147. https://doi.org/10.1016/j.conb.2019.08.005

Sehgal, M., Song, C., Ehlers, V.L., Moyer, J.R., 2013. Learning to learn – Intrinsic plasticity as a metaplasticity mechanism for memory formation. Neurobiology of Learning and Memory 105, 186–199. https://doi.org/10.1016/j.nlm.2013.07.008

Sehgal, M., Zhou, M., Lavi, A., Huang, S., Zhou, Y., Silva, A.J., 2018. Memory allocation mechanisms underlie memory linking across time. Neurobiology of Learning and Memory 153, 21–25. https://doi.org/10.1016/j.nlm.2018.02.021

Shankar, G.M., Bloodgood, B.L., Townsend, M., Walsh, D.M., Selkoe, D.J., Sabatini, B.L., 2007. Natural Oligomers of the Alzheimer Amyloid- Protein Induce Reversible Synapse Loss by Modulating an NMDA-Type Glutamate Receptor-Dependent Signaling Pathway. Journal of Neuroscience 27, 2866–2875. https://doi.org/10.1523/JNEUROSCI.4970-06.2007

Sheng, M., Cummings, J., Roldan, L. a, Jan, Y.N., Jan, L.Y., 1994. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368, 144–147. https://doi.org/10.1038/368144a0

Stein, I.S., Gray, J.A., Zito, K., 2015. Non-Ionotropic NMDA Receptor Signaling Drives Activity-Induced Dendritic Spine Shrinkage 35, 12303–12308. https://doi.org/10.1523/JNEUROSCI.4289-14.2015

Stein, I.S., Park, D.K., Flores, J.C., Jahncke, J.N., Zito, K., 2020. Molecular Mechanisms of Non-ionotropic NMDA Receptor Signaling in Dendritic Spine Shrinkage. J. Neurosci. 40, 3741–3750. https://doi.org/10.1523/JNEUROSCI.0046-20.2020

Steullet, P., Cabungcal, J.-H., Coyle, J., Didriksen, M., Gill, K., Grace, A.A., Hensch, T.K., LaMantia, A.-S., Lindemann, L., Maynard, T.M., Meyer, U., Morishita, H., O'Donnell, P., Puhl, M., Cuenod, M., Do, K.Q., 2017. Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. Mol Psychiatry 22, 936–943. https://doi.org/10.1038/mp.2017.47

Stroebel, D., Paoletti, P., 2021. Architecture and function of NMDA receptors: an evolutionary perspective. J Physiol 599, 2615–2638. https://doi.org/10.1113/JP279028

Tanaka, K.Z., He, H., Tomar, A., Niisato, K., Huang, A.J.Y., McHugh, T.J., 2018. The hippocampal engram maps experience but not place. Science 361, 392–397. https://doi.org/10.1126/science.aat5397

Texidó, L., Martín-Satué, M., Alberdi, E., Solsona, C., Matute, C., 2011. Amyloid β peptide oligomers directly activate NMDA receptors. Cell Calcium 49, 184–190. https://doi.org/10.1016/j.ceca.2011.02.001

Thompson, L.T., Moyer, J.R., Disterhoft, J.F., 1996. Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. Neurobiology of Aging 17, 619–629. https://doi.org/10.1016/0197-4580(96)00026-7

Ting, J.T., Kelley, B.G., Lambert, T.J., Cook, D.G., Sullivan, J.M., 2007. Amyloid precursor protein overexpression depresses excitatory transmission through both presynaptic and

postsynaptic mechanisms. Proceedings of the National Academy of Sciences 104, 353–358. https://doi.org/10.1073/pnas.0608807104

Tononi, G., Cirelli, C., 2014. Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron 81, 12–34. https://doi.org/10.1016/j.neuron.2013.12.025

Tovar, K.R., McGinley, M.J., Westbrook, G.L., 2013. Triheteromeric NMDA receptors at hippocampal synapses. The Journal of neuroscience : the official journal of the Society for Neuroscience 33, 9150–60. https://doi.org/10.1523/JNEUROSCI.0829-13.2013

Viosca, J., Malleret, G., Bourtchouladze, R., Benito, E., Vronskava, S., Kandel, E.R., Barco, A., 2009. Chronic enhancement of CREB activity in the hippocampus interferes with the retrieval of spatial information. Learning & Memory 16, 198–209. https://doi.org/10.1101/lm.1220309

Yashiro, K., Philpot, B.D., 2008. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55, 1081–1094. https://doi.org/10.1016/j.neuropharm.2008.07.046

Zhou, Y., Won, J., Karlsson, M.G., Zhou, M., Rogerson, T., Balaji, J., Neve, R., Poirazi, P., Silva, A.J., 2009. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. Nat Neurosci 12, 1438–1443. https://doi.org/10.1038/nn.2405

Zou, C., Crux, S., Marinesco, S., Montagna, E., Sgobio, C., Shi, Y., Shi, S., Zhu, K., Dorostkar, M.M., Müller, U.C., Herms, J., 2016. Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis. EMBO J 35, 2213–2222. https://doi.org/10.15252/embj.201694085

Appendix 1

PSYCHEDELIC-INDUCED PLASTICITY

Preface

The following chapter contains a figure from published data titled “Psychedelics Promote Structural and Functional Neural Plasticity” that was accepted in Cell Rep on May 7th, 2018. Jonathon M. Wong, Lindsay P. Cameron, and I collected the data shown in Figure 1. This chapter also contains unpublished data characterizing the effects of 5MeO- and 6MeO-DMT on intrinsic excitability in Layer 5 mPFC pyramidal neurons. This chapter was a collaborative work between Lindsay P. Cameron, David E. Olson and John A. Gray. All text and Figure 2 in the following chapter are my own. DEO provided the animals and drugs used for these experiments and LPC injected the animals. JAG was involved in experimental design, interpretation, and editing. The authors report a conflict of interest. DEO has submitted a patent application related to this work (PCT/US2017/054277). This work was supported by R21MH116315 and R01MH117130 (JAG), 5T32MH082174-09 (LPC, EVB), and T32GM099608 (EVB).

Introduction

Psychedelics, also known as hallucinogens, are a powerful class of psychoactive substances capable of altering one's mood, perception, and state of consciousness. Classical psychedelic compounds like psilocybin ("magic" mushrooms), mescaline (peyote), and dimethyltryptamine (DMT) have been consumed as sacraments in religious ceremonies, as well as taken recreationally by indigenous communities in Central and South America for thousands of years (Lowe et al., 2021). The earliest identified record of psychedelic use dates to the 1500s – several centuries before the therapeutic potential of hallucinogenic compounds would pique the curiosity of scientists. The first wave of rigorous psychedelic study in the early to mid-twentieth century led to the synthesis of lysergic acid diethylamide (LAD/LSD) and the extraction/characterization of psilocybin from "magic" mushrooms, as well as distribution and marketing of these compounds as neurotherapeutics (Johnson and Griffiths, 2017; Nichols, 2020). This renaissance of research into these compounds was driven largely by their demonstrated potential to relieve depression, anxiety, and addiction. Yet the excitement these psychoactive compounds generated would prove to be their downfall. By the 1960s and 70s, scientists had grown impatient with restricting the study hallucinogens to research settings or for therapeutic gain. Recreational psychedelic use became more widespread, especially among the hippie (counterculture) movement, and with it, reports of negative side effects, like visual distortions and flashbacks. In response to the furtive use of hallucinogenic substances, the US Drug Enforcement Agency labeled psychedelics (LSD, DMT, psilocybin, mescaline) as Schedule 1 drugs, thus prohibiting their production, distribution, and use (Hendricks et al., 2015). The strong historical association of these compounds with the divisive counterculture has led to a lingering stigmatization, resulting to a rejection of psychedelic research for many years.

The past two decades have seen a resurgence in psychedelic science. This new line of research has led to a greater understanding of the mechanism of action of psychedelic

substances. Classic hallucinogens are predominantly serotonergic, meaning they activate serotonin (5-hydroxytryptamine/5-HT) receptors in the brain, in particular the 5-HT2A subtype (Halberstadt and Geyer, 2011). The serotonin system is involved in cognition, mood and social interaction, while disruptions in this system have been implicated in depression, anxiety, and other neuropsychiatric disorders (Beliveau et al., 2017). The interaction of classical psychedelics and 5-HT receptors suggests that these compounds may have untapped potential for treating these conditions. One of the main objectives of the new era of psychedelic science is to examine psychedelics more rigorously and systematically at a mechanistic level, with the overarching goal of developing safer and more effective treatments for neuropsychiatric disorders.

Therapeutic Potential of Psychedelics

Neuropsychiatric diseases arise from a complex interaction between structural, functional, and genetic changes in the brain. Despite having available therapeutics for neuropsychiatric diseases such as depression, we still find that a large portion (approximately one-third) of those afflicted with depression do not respond to current antidepressants (Rush et al., 2006). Indeed, this may be because most drugs prescribed by psychiatrists were developed when knowledge of the physiological mechanisms of neuropsychiatric disorders was quite limited. Interestingly, one of the most potent antidepressants available today is the dissociative anesthetic, ketamine. The ability to facilitate structural and functional changes within the prefrontal cortex (PFC) is thought to underlie the fast-acting, antidepressant and anxiolytic properties of the compound. Indeed, the efficacy of ketamine as a treatment for depression has been exhibited even in treatment-resistant populations (Berman et al., 2000; DiazGranados et al., 2010; Ionescu et al., 2016; Murrough et al., 2013; Zarate et al., 2012, 2006).

Clinical studies have shown similar antidepressant and anxiolytic effects following a single dose of serotonergic psychedelics, like psilocybin (Carhart-Harris and Goodwin, 2017; Rucker et

al., 2018). The therapeutic activity of ketamine and classic serotonergic psychedelics has also been recapitulated in rodent behavioral tasks that test depression and anxiety-like actions (Cameron et al., 2018). We now know that ketamine and psychedelics are both rapid and effective antidepressants and anxiolytics because they share a common underlying mechanism – facilitation of robust growth of dendritic spines and increased synaptic signaling in the brain through activation of TrkB and mTOR (Ly et al., 2018). It is intriguing that these compounds demonstrate a shared mechanism, considering ketamine and psychedelics do not have the same binding partners – NMDA and 5-HT2A receptors, respectively. However, involvement of TrkB and mTOR activity in both underlying processes suggests these are attractive molecular targets for the development of fast-acting depressants and anxiolytic medicines.

Methods

Drugs

The fumarate salt of *N,N*-dimethyltryptamine (DMT), was synthesized in house as described previously (Cameron et al., 2018) and judged to be analytically pure based on NMR and LC-MS data. For *in vivo* studies, DMT was dissolved in sterile 0.9% saline and administered intraperitoneally at a dose of 1 or 10mg/kg and an injection volume of 1mL/kg.

Animals

Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA, USA). Male and female rats aged ~8 weeks were used. All experimental procedures involving animals were approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC) and adhered to the principles described in the NIH Guide for the Care and use of Laboratory Animals.

Electrophysiology and acute slice preparation

Female Sprague Dawley rats were given an intraperitoneal injection of DMT or vehicle. After 24h, rats were anesthetized with isofluorane and transcardially perfused with ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 11 glucose, 2.5 KCl, 1 NaH_2PO_4, 2.5 $CaCl_2$ and 1.3 $MgSO_4$. Brains were rapidly removed and coronal slices (300 µM) from the mPFC were cut on a Leica VT1200 vibratome (Buffalo Grove, IL) with ice-cold ACSF solution. Slices were incubated in 32°C NMDG solution containing the following (in mM): 93 NMDG, 93 HCl, 2.5 KCl, 1.2 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 10 $MgSO_4$, and 0.5 $CaCl_2$ (Ting et al., 2018) for 10 minutes, transferred to room temperature ACSF, and held for at least 45 minutes before recording. All solutions were vigorously perfused with 95% O_2/5% CO_2. Whole-cell recordings were obtained from CA1 pyramidal neurons under visual guidance (DIC/infrared optics).

Whole-cell voltage clamp

Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded at -70 mV in 32°C ACSF. Cells were patched with 3–5 MΩ borosilicate pipettes filled with intracellular solution containing (in mM): 135 cesium methanesulfonate, 8 NaCl, 10 HEPES, 0.3 Na-GTP, 4 Mg-ATP, 0.3 EGTA, 5 QX-314, pH 7.2, 290mOsm (Sigma, St Louis, MO). Series resistance was monitored throughout experiments; cells were discarded if series resistance varied more than 25%. All recordings were obtained with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Analysis was performed with the Mini Analysis program (Synaptosoft, Decatur, GA) with a 4pA detection threshold. Data represents individual neurons taken from 3 different animals per treatment. Data acquisition and analysis was performed by experimenters blinded to treatment conditions.

Whole-cell current clamp

For all recordings, borosilicate glass pipettes were fabricated with resistances of 4-6MΩ. Pipettes were filled with the following intracellular solution (in mM): 135 K^+ gluconate, 5 NaCl, 2 $MgCl_2$, 10 HEPES, 0.6 EGTA, 4 NaATP, 0.4 NaGTP, pH 7.3, 290 mOsm. Series resistance was monitored and cells in which series resistance varied by more than 20% during a recording were discarded. Recordings were collected with a Multiclamp 700B amplifier (Molecular Devices), filtered at 2 kHz, digitized at 10kHz, and data analyzed using pClamp 10 software (Molecular Devices). Frequency-current relationships for evoked firing were determined by injecting 500ms current steps with amplitudes increasing by 25pA, from 0 to 500 pA from the resting potential. Liquid junction potentials were not corrected.

Statistical Analysis

Appropriate sample sizes were estimated based on our previous experiences performing similar experiments. Data are represented as mean ± SEM. Statistical analyses were performed using Prism 8 software (GraphPad). For analyses involving comparison of three groups, a one-way analysis of variance (Dunnett's *post hoc* test) was utilized. Probability distributions from

electrophysiology experiments were compared using a Kolmogorov-Smirnov test. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$ compared with vehicle control.

Psychedelics Promote Spinogenesis and Synaptogenesis

Hallmarks of depression and other neuropsychiatric disorders include dendritic atrophy and loss of dendritic spines in the prefrontal cortex (Christoffel et al., 2011; Duman and Aghajanian, 2012). To test whether psychedelics affect spinogenesis and synaptogenesis, rat cortical cultures were treated for 24 hr with three distinct serotonergic psychedelic substances: DOI, DMT, and LSD. All three compounds were shown to promote synaptogenesis via increased number of spines. This suggests that chronic psychedelic administration promotes increases of spine and synaptic density *in vitro*. Previous data indicates that a 10mg/kg dose of DMT is sufficient to improve rat performance in depression and PTSD-related behavioral tasks (Cameron et al., 2018). To test the *in vivo* effects of DMT on spine formation in the rat medial prefrontal cortex (mPFC), a single dose of DMT (either 10mg/kg or 1mg/kg) was intraperitoneally injected and 24 hr after administration, cortical pyramidal neurons showed an increase in dendritic spine density, which coincided with functional changes in spontaneous activity. Functional effects were determined using *ex vivo* whole-cell voltage-clamp recordings from Layer 5 pyramidal mPFC neurons. These recordings obtained revealed that acute DMT treatment (10mg/kg and 1mg/kg) increases spontaneous excitatory postsynaptic current (sEPSC) frequency (Figure 1k) and amplitude (Figure 1l). It was surprising that the effect was observed in both doses, despite 1mg/kg and 10mg/kg being sub-hallucinogenic and hallucinogenic doses, respectively (Nair and Jacob, 2016). These significant increases in spontaneous transmission are indicative of increased synapse number and are consistent with the structural changes seen. This work was a part of a much larger story done in collaboration with the Olson lab (Ly et al., 2018), and adds to the emerging body of literature demonstrating that psychedelic compounds promote structural and functional plasticity in the PFC.

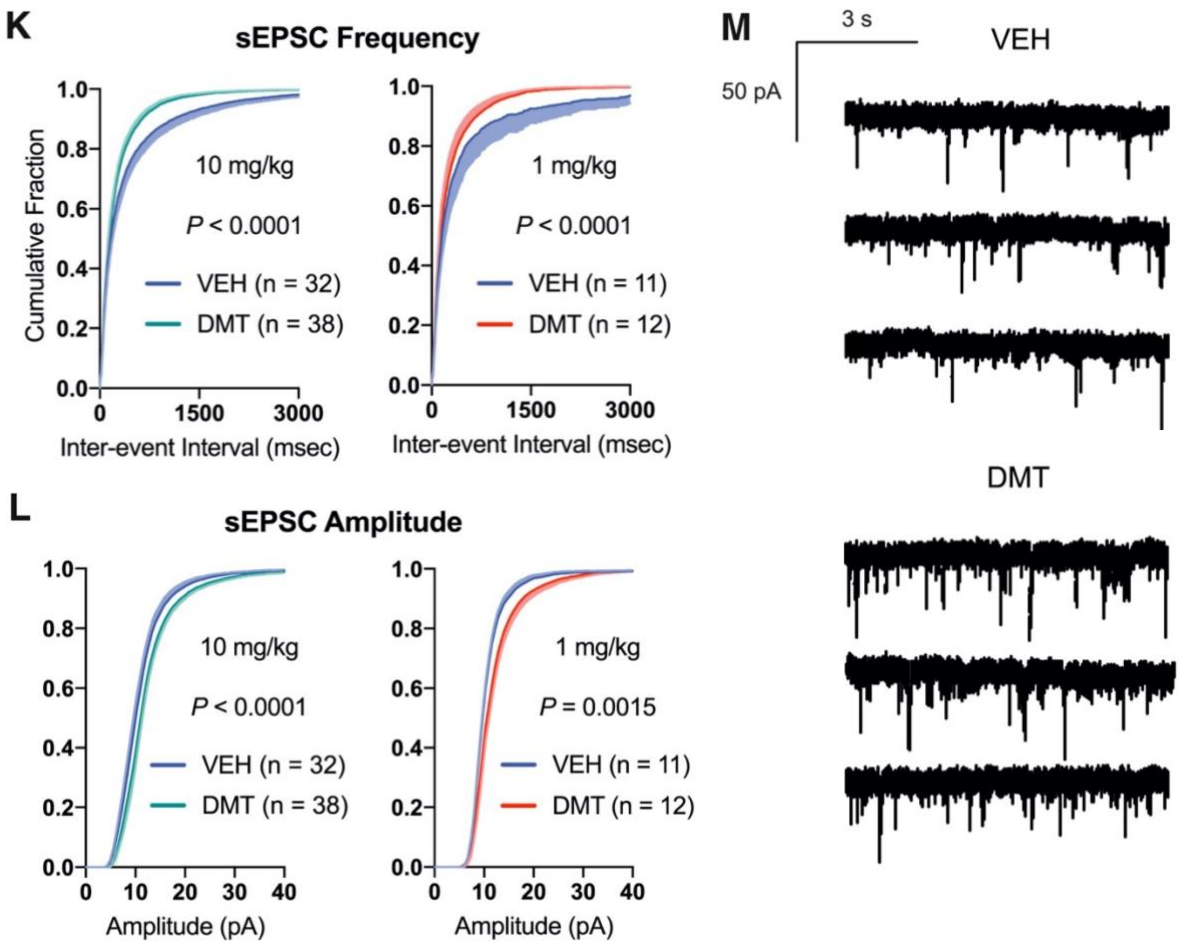

Figure 1. Psychedelics promote functional plasticity. K and L. Whole-cell voltage-clamp recordings of layer V pyramidal neurons from slices obtained 24 hr after DMT treatment (10 mg/kg and 1 mg/kg) demonstrate that DMT increases both spontaneous excitatory postsynaptic current (sEPSC) frequency (K) and amplitude (L) (n=11-38 neurons, 3 animals per group). **M.** Representative traces for the 10mg/kg experiments quantifies in (K) and (L). Data are represented as mean ± SEM. Adapted from Ly *et al*., 2018.

DMT Does Not Affect Intrinsic Neuronal Excitability

Classical serotonergic psychedelics, like DMT, are incredibly effective at promoting neuronal growth and structural change, which is thought to be the process underlying their rapid antidepressant activity. The mechanism by which this occurs involves activation of synaptic AMPA receptors and downstream activation of TrkB and mTOR (Dunlap et al., 2020). Recent work from the Olson lab has shown that the therapeutic properties of a psychedelic compound can be decoupled from hallucinogenic activity (Cameron et al., 2021; Dunlap et al., 2020). A single dose of DMT has been shown to increase the number of functional synapses in PFC pyramidal neurons (Ly et al., 2018), but it is unknown if this enhancement also drives an increase in intrinsic firing capabilities. To test the effects of hallucinogenic (5-MeO-) and non-halluncinogenic (6-MeO-) DMT compounds on intrinsic plasticity, a single dose of drug (10mg/kg) was intraperitoneally injected into adult rats and 24 hr after administration, slices were prepared. Intrinsic cellular properties were assessed using *ex vivo* whole-cell current-clamp recordings from Layer 5 pyramidal mPFC neurons. These recordings showed no change in intrinsic excitability (slope of the spike frequency vs injected current curve) in Layer 5 pyramidal neurons following acute 5-MeO- or 6-MeO-DMT treatment. Other cellular and action potential properties were compared, but any significant differences were minor and did not underlie any major changes in intrinsic firing rate (Figure 2). The results of this set of experiments suggest that changes in intrinsic excitability in mPFC do not underlie the fast-acting therapeutic action of DMT, and most likely other classic psychedelics.

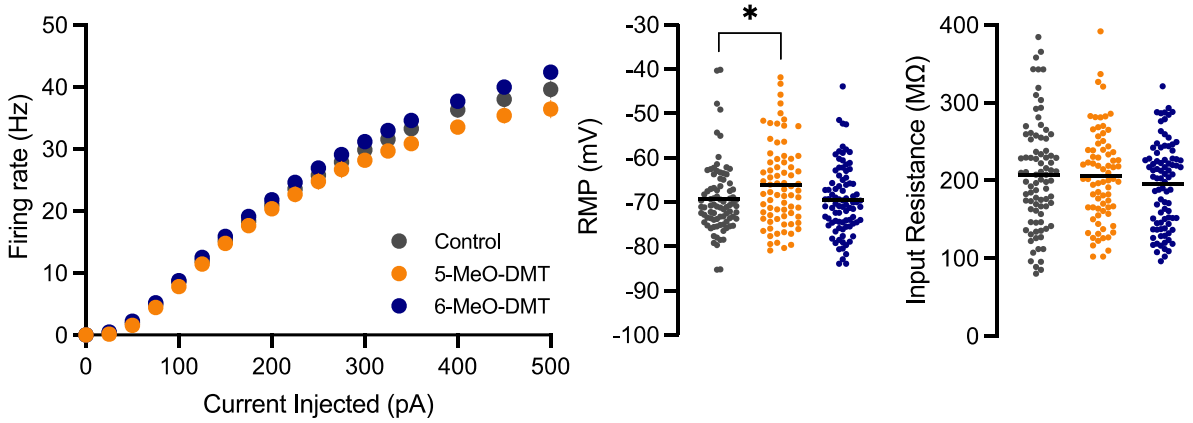

Table: Intrinsic Excitability in Layer V Pyramidal Neurons in the mPFC					
Property	Vehicle n=85	5-MeO-DMT n=75	6-MeO-DMT n=87	P value	F (DFn, DFd)
RMP (mV)	-69.33 ± 0.8677 [-85.24 to -40.18]	-66.09 ± 1.085 [-80.93 to -41.89]	-69.61 ± 0.8344 [-83.95 to -43.88]	0.0150 *	F(2, 244)=4.271
R_{input} (MΩ)	206.4 ± 7.518 [80.06 to 384.6]	205.6 ± 6.859 [102.0 to 391.7]	194 ± 5.905 [96.16 to 321.1]	0.3920	F(2, 244)=0.9400
Rheobase (pA)	94.05 ± 8.136 [3.333 to 380.0]	75.63 ± 4.101 [18.67 to 212.0]	72.11 ± 3.800 [12.00 to 192.7]	0.0153 *	F(2, 244)=4.251
Sag (mV)[A]	0.05880 ± 0.004365 [0.002878 to 0.1644]	0.05484 ± 0.004528 [0.01215 to 0.1882]	0.05827 ± 0.004313 [0.007942 to 0.2116]	0.7973	F(2, 244)=0.2268
Afterdepolarization (mV)	3.710 ± 0.2869 [0.01016 to 12.49]	3.566± 0.2723 [0.01499 to 9.478]	3.664 ± 0.2379 [0.03017 to 10.02]	0.9297	F(2, 244)=0.07296
AP Threshold (mV)[A]	-57.54 ± 0.9322 [-76.92 to -26.55]	-54.74 ± 1.080 [-69.62 to -31.94]	-57.62 ± 0.8698 [-73.72 to -33.91]	0.0622	F(2, 244)=2.809
AP Peak (mV)	133.1 ± 1.802 [91.26 to 181.9]	131.3 ± 1.875 [85.98 to 170.7]	135.0 ± 1.711 [67.20 ± 185.8]	0.3524	F(2, 244)=1.048
AP Half-width (ms)	1.647 ± 0.05928 [0.9267 to 3.174]	1.573 ± 0.05995 [0.8474 to 3.309]	1.590 ± 0.05125 [0.8844 to 3.004]	0.6247	F(2, 244)=0.4714
AHP Peak (mV)	-3.016 ± 0.1783 [-6.729 to 0.1875]	-2.592 ± 0.1712 [-6.569 to 0.0008374]	-3.128 ± 0.1575 [-6.593 to -0.1084]	0.0710	F(2,244)=2.675

Mean ± SEM [range]
* Significant at $p < 0.05$
[A] junction potential not adjusted

Figure 2. 5-MeO- and 6-MeO-DMT do not affect intrinsic cellular excitability. Hallucinogenic (5-MeO-) and non-hallucinogenic (6-MeO-) DMT analogs do not alter intrinsic excitability (n=75-87 neurons, 6 animals per group). Intrinsic cellular properties were largely unchanged by drug treatments. Data represent mean ± SEM, significance was defined as $p < 0.05$.

Discussion

Psychedelics are undeniably some of the most impressive molecules in terms of their ability to stimulate structural and functional changes in the brain. There is now a greater push than ever to utilize these psychoactive compounds as treatments for neuropsychiatric disorders, but there is still much to learn regarding efficacy, safety, and optimal dosing regimen. Additionally, it is still unclear whether the subjective effects, the actual hallucinations, play a large role in the efficacy of psychedelics. Studies in rodents suggest that the therapeutic and subjective effects are dissociable, considering a non-hallucinogenic analog of the psychedelic ibogaine can promote structural plasticity in the brain, attenuate addiction-like behavior, and produce antidepressant-like effects. However, without clinical trials of non-hallucinogenic, plasticity-promoting compounds in humans, it is difficult to determine the relative contribution of altered perception to the therapeutic activity of psychedelics. Despite this, psychedelics represent an incredibly promising avenue for treatment of depression, anxiety, and potentially many other neuropsychiatric disorders, especially in treatment-resistant populations.

References

Beliveau, V., Ganz, M., Feng, L., Ozenne, B., Højgaard, L., Fisher, P.M., Svarer, C., Greve, D.N., Knudsen, G.M., 2017. A High-Resolution *In Vivo* Atlas of the Human Brain's Serotonin System. J. Neurosci. 37, 120–128. https://doi.org/10.1523/JNEUROSCI.2830-16.2016

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biological Psychiatry 47, 351–354. https://doi.org/10.1016/S0006-3223(99)00230-9

Cameron, L.P., Benson, C.J., Dunlap, L.E., Olson, D.E., 2018. Effects of *N* , *N* -Dimethyltryptamine on Rat Behaviors Relevant to Anxiety and Depression. ACS Chem. Neurosci. 9, 1582–1590. https://doi.org/10.1021/acschemneuro.8b00134

Cameron, L.P., Tombari, R.J., Lu, J., Pell, A.J., Hurley, Z.Q., Ehinger, Y., Vargas, M.V., McCarroll, M.N., Taylor, J.C., Myers-Turnbull, D., Liu, T., Yaghoobi, B., Laskowski, L.J., Anderson, E.I., Zhang, G., Viswanathan, J., Brown, B.M., Tjia, M., Dunlap, L.E., Rabow, Z.T., Fiehn, O., Wulff, H., McCorvy, J.D., Lein, P.J., Kokel, D., Ron, D., Peters, J., Zuo, Y., Olson, D.E., 2021. A non-hallucinogenic psychedelic analogue with therapeutic potential. Nature 589, 474–479. https://doi.org/10.1038/s41586-020-3008-z

Carhart-Harris, R.L., Goodwin, G.M., 2017. The Therapeutic Potential of Psychedelic Drugs: Past, Present, and Future. Neuropsychopharmacology 42, 2105–2113. https://doi.org/10.1038/npp.2017.84

DiazGranados, N., Ibrahim, L.A., Brutsche, N.E., Ameli, R., Henter, I.D., Luckenbaugh, D.A., Machado-Vieira, R., Zarate, C.A., 2010. Rapid Resolution of Suicidal Ideation After a Single Infusion of an *N* -Methyl- D -Aspartate Antagonist in Patients With Treatment-Resistant Major Depressive Disorder. J. Clin. Psychiatry 71, 1605–1611. https://doi.org/10.4088/JCP.09m05327blu

Dunlap, L.E., Azinfar, A., Ly, C., Cameron, L.P., Viswanathan, J., Tombari, R.J., Myers-Turnbull, D., Taylor, J.C., Grodzki, A.C., Lein, P.J., Kokel, D., Olson, D.E., 2020. Identification of Psychoplastogenic *N* , *N* -Dimethylaminoisotryptamine (isoDMT) Analogues through Structure–Activity Relationship Studies. J. Med. Chem. 63, 1142–1155. https://doi.org/10.1021/acs.jmedchem.9b01404

Halberstadt, A.L., Geyer, M.A., 2011. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. Neuropharmacology 61, 364–381. https://doi.org/10.1016/j.neuropharm.2011.01.017

Hendricks, P.S., Johnson, M.W., Griffiths, R.R., 2015. Psilocybin, psychological distress, and suicidality. J Psychopharmacol 29, 1041–1043. https://doi.org/10.1177/0269881115598338

Ionescu, D.F., Swee, M.B., Pavone, K.J., Taylor, N., Akeju, O., Baer, L., Nyer, M., Cassano, P., Mischoulon, D., Alpert, J.E., Brown, E.N., Nock, M.K., Fava, M., Cusin, C., 2016. Rapid and Sustained Reductions in Current Suicidal Ideation Following Repeated Doses of Intravenous Ketamine: Secondary Analysis of an Open-Label Study. J. Clin. Psychiatry 77, e719–e725. https://doi.org/10.4088/JCP.15m10056

Johnson, M.W., Griffiths, R.R., 2017. Potential Therapeutic Effects of Psilocybin. Neurotherapeutics 14, 734–740. https://doi.org/10.1007/s13311-017-0542-y

Lowe, H., Toyang, N., Steele, B., Valentine, H., Grant, J., Ali, A., Ngwa, W., Gordon, L., 2021. The Therapeutic Potential of Psilocybin. Molecules 26, 2948. https://doi.org/10.3390/molecules26102948

Ly, C., Greb, A.C., Cameron, L.P., Wong, J.M., Barragan, E.V., Wilson, P.C., Burbach, K.F., Soltanzadeh Zarandi, S., Sood, A., Paddy, M.R., Duim, W.C., Dennis, M.Y., McAllister, A.K., Ori-McKenney, K.M., Gray, J.A., Olson, D.E., 2018. Psychedelics Promote Structural and Functional Neural Plasticity. Cell Reports 23, 3170–3182. https://doi.org/10.1016/j.celrep.2018.05.022

Murrough, J.W., Perez, A.M., Pillemer, S., Stern, J., Parides, M.K., aan het Rot, M., Collins, K.A., Mathew, S.J., Charney, D.S., Iosifescu, D.V., 2013. Rapid and Longer-Term Antidepressant Effects of Repeated Ketamine Infusions in Treatment-Resistant Major Depression. Biological Psychiatry 74, 250–256. https://doi.org/10.1016/j.biopsych.2012.06.022

Nair, A., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharma 7, 27. https://doi.org/10.4103/0976-0105.177703

Nichols, D.E., 2020. Psilocybin: from ancient magic to modern medicine. J Antibiot 73, 679–686. https://doi.org/10.1038/s41429-020-0311-8

Rucker, J.J.H., Iliff, J., Nutt, D.J., 2018. Psychiatry & the psychedelic drugs. Past, present & future. Neuropharmacology 142, 200–218. https://doi.org/10.1016/j.neuropharm.2017.12.040

Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., 2006. Acute and Longer-Term Outcomes in Depressed Outpatients Requiring One or Several Treatment Steps: A STAR*D Report. Am J Psychiatry 13.

Ting, J.T., Lee, B.R., Chong, P., Soler-Llavina, G., Cobbs, C., Koch, C., Zeng, H., Lein, E., 2018. Preparation of Acute Brain Slices Using an Optimized N-Methyl-D-glucamine Protective Recovery Method. JoVE 53825. https://doi.org/10.3791/53825

Zarate, C.A., Brutsche, N.E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C.A., Liberty, V., Luckenbaugh, D.A., 2012. Replication of Ketamine's Antidepressant Efficacy in Bipolar Depression: A Randomized Controlled Add-On Trial. Biological Psychiatry 71, 939–946. https://doi.org/10.1016/j.biopsych.2011.12.010

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A Randomized Trial of an N-methyl-D-aspartate Antagonist in Treatment-Resistant Major Depression. ARCH GEN PSYCHIATRY 63, 9.