

Chapter 1

Introduction



1.1. General introduction

Cells are the fundamental building blocks of life, yet there are innumerable processes that take place within a single cell. On average, the human body contains about 100 trillion cells, each of which has evolved to serve a specific purpose. Amongst the largest and most complex of these cells are those that make up the brain. The brain consists of two primary cell types, neurons and glia. Glial cells are responsible for supporting neurons in a variety of ways and are crucial for axon myelination, cleanup of cellular debris, neuronal migration, and formation of the blood-brain barrier, among other things¹. Neurons are unique in that they communicate with each other at synapses, specialized sites of cell-cell contact of which the average human brain contains 100-500 trillion. The proper establishment of synaptic contacts and synaptic transmission is critical not only for the performance of basic, involuntary tasks such as breathing and the regulation of blood flow, but for carrying out both simple and complex behaviors including walking, talking, and learning. Synaptic transmission depends on the concentration of specific proteins on both sides of the synapse and their segregation between different kinds of synapses. Inhibitory and excitatory synapses, containing different sets of proteins, are present and functional within a few micrometers of each other along the same dendrite. Together, the signals received at these synapses determine the activity level of the entire neuron and ultimately its participation within complex neural networks. Cellular neuroscience as it currently exists owes its foundations to the work of Camillo Golgi and Santiago Ramón y Cajal in the late 1800s. Their discovery that the brain is made up of individual cells which function to signal to one another laid the basis for our understanding of brain organization, neuronal cell types, and intracellular signaling. Research in the years following those initial descriptions of brain cells has led to immense amounts of knowledge regarding the physiological communication between neurons, the roles of different brain regions and cell types in different behaviors, and the processes which take place within a single neuron.

1.2. Cell biology of neurons

During the last two decades major steps have been taken towards understanding the molecular and cellular mechanisms underlying brain function. Much progress has been made by the cloning of neurotransmitter receptors, the determination of SNARE

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function at presynapses, the investigation of postsynaptic density (PSD) proteomics, and the elucidation of receptor trafficking mechanisms involved in long-term potentiation and long-term depression. Electrophysiological techniques such as extracellular and whole-cell patch clamp recordings *in vitro* and *in vivo* have yielded invaluable amounts of information critical to unraveling the mechanisms of synapse function and development. Additionally, genetic approaches have unique benefits to neuroscience research. The phenotypes of knock-out and mutant mice remain the best approximation of gene function in humans, and genetic screening in *C.elegans* and *Drosophila* is a powerful tool for the isolation of genes that affect synapse formation, growth, and stability.

Cell biological approaches have complemented genetics, biochemistry, and electrophysiology in the study of the mechanisms of neuronal function and development. This has led to the development of tools with which to investigate the workings of the brain at the systems and cognitive levels. Already we can visualize GFP-filled dendritic spines being altered by experience in the brain of transgenic mice², view the real-time activation of neuronal circuits in behaving animals³, and visualize synaptogenesis with highly developed molecular tools⁴. Additionally, our understanding of neuronal structure and function has increased tremendously through the use of primary culture techniques that allow brain cells to develop their normal morphological characteristics and the specialization of subdomains, such as growth cones, axon initial segment, dendritic spines, and inhibitory and excitatory synapses, *in vitro*. Primary neuron culture has become an important tool in neuronal cell biology, especially when combined with the ability to transfect, stain, and live image neurons to address the function of specific proteins in their native cellular context.

1.2.1 Neuronal cultures as a model for how the brain works

Like several other specialized cell types, neurons consist of unique organelles (e.g., synaptic vesicles), cellular structures (e.g., chemical synapses), and activities (e.g., synaptic plasticity). But what makes neuronal cell biology so important, and how is it similar and different from general cell biology? The characteristics of the space in which a cell grows are very important for its development. Neurons serve as a prime example of this on account of their expansive morphology and complex intracellular organization. While other cell types establish polarity, neurons are the only cells that develop axons and dendrites which accumulate specific proteins and have distinct

physiological functions. Since axonal and dendritic compartments are easily identified and segregated from one another and neuronal signaling is clearly regulated temporally and spatially and in response to known extracellular signals, neurons are a unique environment that is especially suited to the study of cellular processes that are harder to investigate in non-neuronal cells. For example, most of our knowledge of regulated exocytosis⁵, long distance microtubule-based transport⁶, receptor scaffolding⁷ and diffusion⁸, and local mRNA translation^{9,10} comes from work done in neuronal cells rather than heterologous cell lines.

How useful, though, are neuronal cultures for understanding the function of the brain? Knowing about what goes on inside a single neuron will obviously not explain how the brain processes visual images or how it makes decisions. However, the relationship between cell biology of neurons and ultimate function of the entire brain has been shown on many occasions. It is still exceedingly difficult to study the molecular characteristics of individual neurons in an efficient and controlled manner within an intact and living brain. However, it is feasible to investigate the development and behavior of live CNS neurons in culture¹¹. Some aspects of neuronal communication can be studied with much higher resolution and much greater control in neuron culture systems, where the underlying physiological processes and molecular mechanisms can be examined more precisely and thoroughly than in the intact brain. The more dynamic approaches and applications in neuronal cell biology (e.g., the turnover of synapses and synaptic receptors) allow for more accurate and direct study of the dynamic organization of the brain and remodeling of neural circuits. This has led to tremendous progress in our understanding of synaptic development, transmission, and signaling, which can then be translated into a more reliable and complete interpretation of electrophysiological and behavioral data from more intact model systems.

1.2.2 Development of neurons in culture

Primary culture of neurons has always been more difficult than culturing non-neural cells and did not become routine until the late 1980s¹². Dissociated culture disperses neurons into a two-dimensional layer, making it easier to analyze specific morphological parameters (such as density of synapses, growth of axons and dendrites, and morphogenesis of dendritic spines), to identify the subcellular distribution of specific molecules, and to follow some processes over time by live cell imaging. Hippocampal neurons in culture are most often derived from the hippocampi of embryonic rats a few

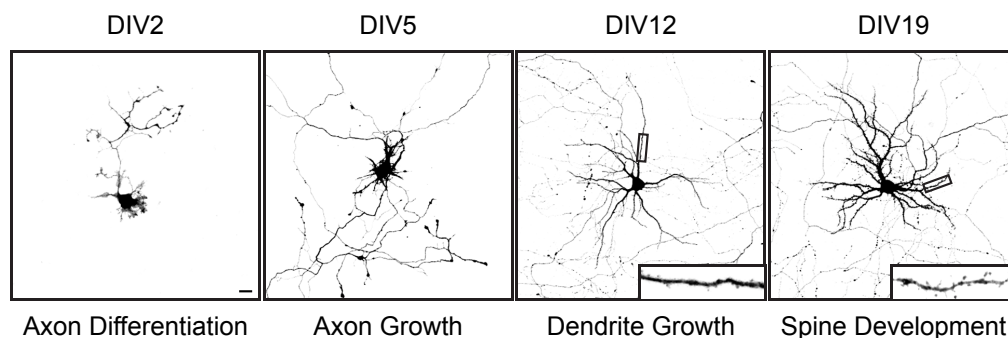


Figure 1.1. *Development of primary hippocampal neuron cultures.*

From left to right, representative images of neurons after 2, 5, 12, and 19 days *in vitro* (DIV2-DIV19). Box indicates zoomed in region. Scale bar, 10 μm .

days before birth (E16-E19). Once they are plated, neurons adhere to the culture dish and almost immediately begin to develop processes that will later become axons and dendrites. After only one to two days *in vitro* (DIV1-2, Figure 1.1), neurons appear asymmetrical, with one neurite having grown significantly more than the others. Over the next few days, this process continues to grow rapidly and begins to branch, eventually displaying the morphological characteristics of an axon. The other processes begin to grow and differentiate into dendrites around DIV4, with dendritic growth peaking from DIV5-7 (Figure 1.1)¹³. After this stage, neuronal polarity is considered to be completely established and neurons continue to grow and mature, forming elaborate networks of axonal and dendritic branches that communicate with each other at functional synapses.

1.2.3 Techniques – transfection, staining, and imaging of cultured neurons

As opposed to the hippocampus *in vivo*, hippocampal neurons in culture are easy to distinguish from one another and can be manipulated in a variety of different ways to facilitate the investigation of all manners of cellular processes that are vital to neuronal function. Exogenous genes can be easily introduced into neurons at any stage in culture, from just plated to fully mature, by electroporation, transfection, or viral infection¹⁴. These genes can encode full length proteins fused to a fluorescent tag to allow for visualization inside the cell, or truncated or mutated proteins that behave differently than the endogenous protein to highlight the importance of particular functions of a given protein. Moreover, the expression of endogenous proteins can be disrupted by the expression of short hairpin (sh) or small interfering (si) RNAs, a process known

as RNA interference (RNAi)¹⁵. Additionally, antibodies designed against native proteins inside the neurons can be used to illuminate their subcellular localizations. Since these tools are useful throughout the lifetime of cultured neurons, they allow for the isolated and rapid examination of most of the processes involved in the development, function, and plasticity of CNS neurons.

Perhaps most advantageous, since primary neuron cultures grow in a monolayer on a glass coverslip, they are easily accessible for fixed and live imaging. In the absence of the depth of tissue surrounding the densely packed neurons inside an intact brain, individual neurons are easily identified and can be imaged at high resolution and for extended periods of time. Confocal microscopy allows for the identification of small clusters of proteins and the observation of their trafficking within the cell with three-dimensional resolution. Furthermore, processes ranging from axon growth and branching to the release of synaptic vesicles in response to membrane depolarization can be imaged in real-time in living neurons. The discovery of fluorescent proteins¹⁶⁻¹⁸ and the continuing development of more versatile and stable variants of fluorescent proteins^{19,20} provides numerous opportunities for the visualization of neuronal growth, development, and function in living cells, and in a reduced system such as primary neuron cultures, the degree of control that can be exerted over the neuron is immense. In all, these techniques make primary neuron cultures an ideal system for studying the fundamental cell biology of neurons, and greater understanding of the way an individual neuron works leads to better interpretation of the data obtained from more *in vivo* preparations.

1.3 Axon Growth, Guidance, and Targeting

As described above, there are multiple processes that characterize the development of a newly differentiated neuron into a fully mature one. Of these, four stand out as major and distinct components of neuronal maturation. In order to function properly as a member of a neuronal circuit, a neuron must send out an axon which can travel long distances and reliably make the correct synaptic contacts and develop a dendritic tree that is capable of integrating the signals received from synaptic contacts. It must build functional pre- and postsynaptic sites that release synaptic vesicles in response to an action potential and respond to the release of vesicles from connected neurons and modify these synaptic contacts in response to their activity.

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The first to occur is the growth of the axon (Figure 1.1)¹³, the neurite which will be responsible for the propagation of action potentials and the sending of signals to other neurons. *In vitro*, axons grow at an average rate of 60 μm per day¹³. Axons *in vivo* have to travel long distances to very specific targets, sometimes more than one meter in humans, thus necessitating rapid growth and efficient pathfinding²¹. The molecular cues responsible for axon growth, guidance, and turning, as well as eventual target selection are still under investigation, though some, for example semaphorins and netrins, are well studied^{22, 23}.

1.3.1 Growth cones

At the tip of each branch of an axon is a growth cone, an actin-rich structure that extends and retracts filopodia while exerting tension responsible for forward growth on the axon itself²¹. Growth cones consist of three domains – the peripheral (P), transitional (T), and central (C) domains (Figure 1.2). The outermost, P, domain is comprised primarily of actin filaments making up lamellipodia and filopodia, while the innermost, C, domain contains a large number of microtubules that are continuous with the rest of the axon, as well as organelles and membranous vesicles. The T domain is a small region at the border between the P and C domains²⁴. Transmembrane proteins on the surface of the growth cone interact with the external environment of the cell and relay information about what they find to the cytoskeleton inside the cell, which in turn stimulates growth, retraction, or turning of the growth cone²¹.

1.3.2 The cytoskeleton and axon growth

The characteristics of axon growth are primarily determined by the interplay and dynamics of the actin and microtubule cytoskeletons within the growth cone. Elongation

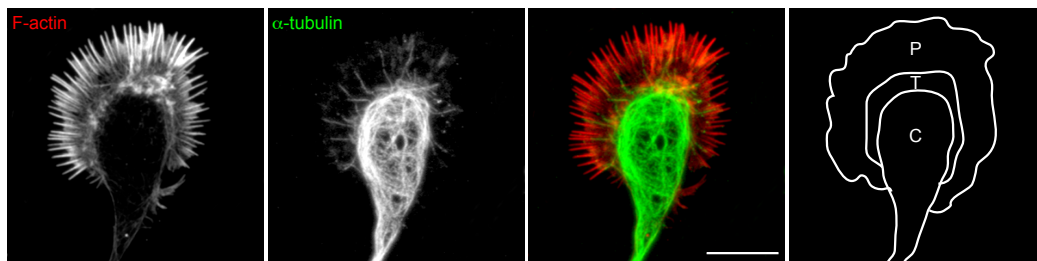


Figure 1.2. Image of an axonal growth cone of a DIV3 neuron.

Neurons were stained for F-actin (far left, red) and α -tubulin (second from left, green). Far right panel shows a cartoon depicting the regions of the growth cone that form the peripheral (P), transitional (T), and central (C) domains. Scale bar, 10 μm .

of an axon is thought to occur by the extensive repetition of three steps – formation of filopodia and lamellipodia at the leading edge of the growth cone (protrusion), thickening at the base of the filopodium and movement of vesicles and organelles into that space (engorgement), and reshaping the morphology of the filopodium to become continuous with the axon proper (consolidation)²⁴⁻²⁶. Importantly, axon growth occurs in a regulated, rather than constitutive, fashion²¹. The interaction of substrate receptors, extracellular matrix receptors, and cell adhesion molecules on the surface of the growth cone with signaling molecules outside of the cell induces an increase in the strength of their connection to the actin network within the growth cone²¹. This, in turn, results in a shift towards forward growth of actin filaments and less inhibition of microtubule polymerization, leading to growth of the entire axon²⁷.

1.3.3 Directed axon growth and guidance cues

In addition to controlling axon growth itself, the extracellular environment controls the direction in which the axon grows. Axon guidance is thought to be mediated by the presence of gradients of attractive and repulsive cues external to the cell²⁴. Receptors in the growth cone respond to these cues by modulating the actin and microtubule cytoskeletons within the growth cone and activating or repressing a variety of secondary signaling pathways²⁸. Some axon growth and guidance cues, such as semaphorins and netrins, are secreted by other cells near the developing axon^{22, 23}. Semaphorins are generally considered to be chemorepellants, meaning that the growth cone will turn away from high amounts of semaphorin protein, while netrins are primarily chemoattractants, although it is important to note that, in both cases, specific axons behave in an opposite fashion^{22, 28}. In addition to secreted guidance cues, axon growth and turning are also modulated by homo- and heterophilic interactions between transmembrane proteins on different cells. For example, both receptor protein tyrosine phosphatases (RPTPs)²⁹ and ephrins and their eph receptors²² are necessary for axon growth, and perhaps more importantly, eventual target selection and synapse formation. Intriguingly, a single growth cone contains a set of receptors that respond to many guidance cues, and the net growth of an axon is dependent on the integration of the signals it receives²². This fact simultaneously increases the possible directions each axon can grow and allows each neuron to interpret the same cues in different ways.

1.4 Dendrite Development

At around DIV5, axon growth begins to slow and the other neurites begin to grow, branch, and develop into dendrites (Figure 1.1)¹³. Mature dendrites are the primary site on which a neuron receives synaptic input from other neurons¹. Each neuron has a unique pattern of dendritic arborization, which, for hippocampal pyramidal neurons, is characterized by a long apical dendrite and many shorter basal dendrites³⁰. In culture, this morphology is not as readily distinguishable, likely due to the absence of a regulated pattern of orienting signals as would be found *in vivo*³¹. However, the dendrites of cultured hippocampal neurons do display the other key morphological features necessary to carry out the process of receiving, distinguishing, and integrating synaptic signals, namely, an extensively branched network of tapered projections characterized by the presence of numerous shorter, mushroom-headed protrusions called spines³².

1.4.1 Dendritic growth and branching

Although dendrites only grow at about one-fifth the speed of axons¹³, they respond to similar growth cues³³⁻³⁵. These combined phenomena indicate that there may be an intrinsic switch within individual neurons between the support of axonal and dendritic growth²¹. However, the growth and maturation of axons and dendrites do differ in some key ways. For example, while axon morphology is fairly similar for most types of neurons, neurons in different parts of the brain often display radically different dendritic branching patterns³⁶. Generally speaking, these patterns are thought to result from a combination of factors intrinsic to the neuron, diffusible growth and guidance cues in the cell's environment, contacts with other cells, and neuronal activity³⁷.

Similar to axon growth, dendritic growth is characterized by the extension and retraction of both the primary neurites originating at the cell soma and secondary neurites emanating from the primary neurites³¹. After a period of tremendous plasticity, certain branches become stabilized, and the structure of the dendritic arbor remains relatively constant for the remainder of the neuron's lifetime (Figure 1.1)³⁷. In maturity, dendritic organization is likely critical for the integration of synaptic signals, as the location of synapses on the dendrite determines the degree to which those synapses influence action potential initiation in the neuron³⁰.

1.4.2 Dendritic spines

The large majority of excitatory postsynaptic sites in hippocampal neurons are found not on the dendritic shaft itself, but on small projections off of the main dendrite called dendritic spines (Figure 1.1)³⁸. Developing neurons possess a large number of motile and dynamic filopodia^{39,40}, which can, in cultured neurons, initiate contact with neighboring axons⁴⁰. Though the mechanism by which they do so varies^{38,41}, filopodia form the basis for eventual dendritic spines^{39,42,43}. The maturation of spines is characterized by the assembly of pre- and postsynaptic specializations in physically adjacent neurons stabilized by transsynaptic adhesion molecules⁴¹, with one spine usually containing one synapse³⁸. However, spine morphology is tremendously variable, even in mature neurons, and changes in spine shape are correlated with changes in synaptic strength^{38,41}. Long-term *in vivo* imaging also revealed activity-dependent plasticity in spine morphology^{2,44}, though to a lesser degree than in cultured neurons.

1.5. Formation and Function of Synapses

The unique morphology of neuronal cells has specifically evolved to facilitate the rapid and reliable transmission of signals between neurons. The actual sites of communication between neurons, though, are synapses, and, molecularly speaking, chemical synapses throughout the brain are remarkably similar⁴⁵. A synapse consists of three major components – the presynapse, which sends signals by releasing neurotransmitter-containing vesicles in response to depolarization, the postsynapse, which receives signals due to the opening of ligand-gated ion channels upon binding of neurotransmitter, and the synaptic cleft, the extracellular space between the pre- and postsynaptic sites (Figure 1.3)⁴⁶. The orderly and reliable assembly, maintenance, and plasticity of synapses is critical to neuronal function, and numerous proteins play important roles in ensuring the fidelity of synaptic transmission.

1.5.1 Synaptic cell adhesion molecules

In addition to their importance in general axon guidance (see above), cell adhesion molecules (CAMs) are crucial mediators of target recognition and synaptic assembly⁴⁷, and the abundance and variety of potential CAMs implies that they play key roles in establishing the specificity of synaptic connections⁴⁸. On a molecular level, most CAMs have related structures that allow them a great deal of functional ver-

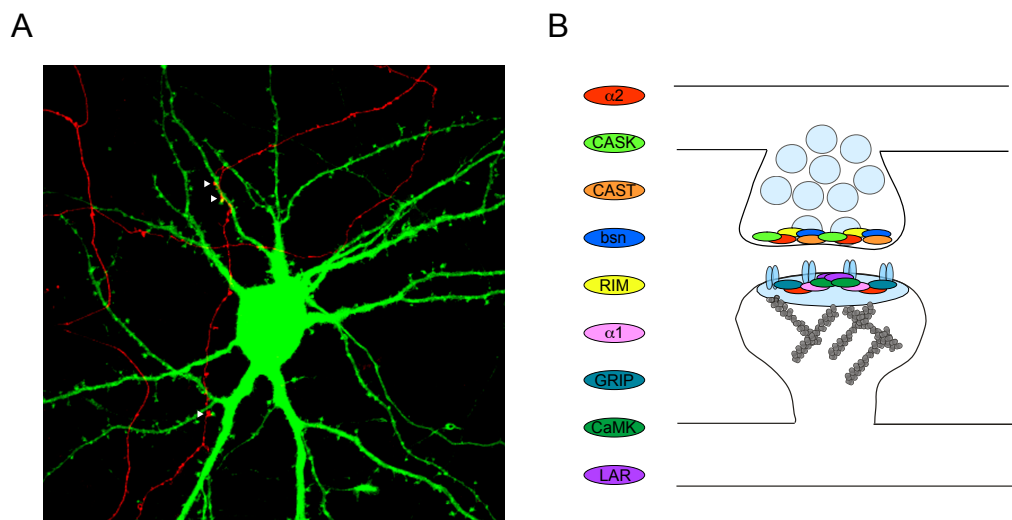


Figure 1.3. Image and model of synaptic sites in cultured hippocampal neurons.

(A) Image of synaptic contacts being made between mature (DIV19) hippocampal neurons in culture. Arrows designate sites where a presynaptic site within an axon (red) is juxtaposed with a dendritic spine (green), forming a putative synapse.

(B) Cartoon representing selected components of a typical synapse. The presynapse contains synaptic vesicles (light blue circles) as well as the proteins liprin- α 2 (red), CASK (green), CAST1/2 (orange), bassoon (blue), and RIM (yellow). The postsynapse contains glutamate receptors and the postsynaptic density (light blue), actin filaments (grey), and the proteins liprin- α 1 (pink), liprin- α 2 (red), GRIP (turquoise), CaMKII (dark green), and LAR (purple).

satility. Many are transmembrane proteins whose extracellular region contains Immunoglobulin (Ig) or Ig-like domains that facilitate homo- and heterophilic interactions with other CAMs and extracellular matrix molecules, while their intracellular region acts as a primary signaling molecule, often a kinase or phosphatase, that serves to initiate diverse signaling cascades inside the neuron⁴⁷⁻⁴⁹. Adhesion molecules are thought to perform four major tasks with regards to the development and function of a synapse: (1) to provide stability by physically linking pre- and postsynaptic cells, (2) to recognize specific synaptic targets, especially in densely packed networks of neurons, (3) to initiate differentiation of the pre- and postsynaptic membrane, and (4) to regulate synapse structure and function⁴⁹. Two recent papers have again shown key roles for cell adhesion molecules in determining synaptic contacts and inducing synaptic differentiation^{50, 51}, further emphasizing their importance in synaptogenesis and synaptic function.

1.5.2 Synaptic scaffolding and signaling proteins

Following cell adhesion, the machinery necessary to conduct synaptic transmission

begins to accumulate at nascent synaptic sites⁵². This leads to two electron dense thickenings of the pre- and postsynaptic membranes, called the cytomatrix at the active zone (CAZ) and postsynaptic density (PSD), respectively (Figure 1.3). These two structures are positioned directly across the synaptic cleft from one another⁴⁷, and are composed of a tightly packed meshwork of scaffolding and signaling proteins that control presynaptic vesicle release^{48, 53, 54} and postsynaptic receptor trafficking⁷. Many molecules, for example CaMKII^{55, 56}, CASK⁵⁷, and liprin- α ⁵⁸, are major components of both the CAZ and the PSD, while others, like bassoon and piccolo (pre)⁵³ and PSD-95⁵⁹ and homer⁶⁰ (post), are only found on one side of the synapse. Proteins in the CAZ and PSD seem to be primarily important in two aspects of synaptic function. First, they provide a stable framework that ensures rapid and reliable synaptic transmission, and second, they act as major signaling molecules to modulate synaptic strength and efficacy in response to the activity of the neuron.

1.5.3 Synaptic vesicles and receptors

Synaptic transmission itself is, however, the release of neurotransmitter from presynaptic vesicles into the synaptic cleft, where it binds and activates postsynaptic receptors (Figure 1.3)¹. In the hippocampus, glutamate is the major excitatory neurotransmitter¹, and ionotropic glutamate receptors mediate the majority of the postsynaptic response to neurotransmitter release⁶¹. The synaptic vesicle cycle consists of a stereotyped set of steps that form the basis of presynaptic function. Neurotransmitters are actively loaded into synaptic vesicles which cluster around the active zone. These vesicles are then docked and primed for release. Neurotransmitter release due to the SNARE-mediated fusion of docked vesicles with the plasma membrane is triggered by Ca^{2+} influx stimulated by an action potential, and is immediately followed by endocytosis and recycling of synaptic vesicles back into the total pool of vesicles at a synapse⁶². In addition to SNAREs such as VAMP2 and SNAP-25, active zone and synaptic vesicle-associated proteins such as munc13, munc18, synaptotagmin, and RIM1 play key roles in the regulation of the synaptic vesicle cycle⁶³. Active zones in cultured hippocampal neurons contain more than 200 synaptic vesicles⁶², on average 4-8 of which are docked⁶⁴. This represents the readily releasable pool of vesicles, or those that are available for immediate release upon high-frequency stimulation⁶². Another ~20 vesicles make up the reserve pool of recycling vesicles⁶⁴, while the remainder populate a large resting pool⁶⁵.

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Once glutamate is released by the presynaptic neuron, it binds to and activates glutamate receptors in the postsynaptic membrane (Figure 1.3). There are two major classes of glutamate receptors found at hippocampal postsynapses, ionotropic and metabotropic glutamate receptors⁶¹. Ionotropic glutamate receptors open after binding glutamate, while metabotropic receptors respond to glutamate activation by initiating G-protein linked signaling cascades⁶¹. N-methyl-D-aspartate (NMDA) type receptors are Ca^{2+} permeable cation channels, while α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) type receptors are largely Ca^{2+} impermeable⁶⁶. Though activation of AMPA receptors is the primary means of postsynaptic depolarization at excitatory synapses, NMDA receptors play a critical role in the regulation of AMPA receptors and contribute to neurotransmission as well^{61, 66}.

1.6. Maintenance and Plasticity of Synapses

Although in fixed light and electron microscopic images, mature synapses appear rather static, they are in fact anything but. Though differentiated neurons are post-mitotic and therefore not replaced over the course of an organism's lifetime, synapses are constantly being created, eliminated, and modified³². This occurs on a number of different levels, from basic maintenance of synaptic structure to long-term alterations of synaptic strength in response to activity.

1.6.1 Protein Turnover

In order to understand the molecular dynamics of synaptic transmission, one must understand the dynamics of the proteins that control synapse function, and while the lifetime of neurons and even single synapses can be on the order of days, months, or years, the lifetime of individual proteins is more properly measured in seconds, minutes, or hours. This means that all synapses are in a constant state of molecular flux, wherein the proteins that make up the synapse exchange in both a constitutive and a regulated fashion. Therefore, the work of building a synapse is never complete, as the former ensures proper maintenance of synaptic function, and the latter allows for extensive modification of synaptic efficacy and strength^{67, 68}. In addition to the exchange of proteins between neighboring synapses⁶⁹, the targeted degradation of proteins by the Ubiquitin Proteasome System (UPS) plays a critical role in both the establishment and maintenance of synaptic contacts^{67, 68}. Key components of both the pre- and post-

synaptic compartments are regulated by the UPS, including presynaptic RIM1, UNC-13, and syntaxin-1 and postsynaptic shank, GKAP, and PSD-95⁶⁸. Interestingly, the synaptic localization of numerous postsynaptic proteins⁷⁰ and the proteasome itself⁷¹ is dependent on synaptic activity, though the extent to which this is the case presynaptically is still under investigation (see Chs. 4 and 5).

1.6.2 Modification of synapses in response to activity

Synaptic plasticity occurs in two forms, short-term and long-term, both of which are heavily dependent on Ca^{2+} concentration at the synaptic site. Short-term plasticity involves changes in synaptic strength that last on the order of minutes and is primarily a presynaptic phenomenon, whereby the synaptic vesicle release probability at a synapse is altered, albeit not permanently, by the prior activity state of that synapse⁷². Short-term synaptic depression is usually caused by a decrease in the number of docked and primed vesicles available for immediate release, and is therefore heavily influenced by synaptic vesicle recycling⁷². Multiple studies have shown that synapses have the capacity to alter the dynamics of the synaptic vesicle cycle⁷², though the ultimate consequences of short-term presynaptic plasticity in terms of overall network activity and behavior are still not clear.

In contrast to short-term plasticity, long-term plasticity refers to changes in synaptic strength that last for hours or even days. Although particular synapses display presynaptic long-term plasticity, the predominant form is expressed postsynaptically⁶⁶. The degree of Ca^{2+} influx through NMDA receptors activates signaling cascades within the dendritic spine, which leads to the regulated insertion or removal of AMPA receptors from the postsynaptic membrane^{61, 66, 74}. High frequency stimulation of NMDA receptors over a short period of time, and therefore a rapid increase in the Ca^{2+} concentration in the spine, results in long-term potentiation (LTP), while less intense but prolonged stimuli lead to long-term depression (LTD)⁶⁶. Interestingly, the distinct morphology of dendritic spines allows for the isolation of these Ca^{2+} signals, ensuring that one synapse can be potentiated while its neighbor is depressed, and therefore altering the contribution each individual spine makes to the overall activity of the neuron⁷⁵.

1.6.3 Learning and Memory

In particular, long-term plasticity has tremendous implications for behavioral process-

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es such as learning and memory. Significant changes in synaptic integration within the dendritic tree result in significant changes in the firing properties of that neuron, and therefore changes in the entire network within which that neuron functions⁷⁵. Intriguingly, genes encoding for proteins that are key in the molecular pathways mediating long-term plasticity have also been shown to be important in determining the performance of mice in learning-dependent behavioral assays^{76, 77}. Though our understanding of the fundamental processes underlying learning and memory has increased tremendously, a complete understanding of the molecular function of the synaptic terminal is still elusive.

1.7. Scope of the Thesis

The establishment and maintenance of synaptic transmission is a highly regulated and choreographed process, and innumerable proteins play key roles at each stage of synaptic development and function. In this thesis, we examine the role of liprin- α proteins in neuronal development and synaptic function. Chapter 2 explores the expression patterns of liprins in the brain on anatomical and subcellular levels. Chapter 3 describes the importance of liprin- $\alpha 2$ as a coordinator of LAR-RPTP signaling in axon growth and branching in hippocampal neurons. In Chapter 4 we show evidence of activity dependent regulation of liprin- $\alpha 1$ protein levels due to degradation by active CaMKII and highlight the importance of this mechanism in LAR-RPTP trafficking and dendrite development. Chapter 5 illuminates the differential roles of liprin- $\alpha 1$ and liprin- $\alpha 2$ in the molecular organization and subsequent function of the presynaptic terminal. Chapter 6 provides a comprehensive description of the experimental procedures involved in this thesis. Chapter 7 consists of a general discussion of the role of liprins in the neuronal development and synapse function and highlights future research directions.